THE 18TH INTERNATIONAL CONFERENCE ON HARMFUL ALGAE

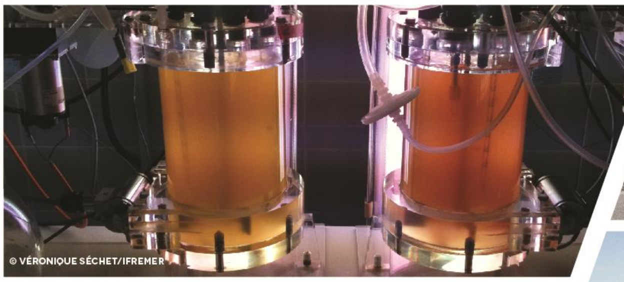
FROM ECOSYSTEMS TO SOCIO-ECOSYSTEMS

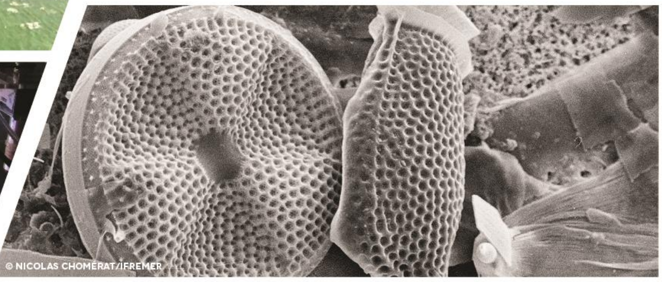
ICHA
2018
21 - 26 OCTOBER
NANTES, FRANCE

ABSTRACT BOOK


© MINYVEL ENVIRONNEMENT/PHENOMER


© FATMA GUELLATI/UPMC-UNIV BADJI MOKHTAR


© VÉRONIQUE SÉCHET/IFREMER


© NICOLAS CHOMÉRAT/IFREMER


© OLIVIER DUGORNAY /IFREMER


© OLIVIER BARBAROUX/IFREMER


© STÉPHANE LESBATS/IFREMER

ORGANIZED BY
Ifremer

CONVENER
ISSHA

WITH SUPPORT OF
Phycotox
Groupement de Recherche
GIS Cyanobactéries
Région PAYS DE LA LOIRE
AMIcroalgae Atlantic

WWW.ICHA2018.COM

WELCOME MESSAGE FROM ICHA 2018 CHAIR

Dear participants,

As chairman of ICHA 2018, and on behalf of the scientific and international advisory committees, I am pleased to present you the abstract book for ICHA 2018.

We are delighted to have a rich set of 623 contributions from 58 countries for which I thank all authors. This edition should be an interesting event with over 700 participants. The abstract book follows the program layout which contains 9 plenary and 255 oral presentations in 45 parallel sessions, as well 45 ignite and 359 poster presentations. While the large interest is probably partly due to the two previous ICHA conferences taking place in the Southern hemisphere, we believe that it is also a testimony to a persistent research effort on harmful algae, a continuing and, in many places, growing problem for society. In line with the design of our French research network GdR PHYCOTOX and other international efforts, e.g. GlobalHAB, we have attempted to include all aspects from the causes of micro-algae and cyanobacteria to their impacts on both society and ecosystems; hence our subtitle FROM ECOSYSTEMS TO SOCIO-ECOSYSTEMS.

While the choice of topics is a continuation from the previous conferences, I would like to take the opportunity to thank all the scientific committee members who accompanied me with a lot of enthusiasm in designing the conference, especially those working on cyanobacteria and those studying impacts. The scientific committee consisted of 45 scientists from the GdR PHYCOTOX and the GIS CYANO research networks and supported the organisation by formulating the large set of topics and by suggesting plenary speakers and international session chairs. The international session chairs, as well as members of the ISSHA Council conference-subcommittee, also helped the scientific committee in the evaluation of submissions. We went through two rounds of evaluation (one blind and one with affiliations known). Criteria for selection as oral or poster presentation were based on scientific novelty, societal interest of the contribution, gender and geographical balance and publication status. We also made every effort to balance contributions from experienced and young scientists as much as possible in the oral and ignite sessions.

I would also like to very heartily thank our 32 sponsors whose participation also testifies toward the societal importance of HAB-research. In particular, the support of ISSHA, NOAA, SCOR, IAEA and GdR PHYCOTOX has helped many (ca. 60) young scientists to attend, and 137 student contributions will be evaluated for the *Maureen Keller Best Student* awards.

I hope you all draw inspiration from this compilation – enjoy the read!

Philipp Hess
Chairman of ICHA 2018, Nantes, France

TABLE OF CONTENTS

All lectures are numbered according to their order in the program grid

PLENARY INVITED LECTURES

OL

Exploring climate impacts on inland and coastal eutrophication

Anna Michalak
Carnegie Institution for Science, Las Altos, California, USA

Abstract: Questions surrounding water sustainability, climate change, and extreme events are often framed around water quantity – whether too much or too little. The massive impacts of water quality impairments are equally compelling, however, and recent years have provided a host of compelling examples of unprecedented harmful algal blooms. Linkages between climate change and water quality impacts are not well understood, however. Several factors explain this lack of understanding, including the relative complexity of underlying processes, the spatial and temporal scale mismatch between hydrologists and climatologists, and observational uncertainty leading to ambiguities in the historical record. Here, we draw on a number of recent studies that aim to quantitatively link meteorological variability and water quality impacts. Focusing on eutrophication and harmful algal blooms, this talk will frame challenges and opportunities related to characterizing water quality, bridging from local to global scales, identifying key drivers, and understanding the role of climate. In each case, the availability of new observational records and modeling tools makes it possible to develop and test novel hypotheses about the role of climate and what the future may hold.

Disclosure of Interest: None Declared

PL1

21st century alternative methods for 21st century safety sciences

Thomas. Hartung[1 2,*]

[1] Johns Hopkins Center for Alternative to Animal Testing (CAAT), Baltimore, USA [2]CAAT-Europe, University of Konstanz, Konstanz, Germany

Abstract: Increasingly, the limitations of animal-based toxicology to predict human health threats are recognized. However, we also recognize more and more shortcomings of traditional (human) cell culture. These include cell identity, differentiation, genetic stability and mycoplasma infection as well as non-homeostatic and non-physiological culture conditions. The increasing pace of technological developments of modern cell culture and their integration leads to what is called “disruptive technologies”. The development of alternatives to traditional approaches for product development and safety assessment benefits from this. The combination of cell culture with bioengineering has led to a number of technologies to make cell culture more organo-typic, such as 3D culture, human stem cell-derived systems, perfusion, co-cultures, combinations with scaffolds and sensors etc.. Increasingly, they lead to “organ-on-chip” or even multi-organ “human-on-chip” solutions. By recreating organ architecture, homeostasis of the cell environment and organ functionality, these models mirror more closely the physiological situation. The example of our human iPSC-derived mini-brain is used to illustrate this. The commercial availability of organoids also improves standardization and reproducibility.

Another disruptive technology is computational toxicology. Local models of QSAR and receptor binding have for long supported drug development. More recently, also machine-learning-based global models based on chemical similarity combined with big data become available. They will have to show their merit and especially are only starting to be applied for systemic toxicities, but they help to flag toxic liabilities early in the development process and the handling of contaminants also by deriving thresholds of toxicological concern.

Such technological advances together with mechanistic approaches, Integrated Testing Strategies and evidence-based methods promise real change how we assess the biological effects of substances.

Disclosure of Interest: None Declared

PL2

A trait-based framework for understanding and predicting harmful algal blooms

Elena. Litchman[1,*]
[1] Michigan State University, East Lansing, Michigan, USA

Abstract: Understanding and predicting the occurrence, severity and composition of Harmful Algal Blooms is becoming more urgent, as their frequency increases around the globe. Using a trait-based framework, where the focus is on functional traits may be a promising way to increase our understanding of HABs and the ability to predict them. Here I highlight some of the key traits that may distinguish the HAB taxa from other phytoplankton and determine their success under specific conditions. I then discuss trait differences and similarities across different HAB groups and individual species and the general patterns in trait distributions along major environmental gradients. I also give examples of different trait classifications that could be helpful in organizing our thinking about traits. Finally, I outline future challenges of trait-based approaches to HABs.

Disclosure of Interest: None Declared

PL3

Cyanobacteria in Danish and Florida lakes and their response to nutrient loading reduction, biomanipulation and climate warming

Erik Jeppesen,
Martin Søndergaard, Lislotte Sander Johansson, Torben L. Lauridsen, Thomas A. Davidson
Aarhus University, Silkeborg, Denmark and Sino-Danish Centre for Education and Research (SDC), Beijing, China
Daniel E. Canfield Jr., Roger W. Bachmann, Mark V. Hoyer
University of Florida, Gainesville, Florida, USA

Abstract

In this talk we will first describe the cyanobacterial dynamics in Danish waters with contrasting nutrient concentration, depth and retention time. Typically a shift occurs from green algae dominance at high N and P loading in shallow lakes, towards cyanobacteria, first non-heterocystous taxa toward those with heterocysts and finally diatoms and a more diverse community. In deep lakes a shift occurs from cyanobacteria and dinophytes towards a more diverse community when moving from high to low nutrient loading. We will also show how cyanobacteria and other algal groups have responded to nutrient loading during the past 20 years. Generally they follow the response pattern expected from the multi-lake data, with some delay due to internal nutrient loading. Using data also from numerous Florida lakes we will further focus on the seasonal dynamics and year-to-year variation as well as how other factors such as climate (+), zooplankton (-), retention time (+) and submerged macrophytes (-) affect cyanobacteria biomass. Finally, we will discuss how cyanobacteria respond to lake restoration by fish manipulation, showing particularly strong decline in growth of these algae after fish removal.

Disclosure of Interest: None Declared

PL4

Ciguatera risk assessment and management: where to from here?

Mireille. Chinain[1,*]

[1] Institut Louis Malardé, Laboratoire des Micro-algues Toxiques - UMR 241 EIO, BP 30, 98713 Papeete-Tahiti, French Polynesia

Abstract: Ciguatera is a well-known illness resulting from the consumption of fish and marine products naturally contaminated with ciguatoxins (CTXs). CTXs are produced by benthic dinoflagellates in the genus Gambierdiscus whose recent expansion to temperate regions has become a major source of concern. This has prompted the implementation of multiple risk assessment and management programs at a local, regional and international scale in order to mitigate the adverse effects of ciguatera.

Climate change is arguably one of the many factors likely to exacerbate the global expansion of ciguatera and its harmful effects on populations. The case of Rapa Iti, an island located in the southernmost part of the Australes archipelago in French Polynesia, is a perfect illustration on how global warming can dramatically affect the health of island communities whose basic nutrition widely relies on lagoon resources.

Globally, the economic impacts from ciguatera likely exceeds that from any other form of hazardous algal blooms, not to mention its major health impacts on Pacific Islands Countries and Territories (PICTs) populations by far the most vulnerable to this threat. In this respect, French Polynesia has been the theatre of almost 5 decades of research on ciguatera and, as such, has witnessed two of the former scientific breakthroughs in this field of research. Several decades later, the significant progress achieved in both the knowledge-base about this complex eco-toxicological phenomenon and the development of novel monitoring and analytical techniques have led to progressively reconsider several of the early paradigms in ciguatera pertaining to, e.g. the existence of not one but multiple species of Gambierdiscus, the link between ciguatoxicity and fish size, the apparent resistance of fish to CTXs, or the novel implication of marine invertebrates in ciguatera outbreaks. How such advances can be translated into practical field interventions in the framework of ciguatera control programs will be discussed.

Despite the extraordinary mobilization of the scientific community and international agencies around this field of research, toxic blooms and ciguatera outbreaks remain difficult to predict and therefore, to manage. The relevance of an integrated approach for an improved assessment and management of ciguatera risk will be discussed in the light of a recent outbreak reported in the Marquesas islands (French Polynesia).

Disclosure of Interest: None Declared

PL5

Chemical Studies on Toxic Cyanobacteria: Poisons as Pharmaceutical Leads

William. Gerwick[1,*]

[1] Scripps Institution of Oceanography and Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, California, USA

Abstract: Macroscopic marine cyanobacteria are globally prevalent in shallow tropical seas. While their delicate protein-rich filaments contribute to reef biomass and biodiversity, occasionally local nutrient, light and temperature conditions will favor a large expansion in the population of a particular cyanobacterium, and such blooms can overgrow corals and other invertebrate species. Remarkably, they are largely not preyed upon by generalist herbivores due to a rich elaboration of toxic anti-predatory natural products. These very same toxic metabolites can offer promising starting points for the development of novel pharmaceutical wherein a toxic phenotype is desired, such as in the treatment of cancer and infectious diseases including those caused by parasites. This presentation will feature two such cases: the anticancer apratoxins from the marine cyanobacterium Moorea bouillonii and the carmaphycins from a Caribbean collection of a Symploca sp. In the former case, an interesting association between a shrimp and the cyanobacterium exists wherein the shrimp cultivates and weaves the cyanobacterium into a protective nest, and derives cryptic and chemical protection by this close association. A number of complex cyclic lipopeptides have been characterized from the cyanobacteirum, known as the apratoxins, which are toxic by virtue of their inhibition of Sec61, a critical protein involved in facilitating protein synthesis. The apratoxin structure has given rise to a number of synthetic compounds that show promising anticancer activity, especially to pancreatic carcinoma. A second example to be presented will describe our discovery of the carmaphycins, exquisitely toxic proteasome inhibitors. The carmaphycins possess a distinctive epoxy-ketone functionality attached to a peptide chain, and show potent cancer cell as well as parasite toxicity. To explore the pharmaceutical utility of this chemotype, we synthesized over 100 analogs and have evaluated these to several targets, including cancer cells and a variety of parasites. Remarkably, we find that modest modifications to the peptide backbone result in selectivity to the proteasomes of different classes of cells and parasites. As a result, we have promising carmaphycin-inspired analogs for the treatment of cancer, rheumatoid arthritis, Plasmodium falciparum, Shistosomaiasis, and Trichomonas. The development status of several of these promising carmaphycin leads will be presented.

Disclosure of Interest: None Declared

PL6

Can we use parasites to control HABs?

Laure Guillou
Station biologique de Roscoff, France

Abstract: The sudden arrival of harmful algal blooms in a previously preserved area is a worldwide observation. Once installed, these microalgae may become a recurrent problem locally. One solution would be to use natural pathogens to help in their regulation. Even with some limitation, this strategy was successfully applied in agriculture (biological pest controls), and in medicine (phage therapy). Is this strategy suitable for HABs? Based on few examples, we will first review the diversity of known pathogens described so far and their peculiarities. We will then review important concepts in parasitology in order to answer that question.

Disclosure of Interest: None Declared

YASUMOTO-AWARD 2016 LECTURES

YL1

Emerging Chemical Ecology Paradigm or New Plankton Paradox: Phycotoxins as Allelochemical Drivers of Harmful Algal Dynamics

Allan D. Cembella* [1]

[1]Ecological Chemistry, Alfred-Wegener-Institut, Helmholtz Zentrum für Polar-und Meeresforschung, Am Handelshafen, Bremerhaven, Germany

Abstract: Marine phytoplankton produce a wide array of potent phycotoxins, with toxicity defined primarily with respect to mammalian cell targets. Among eukaryotic microalgal species a chemically diverse array of potent bioactive secondary metabolites are produced, including linear and macrocyclic polyethers, tetrahydropurine alkaloids and neurotoxic secondary amino acids. These phycotoxins are typically defined in terms of mammalian cell targets, where the bioactive mechanisms as ion-channel effectors or enzyme inhibitors are generally well known. Early hypotheses of their functional importance to the producing species generally stressed their critical role in chemical defence – as agents in the watery arms race for survival and dominance in the plankton.
Laboratory studies have shown that simple bilateral species competition experiments with toxigenic versus non-toxigenic strains often contradict or fail to support the acute toxicity hypothesis as a general mechanism. Many allelochemicals are poorly chemically defined, but are structurally and functionally unrelated to classic phycotoxins, and also exhibit potent biological activity (cell lysis, immobilization, membrane disruption) against co-occurring species. Recent evidence indicates that chemical ecological interactions can affect and even regulate processes such as competition, predator-prey relationships and chemical communication. The bewildering variation in toxin composition and biosynthetic capacity within and among populations of certain species implies a possible alternative resolution to the Hutchinson plankton paradox, as driven by resource limitation and competition. In this scenario, chemical diversity and toxin interactions could contribute to the maintenance of contemporaneous disequilibrium (hence high diversity) but not necessarily by eliciting an acute lethal toxic response. Benthic dinoflagellate species are heavily overrepresented among toxigenic eukaryotic microalgae, particular as producers of polyketide-derived ion-channel effectors. The chemical ecological role of these polyether toxins remains elusive and poorly defined, but competition for substrate space and defensive function against predators or bacterial biofouling are plausible scenarios. The biosynthetic pathways for polyether toxins, synthesised via modular polyketide synthase genes, have been elucidated in a few cases. Further molecular analysis of the respective gene clusters will assist in defining phylogenetic affinities and eventually the functional role of these toxins in pelagic and benthic ecosystems.

Disclosure of Interest: None Declared

YL2

The Evolution of Algal Toxin Measurement Science

Michael A. Quilliam* [1]
[1]National Research Council of Canada, Halifax, Canada

Abstract: There is little doubt over the importance of monitoring algae and shellfish for the presence of toxins – peoples' lives truly depend on a comprehensive and flawless system for such operations. International trade is also dependent upon accurate measurement of restricted substances in shellfish. From the 1950s until recent years, the mouse bioassay was the primary method. The limitations of this method were well recognized, so efforts were started by researchers such as Rapoport, Shimizu, Oshima, Sullivan and Yasumoto in the late 1970s to develop chemical analytical methods with greater selectivity, sensitivity and reliability. A major problem they encountered was the lack of calibration standards for the various toxins, which made it very difficult not only to develop methods but to implement them widely in regulatory laboratories. In 1987, a project was initiated at the National Research Council of Canada (NRCC) in Halifax and a team of researchers was assembled. The main goal of the project was to produce certified reference materials for algal toxins. In late 1987, a new toxic event called Amnesic Shellfish Poisoning occurred and the research team identified domoic acid as the cause. A calibration solution certified reference material (CRM) was quickly produced and a liquid chromatography (LC) method was developed and implemented into monitoring programs worldwide. Also produced was a mussel tissue matrix CRM, which helped with analytical quality control. The success of these developments has been proven by the fact that there have been no cases of human poisoning by domoic acid since that first event. Over the next thirty years, a wide range of CRMs covering most classes of algal toxins have been developed at the NRCC. Following the CRMs was an explosion of new methods, ranging from LC through immunoassays and biochemical assays, developed by many research groups. The development of LC-MS provided a major breakthrough and this technique is now used worldwide in most toxin research and regulatory laboratories. LC-MS also led to discovery of many new toxins, further complicating the challenge of toxin monitoring. This talk will review some highlights over the last thirty years and provide some indications of future directions in the field of toxin measurement science.

Disclosure of Interest: None Declared

ORAL COMMUNICATIONS

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-001

Exploring the molecular basis of responses to light in marine diatoms

M. Jaubert [1], J.-P. Bouly [1], L. Taddei [1], A. Falciatore [1,*]

[1]CNRS, Institut de Biologie Paris-Seine, Laboratory of Computational and Quantitative Biology, Sorbonne Université, Paris, France

Abstract: Light is an essential source of energy for life on Earth and is one of the most important signals that organisms use to obtain information from the surrounding environment, on land and in the oceans. Prominent marine microalgae, such as diatoms, display a suite of sophisticated responses (physiological, biochemical, and behavioural) to optimize their photosynthesis and growth under changing light conditions. However, the molecular mechanisms controlling diatom responses to light are still largely unknown. To fill this important knowledge gap, we are integrating genomic and genetic resources with physiological and biophysical approaches to characterize different light-driven processes in diatoms. These works are unveiling a functional diversification of the diatom photoreceptors (Fortunato et al., Plant Cell 2016; Jaubert et al., Curr Opin Plant Biol. 2017), raising novel hypotheses on the role of these light sensors in controlling growth and adaptive responses in a marine context. We are also characterizing the still largely unknown regulators of diatom photosynthesis. Recent investigations uncovered the key function played by members of the light-harvesting complex stress-related family, LHCXs, in the regulation of photoprotective mechanisms (Bailleul et al., PNAS 2010; Taddei et al., J Exp Bot. 2016 and Plant Physiol. 2018). We found that multiple abiotic stress signals and chloroplast-mediated signals converge to regulate the LHCX content of cells, providing a way to fine-tune light harvesting and photoprotection. Moreover, integrated genomic and biochemical analyses indicate that the expansion of the LHCX gene family reflects functional diversification of its members which could benefit cells responding to highly variable ocean environment.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-002

Proofs for production of sexual resting cyst by the toxic dinoflagellate *Karenia mikimotoi* from clonal cultures and marine sediments

Y. Liu [1 2], Z. Hu [2 3], Y. Deng [2 3], Y. Tang [2 3,*]

[1] University of Chinese Academy of Sciences, [2]CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, [3]Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China

Abstract: The toxic dinoflagellate *Karenia mikimotoi* is known to form large-scale and dense harmful algal blooms (HABs) in coastal waters worldwide and cause serious economic loss in aquaculture and fisheries and other adverse effects on marine ecosystems. Therefore, understanding the ecology of this species is extremely urgent, especially in the mechanisms of overwintering, bloom initiation, and geographic expansion. Resting cyst, as an important survival strategy in life cycle of dinoflagellates, has been proven to play vital roles in the initiation and termination of blooms and geographical expansion in many HABs-forming dinoflagellates. Whether or not *K. mikimotoi* forms resting cyst, however, has been a puzzling issue to the research community of HABs. Here, we provide visual and molecular confirmations of the production of sexual, thin-walled resting cysts by *K. mikimotoi* based on observations of laboratory cultures and detections in marine sediments. Evidences from the light and scanning electron microscopic observations in laboratory cultures include cell pairs in sexual mating, cells in fusion, planozygotes (two longitudinal flagella), thin-walled resting cysts and their germination processes (e.g. new germling with two longitudinal flagella), which together confirmed that *K. mikimotoi* produces sexual resting cysts homothallically. Evidences from marine sediments collected from locations where *K. mikimotoi* bloomed frequently include positive PCR detection using species-specific primers, positive detection with fluorescence in situ hybridization (FISH) using species-specific oligonucleotide probe which targets the LSU rDNA D2 domain of *K. mikimotoi* and was labeled with either FITC or Cys3, light microscopic observation of cysts labeled with FISH probe, and identity confirmation with single-cell PCR sequencing for cysts labeled with FISH probe. The confirmation of sexual resting cyst production by *K. mikimotoi* in laboratory cultures and field sediment samples provides a possible mechanism accounting for the recurrences of annual blooms at certain regions and the global expansion of *K. mikimotoi* blooms during the past decades. Although a postulated population initiation based on the cyst abundance in sediments reflected in our FISH detection may lead to a bloom (e.g. 10^7 cells L^{-1}) in about a month for a shallow water, the extremely low abundance of cysts, however, necessitates more extensive surveys on temporal and spatial distribution of cysts in the field in order to reveal the exact roles played by resting cysts in the population dynamics of *K. mikimotoi*, which is ongoing now.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-003

Influence of light intensity, inorganic nutrients and prey concentration on toxin production and release in *Prymnesium parvum* strains

N. Medić [1,*], K. Anestis [2], R. Anderson [1], E. Varga [3], T. Ostenfeld Larsen [3], U. John [2], P. J. Hansen [1]

[1]Department Of Biology, University Of Copenhagen , Helsingore, Denmark, [2]Ecological Chemistry , Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, [3]Department of Biotechnology and Biomedicine, Technical University of Denmark, Lyngby, Denmark

Abstract: *Prymnesium parvum* is a constitutive mixotroph protist that causes harmful algal blooms in worldwide marine ecosystems. Such phenomena are often associated with massive fish-kills with remarkable damage to fisheries development and subsequent economic losses. It is well known that *Prymnesium parvum* produces allelochemicals that are released to the surrounding water and affect plankton community structure and trophic interactions in the food web, thus playing a crucial role in major biogeochemical cycles. A number of abiotic factors such as nutrient deficiency and light intensity may increase allelochemical production in *P*. *parvum*, however all this information is built on the use of bioassays. Recent progress in the characterization and quantitation of prymnesins (the suspected allelochemicals) allows us for the first time to study the production and release of these compounds under different physical, chemical and biological conditions. The aim of this study is to investigate the role of light intensity, inorganic nutrients and prey concentration in toxin production and release in selected *P. parvum* strains. The hypothesis is that the production and release of the toxins is elevated at high light intensity and that nutrient limitation leads to increased cellular concentrations of prymnesins, but to a lower toxin production. Furthermore, we expect that prymnesin production by *P. parvum* will increase when grown with prey irrespective of light intensity and nutrient limitation. These results will provide important information allowing proper mitigation of *P. parvum* blooms and their effects on the ecosystem.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-004

Physiological, genomic and transcriptomic variation between co-occurring strains of *Raphidiopsis raciborskii*

A. Willis [1 2,*], J. Woodhouse [3], X. Man [1], A. Chuang [1], A. Jex [4], B. Neilan [5], M. Burford [1]

[1]Australian Rivers Institute, Griffith University, Nathan, [2]Australian National Algae Culture Collection, CSIRO, Hobart, Australia, [3]Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Stechlin, Germany, [4]Faculty of Veterinary and Agricultural Sciences, Melbourne University, Parkville, [5]School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, Australia

Abstract: Co-occurring strains of the cyanobacterium *Raphidiopsis raciborskii* (basionym *Cylindrospermopsis raciborskii*) vary in their morphology and physiology, resulting in populations that are quick to adapt to a changing environment. Understanding how strain variation arises, and the magnitude of variation between strains, is needed to improve prediction and management of harmful blooms.

Strains isolated from a single population were initially revealed to exhibit a range of different growth rates and toxin cell quotas under the same conditions. Strains exhibited equally variable genomes and transcriptomes which themselves were not sufficient to explain one another nor the variability within physiological responses. Whilst we observed a relatively large core genome and the transcriptome analysis indicated some common responses to nutrient stress, demonstrating a broad similarity between strains, the transcriptome analysis also showed strains used different genetic pathways to arrive at similar physiological responses. The results indicate that the physiological variation between strains is a result of a complicated mix of genome and transcriptome-level variation, which may lead to fluctuating strain dominance in natural systems and an adaptive population. An understanding of strain variation is needed to optimise strain choice for laboratory studies, for comparison to field responses, and data inputs for models. In conclusion, strains vary in their genomes, transcriptomes and physiology, therefore it is necessary to characterise multiple strains to be able to understand population responses to environmental variables.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-005

Differences in nutrient physiology and toxin production among three *Pseudo-nitzschia* species: influence of life cycle stages

A. SAUVEY [1,*], J. FAUCHOT [1], P. CLAQUIN [1]

[1]Biologie des Organismes et Ecosystèmes Aquatiques, Normandie Univ, UNICAEN, CNRS, BOREA, 14000 Caen, France

Abstract: Several species belonging to the genus *Pseudo-nitzschia* produce a neurotoxin, domoic acid (DA), that bioaccumulates in marine food webs resulting in Amnesic Shellfish Poisoning (ASP) events. ASP events have negative impacts on coastal ecosystem services especially through their impact on fisheries. In many ecosystems, variability in toxicity during *Pseudo-nitzschia* blooms has been linked to differences in species diversity, domoic acid production being very different as a function of *Pseudo-nitzschia* species. Understanding the factors controlling *Pseudo-nitzschia* species-specific phenology in natural populations is therefore necessary to provide scenarios of toxic blooms for ecosystem management. Among the environmental factors controlling phytoplankton blooms, nutrients have been hypothesized to influence both *Pseudo-nitzschia* diversity and toxicity level. The objective of our study was therefore to characterize the physiology of three *Pseudo-nitzschia* species (*P. australis*, *P. fraudulenta* and *P. pungens*), focusing on their nutrient uptake capacities and the influence of nutrient limitation on several physiological indicators. Growth rate, cell quotas, photosynthetic efficiency (PAM), nutrient uptake kinetics and domoic acid production were studied in batch cultures in controlled conditions. The heterothallic life cycle of *Pseudo-nitzschia* was also considered by studying how cell size reduction affect cellular physiology and domoic acid production. These studies were performed on multiple strains for each species which allowed us to characterize the variability of physiological indictors du to intraspecific diversity. Singular behavior regarding nutrient uptake (especially for Si) and in photosynthetic efficiency were observed in *P. australis*. Moreover, our results reveal that life cycle has a strong influence on the physiology of *P. australis* since cellular metabolism (including toxin production) is modified when cells acquire the ability to sexually reproduce becoming gametangia.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

O-006a

Harmful algal blooms in the Baltic Sea and in the Kattegat-Skagerrak area investigated using novel *in situ* and remote sensing methods

B. Karlson [1,*], L. Arneborg [2], L. F. Artigas [3], M. L. Brosnahan [4], A. Cembella [5], W. Eikrem [6], A. Godhe [7], J. Johansson [8], M. Johansson [9], S. Lehtinen [10], F. Lizon [11], A. Louchart [12], M. Mohlin [13], J. Seppälä [14], L. Stemmann [15] and JERICO-NEXT

[1]Research and development, oceanography, Swedish Meteorological and Hydrological Institute, [2]Research and Development, Oceanography, Swedish Meteorological and Hydrological Institute (SMHI), Västra Frölunda, Sweden, [3]Laboratory of Oceanology and Geosciences, CNRS-ULCO-UDL, Wimereux, France, [4]Woods Hole Oceanographic Institution, Woods Hole, USA, [5]Ecological Chemistry, Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar-und Meeresforschung, Bremerhaven, Germany, [6]Norwegian Institute for Water Research and the University of Oslo, Oslo, Norway, [7]Department of Marine Sciences, University of Gothenburg, Gothenburg, [8]Oceanography, Swedish Meteorogical and Hydrological Institute, Västra Frölunda, Sweden, [9]The Arctic University of Norway, Tromsø, Norway, [10]Marine Research Centre, Finnish Environment Institute (SYKE), Helsinki, Finland, [11]CNRS Laboratory of Oceanology and Geosciences, University of Lille, Lille, [12]CNRS Laboratory of Oceanology and Geosciences, ULCO, Wimereux, France, [13]Oceanographic unit, Swedish Meteorological and Hydrological Institute (SMHI), Västra Frölunda, Sweden, [14]Marine Research Centre, SYKE Finnish Environmental Institute, Helsinki, Finland, [15]Institut de la Mer de Villefranche, Sorbonne University, Villefranche sur Mer, France

Abstract: Recent developments in *in situ* instrumentation and remote sensing makes it possible to study harmful algal blooms in higher detail than earlier. The presentation includes examples of this and discusses advantages and disadvantages of the different approaches. In the seas surrounding Sweden several different types of harmful algae are observed. In the Kattegat-Skagerrak, adjacent to the North Sea, algae from the genera *Prymnesium, Dictyocha* and *Pseudochattonella* have caused fish mortalities while the genera *Dinophysis, Alexandrium, Pseudo-nitzschia, Protoceratium* and *Lingulodinium* produce phycotoxins that may accumulate in bivalves. During a study close to a mussel farm on the Swedish Skagerrak coast the Imaging Flow Cytobot, an imaging flow cytometer, was used to investigate the dynamics of the phytoplankton community including harmful algae. Also, frequent water sampling with traditional microscopic analysis was carried out together with observations of physical oceanographic conditions etc. High frequency observations during twelve weeks in autumn 2016 revealed fast changes in the abundances of harmful algae, in part a result of water exchange. Nitrogen fixing (diazotrophic) cyanobacteria are common in the Baltic Sea, especially in summer. Surface scums of cyanobacteria represent a nuisance and cause problems for tourism. *Nodularia spumigena* produces the toxin nodularin. *Dolichospermum* spp. and *Aphanizomenon* spp. are other common diazotrophic taxa. In a study carried out in July 2017 the distribution of these microplanktonic (filament length >20 µm, width ca. 5-10 µm) cyanobacteria was investigated in the Baltic Sea and the Kattegat-Skagerrak. Ocean colour and radar-based satellite remote sensing images provided a high horizontal resolution of surface accumulations. Flow-through systems on a research vessel and on two merchant ships were used to investigate the near surface distribution based on fluorescence properties of the organisms. The vertical distribution was investigated by water sampling and microscopy as well as using *in situ* imaging of cyanobacteria colonies (UVP5 instrument), multi wavelength fluorometry (Fluoroprobe) and by analysing samples using automated flow cytometry (CytoSense). Horizontal and vertical small-scale variability was substantial. The different cyanobacteria had dissimilar distribution, and all the filamentous cyanobacteria taxa were absent in the Kattegat-Skagerrak. These studies highlight the need for multi-method approaches when investigating harmful algal bloom dynamics.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

O-006b

Automated *in vivo* approaches for characterizing HAB in coastal waters: the JERICO-Next European Research Infrastructure.

L. F. Artigas[1,*], B. Karlson[2], M. Brosnahan[3], P. Claquin[4], V. Créach[5], R. De Blok[6], E. Debusschere[7], K. Deneudt[7], F. Gómez[1], G. Grégori[8], P.-A. Hébert[9], J. Kromkamp[10], A. Lefebvre[11], S. Lehtinen[12], F. Lizon[1], A. Louchart[1], K. Möller[13], E. Poisson-Caillault[9], M. Rijkeboer[14], M. Thyssen[8], J. Seppälä[12], L. Stemmann[15], A. Veen[14], G. Wacquet[1]

[1]Laboratory of Oceanology and Geosciences (LOG), CNRS-ULCO-UDL, Wimereux, France, [2]Swedish Meteorological and Hydrological Institute (SMHI), Norrköping, Sweden, [3]Woods Hole Oceanographic Institution, Woods Hole, USA, [4]Biologie des Organismes et Ecosystèmes Aquatiques (BOREA), CNRS-University of Caen, Caen, France, [5]Center for Environment, Fisheries and Aquaculture Science (CEFAS), Lowestoft, United Kingdom, [6]Protistology and Aquatic Ecology, Ghent University, Ghent, [7]Vlaams Instituut voor de Zee (VLIZ), Ostende, Belgium, [8]Institut Méditerranéen d'Océanologie, CNRS-AMU, Marseille, [9]Laboratoire d'Informatique Signal et Image de la Côte d'Opale (LISIC), ULCO, Calais, France, [10]Royal Netherlands Institute for Sea Research (NIOZ), Yerseke, Netherlands, [11]Laboratoire Environnement Ressources, IFREMER, Boulogne sur Mer, France, [12]SYKE Finnish Environmental Institute, Helsinki, Finland, [13]Institute for Coastal Research, Helmholtz-Zentrum Geesthacht (HZG), Geesthacht, Germany, [14]Centre for Water Management - Laboratory for hydrobiological analysis, Waterdienst - RWS, Lelystad, Netherlands, [15]Laboratoire d'Océanographie de Villefranche, Sorbonne Universités, Villefranche sur Mer, France

Abstract: In order to better understand phytoplankton temporal and spatial distribution in coastal systems, automated *in vivo* approaches are being deployed since the last decade. These innovative techniques provide new insights into the detection of phytoplankton community changes affecting growth rates, size structure, taxonomic and/or pigmentary composition, which can occur at different time and spatial scales, evidencing rapid as well as long-term changes in environmental conditions. When implemented in automated environmental monitoring platforms, as fixed stations, moorings, research vessels and/or ships of opportunity, these techniques can represent valuable early-warning systems of community changes, as the occurrence of blooms and, in particular, of harmful algal blooms (HAB), which can lead to disruption of marine food webs and mass mortalities of marine organisms and which are of special interest in areas of fishing, aquaculture and tourism. Therefore, there is an urgent need to improve the discrimination and operability of automated techniques addressing phytoplankton diversity (at nearly taxonomical and/or functional levels) and productivity in order to provide early warning platforms for the occurrence, duration and spatial extent of phytopankton blooms and, by extent, of noxious episodes. The Joint European Research Infrastructure network for Coastal Observatories – Novel Expertise for coastal observatories (JERICO-Next – H2020, 2015-2019), scientists inter compare, work on technical and analytical improvements and apply a combination of phytoplankton automated observation approaches, based on single cell/particle or bulk optical characteristics, in several European coastal systems ranging from oligotrophic (West Mediterranean) to mesotrophic (southern Bay of Biscay, Celtic seas) and eutrophic systems (eastern Channel, southern North Sea, Skagerrak/Kattegat, Baltic Sea), characterised by different types of potential HABs developments (dinoflagellates, diatoms, haptophytes, cyanobactetria). Three main groups of techniques, image in-flow or *in situ* acquisition and analysis, pulse shape-recording automated flow cytometry, as well as multispectral and variable fluorometry and spectrophotometry, are being critically explored and implemented in different sites and platforms. A summary of the main results gathered by a combination of these techniques is presented. Moreover, recommendations on future applications of these techniques and remaining challenges are discussed in the frame of their implementation in operational marine observing systems.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

O-007

Diversity and toxicity of *Pseudo-nitzschia* species in Monterey Bay, California (USA): perspectives from targeted and adaptive sampling

H. Bowers [1,*], J. Ryan [2], K. Hayashi [3], A. Woods [1], R. Marin [2], G. J. Smith [1], K. Hubbard [4], G. Doucette [5], C. Mikulski [5], A. Gellene [6], Y. Zhang [2], R. Kudela [3], D. Caron [6], J. Birch [2], C. Scholin [2]

[1]Moss Landing Marine Laboratories, [2]Monterey Bay Aquarium Research Institute, Moss Landing, [3]Department of Ocean Sciences , University of California at Santa Cruz, Santa Cruz, [4]Fish and Wildlife Research Institute, Saint Petersburg, [5]National Centers for Coastal Ocean Science, NOAA/National Ocean Service, Charleston, [6]Department of Biological Sciences, University of Southern California, Los Angeles, USA

Abstract: Near-annual blooms of *Pseudo-nitzschia* in Monterey Bay, California (USA) lead to impacts on tourism, commercial/recreational fisheries, and marine animal health. The most toxic events have been from the proliferation of *P. australis*, however other species within the genus can contribute to widespread variability in toxicity and community structure across years. Current monitoring methods are limited in their ability to capture the full breadth of species diversity and spatial coverage, thereby hindering our understanding of events and limiting predictive efforts. During two divergent bloom years in the bay, we deployed an integrated network of in situ platforms, some with autonomous adaptive sampling capabilities, which allowed us to discover detailed aspects of population and toxicity dynamics. In 2013, the bloom was characterized by spatial differences in *Pseudo-nitzschia* populations, with the low-toxin producer *P. fraudulenta* dominating the inshore community and toxic *P. australis* dominating the offshore community. In 2015, an exceptionally toxic bloom developed as a diverse *Pseudo-nitzschia* community abruptly transitioned (within a week) into a bloom of highly toxic *P. australis*. Increases in cell density and proliferation were associated with strong upwelling of nutrients, while high toxicity was driven by silicate limitation of the dense bloom. The temporal shift in species composition mirrored observations further north in the California Current System off Oregon and Washington. The broad scope of sampling and unique platform capabilities employed during our studies revealed important patterns in bloom formation and persistence for *Pseudo-nitzschia*. These results underscore the benefit of expanded biological observing capabilities and targeted sampling methods to capture more comprehensive spatial and temporal scales for studying and predicting future events.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

O-008

New insights into the diversity of *Azadinium* spp. and distribution of azaspiracids along the South and West coasts of Ireland

S. M. Mcgirr[1,*], D. Clarke[2], J. Kilcoyne[3], H. Koehler[4], J. Silke[2], N. Touzet[4]

[1]Centre for Environmental Research, Innovation and Sustainability, Institute of Technology, Sligo, Sligo, [2]Shellfish Safety, Marine Environment and Food Safety Services, [3]Biotoxin Chemistry, Marine Environment and Food Safety Services, Marine Institute, Galway, [4]Centre for Environmental Research, Innovation and Sustainability, Institute of Technology Sligo, Sligo, Ireland

Abstract: Harmful algal blooms have become a pressing global issue and the apparent increase in their distribution, frequency, and intensity has threatened our capacity to sustainably exploit aquatic resources. Some HAB taxa, such as members of the dinoflagellate genus *Azadinium*, can produce potent biotoxins that present a health risk towards humans who consume seafood products. To date, three *Azadinium* species have been identified in Irish coastal waters, including both toxic and nontoxic forms.

A coastal survey was undertaken around the South and West of Ireland in August 2016 to document the distribution of *Azadinium* spp. Over 130 samples were collected for analysis using previously developed molecular assays including the SYBR Green based real-time PCR method for detecting members of the *Amphidomataceae* family and species-specific Taqman assays for *A. spinosum, A. obesum* and *A. poporum*. Analytical chemistry based on UPLC-MS/MS was also used to detect and quantify associated azaspiracids.

Results showed the positive detection of *Amphidomataceae* family members by real time PCR in 95% of the samples. Signals for both *A. poporum* and *A. obesum* were negative in all the samples tested. *A. spinosum* was detected in only 10% of samples (highest Ct value of 27.50, ~ 1000 cells l^{-1}). Azaspiracid toxins were detected in all the samples where *A. spinosum* was found positive by qPCR. The samples which proved negative for *A. spinosum* yet positive using the *Amphidomataceae* assay showed varying levels of AZA-1 and -2. Noticeably, the highest levels of AZA-1 and AZA-2 (1050 and 925 fg^{1} ml^{-1}, respectively) were found in samples where *A. spinosum* was apparently absent. Denaturant gradient gel electrophoresis was applied to the PCR amplicons generated using the primers of the SYBRGreen based *Amphidomataceae* family assay. Bands were excised from the gel then sequenced, returning sequences identical to *Azadinium* spp., even though the three species-specific assays returned negative outcomes.

Results of the sampling campaign not only suggest that there is a greater than previously thought diversity of *Azadinium* spp. in Irish coastal waters, but these yet to be identified 'forms' may be possibly able to synthesise azaspiracids. Further investigation is hence urgently needed in the context of the shellfish safety and phytoplankton monitoring programmes operated in Europe.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

O-009

Operational tools to improve the prediction capacity of the HABs in Galician mollusc production areas

Y. Pazos [1,*], F. BellasAlaez [2], L. González Vilas [2], J. M. T. Palenzuela [2]

[1]Oceanography and Phytoplankton, INTECMAR, Vilagarcía, [2]Applied Physics Department, University of Vigo, Vigo, Spain

Abstract: Galician *rias* is located in the northern part of the Northeast Atlantic Upwelling System, which cause a high primary production, support important fishing and aquaculture activities, mainly mollusc culture in rafts. These activities are seriously hindered by harmful algae blooms (HABs). The study requires not only an understanding of the ocean processes over the continental shelf, but also of the effects of rivers and freshwater runoff, the anthropogenic pressure, the biology of species, and tools integrating information. In addition to the program for shellfish safety and *in situ* weekly oceanography and phytoplankton monitoring, images from new satellites provide spatial information about optical and thermal properties of coastal waters using local-based algorithms for chlorophyll *a*, coloured dissolved organic matter, total suspended matter and sea surface temperature. Finally, statistical techniques, machine learning methods and hydrodynamic models are useful for predicting HABs using both environmental parameters and distribution patterns of toxic species. In this work, we show monitoring and prediction results of paralytic shellfish toxins (PSP) and amnesic shellfish toxins (ASP) toxic events on the Galician coast using products derived from Ocean and Land Colour Instrument (OLCI) on-board Sentinel 3 satellite, *in situ* oceanographic, phytoplankton and toxicity data and predictive models based on Support Vector Machines (SVM) and AdaBoost/ RandomForest architectures.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

O-010

Dinoflagellate cysts distribution in recent sediment of a southern Atlantic coastal zone (Oualidia lagoon, Morocco)

K. Chaira [1,2], H.Rhinane[2], B.Ennaffah[1], S.Maimouni[3], R.Sagou[1], S.Loulad[2], A. Ben Mohamed[1], A. Agouzouk[1], S. Benbrahim[1] and M. Laabir[2].

[1]Toxic Microalgae Laboratory, Institut National de Recherche Halieutique, Casablanca, Morocco.
[2]Laboratory of Geosciences, Department of Geology, Faculty of Sciences, University Hassan II, Casablanca, Morocco
[3]Department of Geology, Faculty of Sciences Ben M'sick, University Hassan II, Casablanca, Morocco
[4]Center for Marine Biodiversity, Exploitation and Conservation (MARBEC), Montpellier University, CNRS, IRD, Ifremer, Place E. Bataillon, CC93, 34095 Montpellier cedex 5, France.

Abstract: Abstract

The Oualdia lagoon, located along the Western Atlantic Coastline of Morocco holds important shell-farming companies. This lagoon is subject to sporadic HABs species. 51 stations covering all of this water system were sampled by coring in February-March 2017 altogether with the measurement of the main environmental parameters. Our results on dinoflagellate cysts assemblage in Oualidia lagoon revealed the presence of fourteen morphotypes belonging to six genera. The highest total cyst abundance was 293 cysts.g^{-1} dry sediment (DS) at station 25 located at the center of the lagoon. Two species dominated the cystic assemblage, *Lingulodinium poleydrum* (49.74%) and *Gonyaulax spinifera* (21.5%). *L. polyedrum* cysts were widely distributed reaching a maximum density of 959 cysts g^{-1} DS. By applying the Geographic Information System tool, two zones of preferential accumulation of cysts have been identified representing areas at risk. The Pearson statistical test revealed a positive and significant correlation between cyst abundance and water content, organic matter and of fine sediment (<63µm) percentages. Our study showed the presence of morphotypes of potentially toxic species (*Alexandrium minutum, Alexandrium catenella/tamarense complex, L. polyedrum, Gymnodnium catenatum* and *Gonyaulax cf spinifera*). The cysts of these species present in the sediment of the Oualidia lagoon could germinate, when environmental conditions become favorable, and in turn could inoculate the water column with the subsequent bloom formation. This could explain the recurrence of the blooms observed in this ecosystem and the related intoxication of the mollusks measured by RSSL survey implemented by INRH (Institut National de Recherche Halieutique). This study confirms the usefulness of cyst analysis in the assessment of harmful bloom risk in this area important for oyster's culture.

Disclosure of Interest: None Declared

Optical sensors and drone systems for the monitoring of harmful blooms

O-011

Development of a low cost optical sensor and of a drone system for the monitoring of cyanobacteria in freshwater ecosystems

J.-F. Humbert [1,*], K. Soudani [1], G. Hmimina [1], L. Audebert [2], S. Buttigieg [3], P. Chatelier [3], S. Chollet [4], B. Decencière [4], F. Derkx [3], C. Freissinet [5], Y. Hong [6], F. Hulot [2], B. Lemaire [6], A. Millot [4], A. Perrin [5], C. Quiblier [7], G. Six [3], J.-L. Sorin [3], K. Tambosco [1], V. Tran Khac [6], B. Vinçon-Leite [6]

[1]iEES Paris-INRA-Sorbonne Université, Paris, [2]ESE-Université Paris-Sud, Orsay, [3]IFSTTAR Nantes & Champs/Marne, Nantes, [4]CEREEP, Foljuif, [5]ARTELIA Grenoble, Grenoble, [6]LEESU-ENPC Champs/Marne, Marne La Vallée, [7]MNHN Paris, Paris, France

Abstract: Cyanobacteria blooms frequently disturb the functioning of freshwater ecosystems and their uses, due to the toxins dangerous to health that cyanobacteria are able to synthesize. Therefore, many countries have implemented monitoring programs aimed at reducing the risk of human exposure to these toxins. The main limitation is related to the heterogeneity of the spatial distribution of cyanobacteria. In the vertical dimension, these organisms can stay in different layers in the water column and in the horizontal scale, the cells may accumulate in some area of the water body, under the action of winds or currents. In an attempt to improve monitoring, many research projects have been undertaken in order to develop new tools, like buoys equipped with various underwater sensors. This tool is highly relevant but it does not allow assessing the horizontal distribution of cyanobacteria and its cost remains expensive. Moreover, if satellite remote sensing can be considered very useful for estimating biomass and horizontal distribution of cyanobacteria in a water body, the cost of this technology and the lack of satellite availability make it unaffordable for routine monitoring. In this context, our project named OSS-CYANO (Development of optical sensors and drone system for the survey of cyanobacteria) project aims in a first time to develop and validate a new, low-cost aerial sensor. In this goal, we developed a framework for performing high-throughput measurements of the absorbance and reflectance of small volumes of controlled mixtures of phytoplankton species and studied the potential of this framework to validate remote-sensing proxies of cyanobacteria concentration. A systematic test of every possible two-band and three-band normalized difference between optical indices was then performed, and the coincidental correlation with chlorophyll-b (absent in cyanobacteria) was used as an indicator of non-specificity. Two-band indices were shown to suffer from non-specificity issues and could not yield strong and specific relationships with phycocyanin or phycoerythrin (maximum $R^2$0.8). The second part of our program aimed to couple the high-frequency measurements and a physically-based three-dimensional hydro-ecological model for predicting cyanobacteria blooms. Finally, the third part of our program aimed to implement a drone capable of carrying the sensor to perform spatial measurements on large water bodies, and other instruments for water sampling or for performing underwater measurements. Six public research laboratories and one private company have been involved in this project.

Disclosure of Interest: None Declared

Optical sensors and drone systems for the monitoring of harmful blooms

O-012

COUPLING HIGH-FREQUENCY MEASUREMENTS AND PREDICTIVE MODELLING IN A MONITORING AND EARLY WARNING SYSTEM OF CYANOBACTERIA BLOOMS

B. Vinçon Leite[1], Y. Hong[1,*], V. Tran Khac[1], D. Furstenau Plec[1 2], C. Li[1], P. Dubois[1], M. Saad[1], B. J. Lemaire[1], G. Hmimina[3], K. Soudani[3], C. Quiblier[4], K. Tambosco[5], G. Six[6], C. Freissinet[7], B. Decencière[8], J.-F. Humbert[5]

[1]LEESU, Ecole des Ponts ParisTech, AgroParisTech, UPEC, Univ Paris Est, Marne La Vallée, France, [2]Department of Hydraulic and Water Resources, Federal University of Minas Gerais (UFMG), Pampulha, Brazil, [3]Ecologie Systematique Evolution, CNRS, AgroParisTech, Université Paris-Saclay, Orsay, [4]UMR 7245 MNHN-CNRS, Museum National d'Histoire Naturelle, [5]iEES Paris-INRA, Sorbonne Université, Paris, [6]IFSTTAR, Univ Paris Est, Marne La Vallée, [7]ARTELIA, Echirolles, [8]ENS, CNRS, CEREEP Ecotron IDF, UMS 3194, St Pierre Les, Nemours, France

Abstract: Anthropogenic activities have major impacts on the ecological quality of downstream water bodies. The degradation of the water quality can lead to toxic cyanobacteria blooms, which in turn may cause serious health risks to people doing water sports. Due to the complexity of natural processes in water bodies, physically-based and spatially distributed models are valuable tools for a better understanding of the interactions between variables driving cyanobacteria blooms, as well as for helping stakeholders determine their management strategies. However, traditional in situ measurements have too limited temporal and spatial resolution to make such a numerical model reliable. Efforts must be made to use innovative monitoring to overcome these limitations. Continuous in situ measurements provided by automated high-frequency monitoring can decisively improve model performances.

Therefore, the main objectives of the study were to (i) set up a full-scale experimental site for high-frequency monitoring of cyanobacteria biomass in an urban lake; (ii) couple the high-frequency measurements and a physically-based three-dimensional hydro-ecological model for predicting cyanobacteria blooms; (iii) establish a transfer platform for real-time data management; and (iv) develop a web platform for communicating information with lake managers, other stakeholders and the public. In the framework of the OSS-cyano project, the study site is Lake Champs-sur-Marne (0.12 km^2 surface, 3.5 m maximum depth), located in Greater Paris. The field monitoring includes measurements of water temperature, dissolved oxygen, chlorophyll fluorescence and phycocyanin fluorescence every fifth minute, and fortnightly vertical profiles of temperature and of the fluorescence of the main phytoplankton groups. The Delft3D hydrodynamic and ecological models, respectively Flow and Bloom, were implemented. Using continuous measurements and short-term meteorological forecast, a predictive modelling of the cyanobacteria biomass evolution over a week was performed. Simulation results were then communicated to the lake manager and the public through a web platform for sanitary risk warning.

Disclosure of Interest: None Declared

Optical sensors and drone systems for the monitoring of harmful blooms

O-013

Being in the right place at the right time: reflections on a decade of in situ HAB sensor deployments

D. Anderson[1,*], M. Brosnahan[1], H. Sosik[1], R. Olson[1], B. Keafer[1], G. Doucette[2]

[1]Biology Department, Woods Hole Oceanographic Institution, Woods Hole MA, [2]National Ocean Service, NOAA, Charleston SC, USA

Abstract:

The dinoflagellate *Alexandrium catenella* (=*A. fundyense*) and diatoms in the genus *Pseudo-nitzschia* cause frequent harvesting closures of shellfish resources in the Gulf of Maine region of the U.S. due to the threat of paralytic shellfish poisoning (PSP) and amnesic shellfish poisoning (ASP). Blooms can be widespread, covering hundreds of km of coastline, or localized in small embayments and estuaries. In recent years, our studies of harmful algal bloom (HAB) dynamics in both of these systems have incorporated two novel biosensors – the Environmental Sample Processor (ESP) and the Imaging FlowCytobot (IFCB). The ESP uses molecular assays to detect and analyze cells and toxins whereas the IFCB is an automated underwater microscope. Each has presented logistical challenges of different types, requiring new mooring and platform designs, communication protocols, and deployment strategies. This talk will review a decade of deployments, highlighting how these instruments have augmented HAB research in the two hydrographic systems over multiple bloom seasons, emphasizing the unique nature of the data each sensor can provide, correlations with other data such as shellfish toxicity measurements, as well as the engineering and scientific obstacles that were overcome. These studies have taught us that previous laboratory studies produced surprisingly bad estimates of rates and behaviors that are fundamental to predicting bloom dynamics. Nonetheless, realistic estimates of these dynamics are crucial for predicting how these species will respond to a changing world and how we should respond to the evolution of HAB threats facing coastal communities. We thus need to expand and refine the use of these instrumental approaches in our HAB science. While in situ tools hold great promise for improving our understanding of HABs across all habitats, we now believe that the most rapid progress can be achieved by concentrating on inshore populations. Furthermore, given the temporal and spatial patchiness of HABs, we need higher-level strategies that can place sensors where the cells are, rather than putting the instruments in fixed locations, hoping that the HABs will come to us. Mobile and portable platforms, particularly those that enable adaptive, targeted sampling for addressing temporal as well as spatial patchiness, offer great promise for characterizing the evolution of populations through time, and several of these will be described here. In situ sensors can play a major role in HAB research and monitoring, but approaches need to be strategic and flexible in order to meet the challenges posed by highly dynamic blooms.

Disclosure of Interest: None Declared

Optical sensors and drone systems for the monitoring of harmful blooms

O-014

BloomAlert: early detection and monitoring of algal blooms in small lakes and rivers

C. J. S. Bolch [1,*], J. Bramich [1], C. Mabin [1], A. Fischer [1]
[1]Institute for Marine and Antarctic Studies, University of Tasmania, Launceston, Australia

Abstract: Drinking and irrigation water in rural areas are often drawn from many small lakes/rivers which suffer regular harmful and toxic algal blooms, have limited water treatment, and represent significant risk to both animal and human health. The large number of storages mean that water quality/bloom management is both expensive and reactive. The small water area of storages also creates challenges for application of existing remote sensing products for bloom detection. Here we describe the design and performance of an *in-situ* bloom monitoring network using commercial fluorescence probes providing real-time assessment of algal communities to water managers/regulators. Fluorescence and environmental parameters are measured at high frequency (15 min.) sent via telemetry to a central server for data processing prior to reporting to water managers and field staff via a mobile-responsive web page. Data outputs include general water quality (temperature, conductivity, pH, turbidity), indicators of algal and cyanobacteria biomass, and an overall bloom alert value to guide management responses according to algal bloom management plans. The i*n-situ* data is then used to validate improved remote-sensing models for chlorophyll-a and phycocyanin retrieval; initially from LandSat 8 OLI and now newer high resolution products (e.g. Sentinel 2) better suited to small water bodies that alllows us to extend monitoring to water bodies beyond the *in-situ* sensor network. In the future we aim to combine BloomAlert monitoring data with data from other dispersed sensor networks (meteorological and farm-based sensors) to develop seasonal risk profiles and bloom forecasting capacity.

Disclosure of Interest: None Declared

Optical sensors and drone systems for the monitoring of harmful blooms

O-015

High-resolution satellite remote sensing of coastal red tides using laboratory measurements of *Mesodinium rubrum* optical properties

P. Gernez [1,*], T. Lacour [2], V. Martinez-Vicente [3], V. Raimbault [2], V. Séchet [4], T. Harmel [5]

[1]Mer Molécules Santés, University Nantes, [2]Laboratoire Physiologie Biotechnologie Algues, Ifremer, Nantes, France, [3]Plymouth Marine Laboratory, Plymouth, United Kingdom, [4]Laboratoire Phycotoxines, Ifremer, Nantes, [5]solvo, Antibes, France

Abstract: *Mesodinium rubrum* is a globally-distributed photosynthetic marine ciliate known to form ephemeral and massive red tides in coastal areas, such as estuaries, fjords, and upwelling zones. Though *M. rubrum* does not produce toxins, it has been identified as prey for *Dinophysis spp.*, a dinoflagellate responsible for the diarrheic shellfish poisoning toxin. *M. rubrum* blooms are generally classified as Harmful Algal Blooms (HABs) due to their impact on water quality (i.e., oxygen depletion, modification of food-web dynamics). Detection, sampling, and quantification of such HABs is notoriously challenging due to the speed at which this ciliate can grow, swim, aggregate, disaggregate, and/or be consumed. Here, we present a novel detection and quantification method based on ocean colour satellite remote sensing. The inherent optical properties (absorption and backscattering coefficients) of *M. rubrum* were first characterized using laboratory measurements. Second, a simplified radiative transfer model was used to simulate the specific signature of *M. rubrum*, in terms of remote sensing reflectance (R_{rs}). Third, a detection and quantification algorithm was developed based on the specific shape of the simulated R_{rs} spectra: a 705 nm peak associated with high Chlorophyll-*a* biomass and a green trough associated with Phycoerythrin absorption. Fourth, the algorithm was applied to the 2016-2018 archive of Sentinel-2 satellite data. Several red tides were successfully detected, allowing us to study the spatio-temporal dynamics of *M. rubrum* blooms at high resolution (20 m, 5 days revisit) over a wide coastal area. Massive blooms of other species (e.g., *Lepidodinium chlorophorum*) were also observed and successfully discriminated.

Disclosure of Interest: None Declared

Socio-economic impacts of HABs

O-016

Socioeconomic impacts of the 2015 U.S. West Coast domoic acid event in fishing communities

S. Moore [1,*], E. Allison [2], S. Dreyer [3], J. Ekstrom [4], S. Jardine [2], T. Klinger [2], K. Moore [2], K. Norman [1]

[1]Northwest Fisheries Science Center, National Oceanic and Atmospheric Administration, [2]School of Marine and Environmental Affairs, University of Washington, Seattle, [3]School for the Future of Innovation in Society, Arizona State University, Tempe, [4]Coastal and Marine Sciences Institute, University of California, Davis, Davis, USA

Abstract: In 2015, a massive *Pseudo-nitzschia* bloom occurred along the entire U.S. West Coast that was unprecedented in its toxicity and geographic extent. Shellfish acquired high levels of the toxin domoic acid, necessitating extended and widespread closures of the lucrative commercial Dungeness crab and popular recreational razor clam fisheries. The fisheries closures successfully prevented human illnesses, but generated an economic shock that was disastrous for coastal communities. Here we report on the socioeconomic impacts of the 2015 bloom across 17 fishing communities on the U.S. West Coast using primary interview and survey data along with catch data from the Pacific Fisheries Information Network. The survey instrument, deployed in the summer of 2017, collected sociodemographic and economic factors hypothesized to confer resilience to toxic blooms as well as data that quantifies individual impacts. Community members in fishing-related occupations had 5 times greater odds of reporting higher income losses compared to those in other occupations. Resilience, as measured by the recovery of financial losses, was conferred by mitigative actions. Individuals who were able to fish in alternate areas or for alternate species had greater odds of recouping losses. Advertising business more widely was also associated with greater odds of loss recoupment. The respondents who increased advertising tended to have higher levels of education. In an analysis of ex-vessel price data, we find that following the 2015/2016 closures in California, ex-vessel prices for Dungeness crab were lower than expected, even after accounting for quantity landed and other drivers of ex-vessel prices. The result suggests that the economic impacts for California fishermen were greater than the reductions in landings alone would suggest. These results will inform efforts to prepare for harmful algal blooms, mitigate their impacts, and aid recovery of impacted communities.

Disclosure of Interest: None Declared

Socio-economic impacts of HABs

O-017

Understanding the preferences of water users in a context of cyanobacterial blooms in Quebec

C. L'Ecuyer-Sauvageau [1 2,*], C. Kermagoret [1], J. Dupras [1], J. He [3], T. Poder [3], J. Leroux [4], M.-P. Schinck [4]

[1]Département des sciences naturelles, Université du Québec en Outaouais, Ripon, [2]Centre des Sciences de la Biodiversité du Québec, Montréal, [3]Département d'économique, Université de Sherbrooke, Sherbrooke, [4]Département d'économie appliquée, HEC Montréal, Montréal, Canada

Abstract: Algal blooms, whether they are harmful or more akin to a nuisance, do pose negative impacts on human well-being. In the province of Quebec, excessive phosphorus that contributes to the problem of cyanobacterial blooms comes mainly from non-point sources. Limited regulation on the management of this nutrient, its capacity to accumulate in soils, and climate change all contribute to increasing the magnitude of the effects of algal blooms on humans and the environment. These effects include eutrophication of waterbodies, the release of toxins that can affect human health, but also the closing of beaches due to potential release of toxins, foul smell coming from waterbodies, a change in water colour and texture, all affecting the ability of individuals to enjoy waterborne activities. Since algal blooms occur mainly during the summer and at the beginning of autumn, this research focused on studying the impacts of algal blooms events on recreationists and people living near affected waterbodies. More specifically, we wanted to understand the trade-offs individuals make between different ecosystem services (ES), mainly cultural ES, provided by waterbodies (recreational activities, aesthetic aspects, and ecological health). We also wanted to estimate the average willingness to pay of individuals to finance mechanisms that would allow the resolution of the issue. To achieve our objectives, we used a choice modelling approach enclosed in a questionnaire that was conducted in person and filled out in whole by 252 people. For our analysis, we used conditional logit with and without interactions, and a random parameter logit (mixed logit). The results show that individuals value primarily their ability to perform recreational activities, the ecological health of waterbodies, and aesthetic aspects (odour and visual aspects), in that order. Interestingly, the fact that people reported performing the activities of walking and of fishing influenced the way they prioritized ES in the choice modelling exercise. Based on the most robust model, we estimated an average willingness to pay of $88 per year in tax to finance mechanisms to resolve the algal bloom issue.

Disclosure of Interest: None Declared

Socio-economic impacts of HABs

O-018

Integrated management of HABs along the French Channel area. A system approach to assess and manage socio-economic impacts of HABs.

P. Raux[1,*], S. Chenouf[1], J. A. Pérez Agúndez[2]

[1]UMR AMURE, Institut Universitaire Européen de la Mer, Université de Bretagne Occidentale, Plouzané, [2]UMR AMURE, Institut Universitaire Européen de la Mer, Unité d’Economie Maritime, Ifremer, Nantes, France

Abstract: HABs are natural events. However, ecosystem disturbances can play an important role in their more frequent occurrence, intensity and spatial extent (Hallegraeff et al., 2004). Several drivers contribute to this increase such as climate change and human activities leading to the increase of nutrient loads and pollution, food web alterations, introduced species or water flow modifications (NOAA, 2018). As a result, the impacts of HABs have also increased in recent decades with the intensification of uses in coastal and marine areas (fisheries, aquaculture, tourism and recreational activities for the most important ones).

Research and knowledge about factors that contribute to HABs have increased significantly with the development of monitoring systems. But the mechanisms which generate algal blooms are still not well understood (NOAA, 2018). As a consequence, HABs cannot be properly forecasted and are managed on a crisis basis. Their detection by in situ monitoring and alert systems can result in the closure of impacted areas, commercial ban of seafood and temporary ban of recreational activities. Such alert systems are expensive, spatially limited and unreactive due to time constraints for toxicological analyses.

In such context, developments in remote sensing science can bring a significant added value to the existing monitoring systems. Using material from the Copernicus S-3 satellite, the Interreg France (Channel) England S-3 EUROHAB project will track the growth and spread of HABs in the Channel. This data will then be used to create a web based alert system to alert marine managers and fishing industries of the growth of potentially damaging algal blooms. The alert system will result in much faster response times. Data gathered will also help to better understand why, how and when HABs occur.

This work focuses on the analysis of consequences that can be associated to the implementation of this new alert system. To achieve this, a system approach is proposed to analyze the relationships between the existing monitoring systems, the decisions associated to HAB events and the responses that can be implemented by stakeholders in order to mitigate the socio-economic impacts of HABs (monitoring and management costs, avoided economic losses, risk management by stakeholders…). This holistic vision allowed by a system approach will be also used to explore scenarios for alternative management of HABs and to refine the design of the web based alert system.

Hallegraeff G. M. et al, 2004. Manual on Harmful Marine Microalgae, Second revised edition, Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. (Eds), Intergovernmental Oceanographic Commission UNESCO Publishing, 793p. NOAA, 2018. https://oceanservice.noaa.gov/facts/why_habs.html

Disclosure of Interest: None Declared

Socio-economic impacts of HABs

O-019

Socio-economic determinism of cyanobacterial blooms in Victoria Lake (Uganda)

O. Mark [1 2,*], V. Mitroi [3], M. Troussellier [4], J.-F. Humbert [3], R. Semyalo [5], C. Quiblier [6 7], B. Cécile [6], W. Okello [2]

[1]Makerere University Institute of Environment and Natural Resources (MUIENR), P. O. Box 7062, Kampala, [2]National Fisheries Resources Research Institute, P. O. Box 534, Jinja, Uganda, [3]INRA- Institute of Ecology and Environmental Sciences of Paris, Paris, [4]Place Eugène Bataillon, 34095 Montpellier cedex 5, UMR UMR 9190, MARBEC, CNRS-Université de Montpellier – IRD - IFREMER, , Montpellier, France, [5]Department of Zoology, Entomology and Fisheries Sciences, Makerere University, P. O. Box 7062, Kampala, Uganda, [6]UMR 7245 MCAM CNRS-MNHN, Muséum National d'Histoire Naturelle, [7]Université Denis Diderot, 75013 , Paris, France

Abstract: Lake Victoria (LV) is a good example of the huge challenge facing the South countries concerning the sustainable management, monitoring and protection of their surface water resources. High human population growth rates and related activities around LV has led to progressive eutrophication since the 1950s, leading to recurrent proliferations of floating plants - water hyacinth and cyanobacteria blooms. These blooms mainly occur in the numerous bays and gulfs (B&Gs) of LV surrounded by big cities - Kampala, Kisumu, Mwanza etc. Although the lake remains a vital resource, this increasing eutrophication is a major threat to its ecological functioning and support to the growing populations. Several surveillance programmes have collected water quality data in the framework of the Lake Victoria Environmental Management Projects (LVEMP I and II) in addition to other research programs in the past 20 years. Based on this extensive literature, we aim to present a review of the direct and indirect relationships between socio-economic factors and cyanobacterial blooms (and more globally eutrophication). On one side, an analysis of the history of eutrophication in LV and the current available knowledge on cyanobacterial toxins. More attention will be drawn on B&Gs, which experience huge cyanobacterial blooms and high level of toxicity. In B&Gs, high concentrations of microcystins (up to 81 µg/L) have been found, which is not a common occurrence in Africa and the transfer of these toxins to fishes has been reported, increasing the potential exposure of humans. On the other hand, the demographic data of populations in the LV watershed will be related to land use changes, and to eutrophication process of the main lake as well as the consequences of growing urbanization near B&Gs, which lead to the hyper eutrophication of these sub-ecosystems. A synthesis screen will permit the discussion of all these multiples stressors, factors and processes involved in the determinism of the blooms and related interactions. The last part of the presentation will concern (i) the potential impact of these cyanobacterial blooms on the ecosystem services (drinking water, food provision, climate regulation, etc.) provided by the lake; including the economic costs of cyanobacterial blooms for the water supply, and (ii) a global analysis of the efficiency of the numerous actions that have been undertaken with the goal to reduce nutrient loads and consequently cyanobacterial blooms in the main lake and in B&Gs. In conclusion, we will discuss the importance of the B&Gs for the human populations but also for the functioning of the main lake. These highly polluted areas that collect wastewater originated from large urban areas appears to constitute buffer areas, probably limiting the pollution of the main lake, while they remain used for the water and food supplies.

Disclosure of Interest: None Declared

Socio-economic impacts of HABs

O-020

Co-producing knowledge and local empowerment for managing harmful algae. A citizen science experience on Aghien Lagoon in Ivory Coast

V. Mitroi [1,*], P.-Y. Bulot [1], J.-F. Deroubaix [2], A. Chrislain Kouadio [3], J. Kalpy Coulibaly [4], M. Koné [3], I. Jonas [3], F. Tra [3], O. Kam [3], A. Ouattara [5], J.-F. Humbert [6] and Wasaf

[1]CNRS - iEES Paris, [2]Leesu-ENPC, Paris, France, [3]Université Félix Houphouët-Boigny, Abidjan, [4]Institut Pasteur, Abijdan, [5]Institut Pasteur, Abidjan, Côte d'Ivoire, [6]INRA, Paris, France

Abstract: Numerous Sub-Saharan countries confronted with accelerate eutrophication of surface water resources, which are essential for water supply, need urgently to implement long term monitoring programmes of their freshwater resources. Until recently, the monitoring of freshwater ecosystems only involved research or institutional laboratories. Our talk will present an example of a citizen science interdisciplinary project implemented in Ivory Coast where riverine populations of the Lagoon Aghien have been involved in its survey, in addition to the monthly monitoring performed by the Institut Pasteur of Abidjan.

The participation of the villagers is based on the use of a free mobile application (EpiCollect5) that allows to complete a short questionnaire and to take geo-localized and dated photo. The application was proposed to three villages selected according to a longitudinal transect of the lagoon. In order to involve local populations into the monitoring two strategies were employed: i) focus-groups presenting the application to different groups of stakeholders (young people, local leaders representing various economical activities and women); and ii) training of one “local sentinel” in each village to whom a smartphone was provided. After six months of use, more than 300 data sets have been registered, most of them showing: water hyacinth, water troubled water, detritus and green algae (or green water). The presence of cyanobacteria in the lagoon was confirmed by the Institut Pasteur when “green water” photos were provided on the web site. Each three months a "health bulletin" of the lagoon informs villagers about both scientific and non-scientific monitoring data and water use precautions.

As first results of participatory monitoring of harmful algae, we can highlight two major benefits: i) maintaining affordable data costs on cyanobacteria monitoring; and ii) social benefits aspects related to local participation. Concerning the first point, real-time monitoring of cyanobacteria is still very costly and requires technological instrumentations and scientific competences, which are not available in developing countries. The involvement of local populations produces reliable, low-cost, and real-time data. The second type of benefits concerns local awareness with ecological and heath risks related to cyanobacteria proliferation (new learnings and understandings of water ecosystem and of eutrophication risk, new capacities to identify pollution sources and sanitary and ecological risks, etc.). By bringing together scientific ecological knowledge and local knowledge and perception of water quality, citizen science projects can also contribute to the development new collective capacities of planning and action adapted to local contexts.

Disclosure of Interest: None Declared

Socio-economic impacts of HABs

O-021

Saxitoxin and the Cold War

P. Tester [1,*], J. Matweyou [2], S. Kibler [3], B. Wright [4], B. Himelbloom [5], R. W. Litaker [3]

[1]Ocean Tester, LLC, Beaufort, [2]Alaska Sea Grant, College of Fisheries and Ocean Sciences, University of Alaska, Kodiak, [3]Plankton Ecology & Physiology, National Ocean Service, NOAA, Beaufort, [4]Marine Ecology, Aleutian Pribilof Islands Association, Anchorage, [5]Seafood Science & Technology, College of Fisheries and Ocean Sciences, Kodiak, USA

Abstract: In November 1940, the Fishery Products Laboratory was established in Ketchikan Alaska. By 1943, staff members were tasked with investigating marine species as emergency food sources in the event of protein shortages brought about by World War II. The vast shellfish resources on the Alaska coast were recognized and significant efforts were directed toward shellfish utilization through the early 1960s. By the end of the decade the Ketchikan Lab (renamed Ketchikan Technological Laboratory) was closed and in 1971 personnel and equipment were relocated to a newly constructed facility at Kodiak AK. More than 5,000 records of mouse bioassays and associated data from work completed at the Ketchikan Lab were archived and forgotten until 2013-2014 when rumors about "lost PSP" data began to circulate. In the summer of 2014 multiple lab notebooks were scanned along with data associated with the early studies including a stellar thesis that was never published. Among the data was a contract for clams and a shipping receipt to the US Army's Biological Warfare Laboratories at Camp Detrick (later renamed Ft. Detrick) MD. Were the Alaskan clams a source of saxitoxin for the Biological Warfare Lab? On 25 November 1969, President Nixon asked for all biological warfare agents to be destroyed and for the US Senate to ratify the 1925 Geneva Protocol prohibiting the use of chemical and biological weapons. Information from the Church Committee Hearings* in 1975 revealed activities of the Central Intelligence Agency (CIA) and the US Army's Biological Laboratory at Ft. Detrick regarding retention of a "small" amount (11 grams) of shellfish toxin. "A major early requirement of the Agency was to find a replacement for the standard cyanide L-pill issued to agents in hazardous situations during WW II. This was the basis on which eventually we discovered the shellfish toxin." Was saxitoxin ever provided by the CIA to those in hazardous situations? Yes, it was. Join me to learn more about the history of saxitoxin in WWII and during the Cold War that followed.

---

*Intelligence Activities Senate Resolution 21, Hearings before the Select Committee to Study Governmental Operations with Respect to Intelligence Activities of the United States Senate, 94th Congress, First Session, (Vol 1) – Unauthorized Storage of Toxic Agents September 16-18, 1975

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

O-022

Local atmospheric and oceanographic factors mediating an *Alexandrium catenella* bloom in Southern Chile

O. Espinoza-González [1,*], L. Guzmán [1], L. López [1], E. Cascales [1], V. Besoaín [2]

[1]Centro de Estudios de Algas Nocivas (CREAN), Instituto de Fomento Pesquero, Puerto Montt, [2]Centro Putemún, Instituto de Fomento Pesquero, Castro, Chile

Abstract: This is the first report of a bloom of the toxic dinoflagellate *Alexandrium catenella* along the Pacific Ocean coastline of Chiloé Island. During the 2018 summer (January – February) the favorable atmospheric – oceanographic conditions allowed the proliferation of *A. catenella* and their progressive presence towards the North. To evaluate the development of this proliferation along the Pacific Ocean, we conducted a study from Zorra cove (43,1°S) to Mansa bay (40,5°S) in the north of the Los Lagos region encompassing up to 10 miles from de coast. Results showed that the *A. catenella* bloom was restricted to the southern studied area, reaching up to 300 cells mL^{-1} at five meters depth at Cucao zone (42,5°S) on February 3rd, in conjunction with a well stratified water column above to 25 meters, water temperatures >15°C and northwest winds favouring coastal accumulation. The phytoplankton assemblages were dominated by *Thalassiosira cf.minúscula* and *Leptocylindrus minimus* (37% of total abundance) and *A. catenella* reaching values ~10% of total phytoplankton abundance. While, in the central studied area, the stations located near Chacao channel (41,8°S), the local winds induced a cold water upwelling (<11°C), a more unstable mixed water column and a clear change in the phytoplankton assemblage, being *A. catenella* replaced by diatoms and *Asterionellopsis glacialis* reached ~20% (up to 400 cells mL^{-1}) of total abundance. Towards the north, in Mansa bay we found a stratified waters (>13°C) with dominance of *Pseudo-nitzschia* spp. (25% of total abundance) and *Pseudo-nitzschia* cf. *pseudodelicatissima* reaching values ~6% of total phytoplankton abundance.

From these data we propose that the intensity and direction change of local winds, the upwelling of cold waters and the mixing of the water column were the mechanisms that prevented a bloom of *A. catenella* in this area and its displacement to the inner waters of Chiloé island through the Chacao channel, showing a clear difference with 2016 *A. catenella* bloom which strongly entered through the channel during Spring tides.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

O-023

Population level dynamics of PSP toxin production by an inshore bloom of *Alexandrium catenella*

M. Brosnahan[1,*], J. Smith[2], G. Doucette[3], A. Fischer[4], D. Kulis[1], S. Lim[5], D. Ralston[6], R. Olson[1], H. Sosik[1], D. Anderson[1]

[1]Biology, Woods Hole Oceanographic Institution, Woods Hole, MA, [2]Aquatic Health Sciences, Virginia Institute of Marine Science, Gloucester Point, VA, [3]NOAA/National Ocean Service, Charleston, SC, [4]Ocean Sciences, University of California - Santa Cruz, Santa Cruz, CA, [5]Scripps College, Claremont, CA, [6]Applied Ocean Physics and Engineering, Woods Hole Oceanographic Institution, Woods Hole, MA, USA

Abstract: Paralytic shellfish poisoning (PSP) is among the most severe toxin syndromes caused by harmful algal blooms yet there are still many unknowns about the transfer of PSP toxins into coastal ecosystems and the food supply. The dinoflagellate *Alexandrium catenella* (=*A. fundyense*), the most widespread PSP-causing species globally, has been studied intensively within the Nauset Marsh estuary (Cape Cod, MA USA) through deployments of new phytoplankton sensor technologies. Observations of *A. catenella* are enabled in part by the retentive nature of Nauset's terminal kettle ponds, which facilitates effective monitoring from fixed observatory sites. Selective retention also often results in near monospecific blooms. Our sensor installations are producing unprecedented insights into this species' behavior and physiology in situ. Among several noteworthy results are that naturally occurring *A. catenella* blooms develop more quickly, are more readily converted to cysts, and produce more toxins than had previously been known or thought possible. With high-frequency profiling through the full depth of one of these kettle ponds, the overall size of the *A. catenella* population in total cell number and biovolume was characterized through two cycles of bloom development and decline. In combination with time-series of cell toxin quotas and cell imaging-derived estimates of division rate, population size estimates were used to characterize the dynamics and scale of toxin production at a population level. Such whole population estimates provide a foundation for quantitative exploration of the supply and fate of PSP toxins within the Nauset ecosystem.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

O-024

Atmospheric and oceanographic processes on the distribution and abundance of *Alexandrium catenella* in the North of Chilean fjords

L. Guzmán [1,*], O. Espinoza-Gonzalez [1], E. Pinilla [2], V. Besoain [2], M. J. Calderón [1], J. Caceres [1], L. Iriarte [3], V. Muñoz [3], R. Martinez [1], C. Hernández [4], M. A. Tocornal [5], P. Carbonell [1]

[1]CREAN, Instituto de Fomento Pesquero (IFOP), Puerto Montt, [2]Centro Putemun, Instituto de Fomento Pesquero (IFOP), Castro, [3]CREAN, Instituto de Fomento Pesquero (IFOP), Puerto Aysén, [4]Laboratorio Ambiental, Secretaria Regional Ministerial de Salud, Puerto Montt, [5]Laboratorio Ambiental, Secretaria Regional Ministerial de Salud, Puerto Aysén, Chile

Abstract: Since 1972, *Alexandrium catenella* has bloomed and caused Paralytic Shellfish Toxin (PST) outbreaks in southern Chile. In the fjords central area, i.e. Aysén region (43° - 48°S), since 1994 the blooms have been annually recurrent, while in the northern area i.e. Los Lagos region (41° - 43°), on 2002, 2006, 2009, 2016 and 2018, sporadic blooms have occurred. The 2006 bloom showed that the *A. catenella* abundance, suddenly in a week, locally increased, increment that cannot be explained by asexual reproduction. We hypothesized that passive physical transport by dynamical processes could be responsible of the observed abundance. Here we present the 2018 *A. catenella* bloom along 42°- 47°, including the changes in distribution and abundance, the PST evolution and the atmospheric-hydrodynamics features. The bloom was preceded and developed under atmospheric - oceanographic favorable growth conditions for *A. catenella* and other harmful dinoflagellates. Using particle tracking modeling, the southeast winds mediated connectivity in mid-January, between the northern area of the Aysén region with the southeast of Chiloé island. This process would explain: a) the increase of *A. catenella* density in 5-7 days in the southeast of Chiloé island; b) the northward cell dispersion by the inland sea, mainly along the east coast of Chiloé island, keeping cells almost absent from the mainland coastline and c) the exit of vegetative cells from fjords, to the Pacific Ocean. Also, these hydrodynamic processes allows the understand of the microalgae distribution and abundance in the central area fjords, where bloom lasted at least eight weeks, covering from 43° to 47°; and, a toxicity record in mussels (143,000 µg STX.eq. 100 g^{-1}) was registered. *A. catenella* bloom densities were around 300 cells mL^{-1}, but, reached up to 1,000 cells mL^{-1}. It cannot be discarded that *A. catenella* blooms originate in the Pacific Ocean, but results support that these could originated within fjords.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

O-025

Predicting bloom initiation on the Texas coast: Combining satellite imagery with an individual-based model

D. Henrichs [1,*], M. Tomlinson [2], L. Campbell [1]

[1]Oceanography, Texas A&M University, College Station, [2]National Centers for Coastal Ocean Science, National Oceanic and Atmospheric Administration, Silver Spring, USA

Abstract: A key question when modeling harmful algal blooms is: where do blooms start? Here we combined a previously developed, spatially explicit individual-based model (IBM) for *Karenia brevis* with a satellite imagery ensemble model to identify patches of *K. brevis* that may be seed populations in the southern Gulf of Mexico. We focused on the region north of the Yucatan peninsula, Yucatan, Mexico to identify potential *K. brevis* populations, which were then used to seed the IBM and track cells through time. Individuals were tracked from ~1 Jul through 31 Dec (~180 days) and the arrival of a sustained presence of cells (i.e. consecutive days with cells present) at the coast of Texas was compared with a 10-year time series of abundance captured with an Imaging FlowCytobot (IFCB) at Port Aransas, Texas, USA. The IFCB captures images of all phytoplankton cells (10-150 µm) and has successfully provided early warning for 8 harmful algal blooms. Modeled cells were allowed to vertically migrate in the water column to maximize their access to light and nutrients, but there was no cell division and no cell death. Two, highly simplified, nutrient fields were tested, one based on salinity (concentration increases with decreasing salinity; SALT) and one based on mixed layer depth (concentration increases with depth; MLD). Due to randomness associated with each individual cell, results were averaged from multiple model runs for each year. In 2015, a bloom of *K. brevis* was identified at Port Aransas on 14 Sept. Retrospective analysis of the satellite imagery indicated the potential presence of a patch of *K. brevis* north of the Yucatan Peninsula on 12 Jul. Cells were seeded in this region and tracked forward in time. The first sustained presence of modeled cells in the region near Port Aransas occurred between 7 - 18 Sept, dependent upon how the nutrient field was derived. Model runs utilizing the MLD-based nutrient field were better at capturing the timing of arrival while those utilizing the SALT-based nutrient field appeared to better capture the bloom dynamics once the bloom had arrived. Results varied by year, with the starkest difference being 2010, when no large bloom of *K. brevis* was observed along the coast of Texas. Results from the model utilizing a SALT-based nutrient field indicated a large bloom, with cells arriving in mid-September. In contrast, the results utilizing a MLD-based nutrient field indicated no cells until mid-November, well beyond the typical start period for a bloom in Texas (Sept-Oct). As the true nutrient field is much more complex than those used here, the incorporation of better nutrient fields could greatly improve future results and the potential for forecasting of blooms. Using satellite imagery to identify potential seed populations for subsequent blooms in Texas looks promising but additional field samples are necessary.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

O-026

TRACKING THE FLOATING ECOTYPE OF ULVA PROLIFERA, THE CAUSATIVE SPECIES OF GREEN TIDE IN THE YELLOW SEA

P. Jiang [1,*]

[1]Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China

Abstract: A unique floating ecotype of *Ulva prolifera* (Ulvophyceae, Chlorophyta) has been confirmed to be responsible for the world-largest green tide occurred in the Yellow Sea, and a SCAR genetic marker specific to this ecotype has been developed. To re-evaluate the Subei Shoal as the origin of *Ulva* bloom, a SCAR-based investigation was conducted in this research, with thousands of green seaweed individual samples collected from two stages, *i.e.* the attaching stage on *Pyropia* aquaculture rafts and the subsequent free drifting stage northward. Here we showed that there were diversity and succession of *Ulva* species attached on the *Pyropia* rafts, in which the floating ecotype of *U. prolifera* appeared in late March when water temperature increased. Despite a low species proportion close to 5%, this floating ecotype dominated the *Ulva* bloom soon after being discarded to the sea. In the drifting stage, the ratio of floating ecotype reached to almost 100% in 2016, and nearly 70% in 2017 when a golden tide by *Sargassum* happened in same region. Based on the synchronous investigation to the diversity of attached *Ulva* along the Yellow Sea coastal line, there were no notable settlement of floating ecotype by far. In conclusion, the population dynamics for this floating ecotype during green tide was described for the first time. It was not the coastal line but the Subei Shoal that served as the seed origin for the *Ulva* bloom in the Yellow Sea.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

O-027

Spatial distribution of *Microcystis* and microcystins in lake sediment

B. M. Weisbrod [1,*], S. Wood [2], J. Puddick [2], D. Dietrich [1]

[1]Human and environmental toxicology, University of Konstanz, 78457 Konstanz, Germany, [2]Cawthron Institute, Nelson, New Zealand

Abstract: Harmful cyanobacterial blooms (HCBs) occur worldwide in increasing numbers. Those blooms can be composed by several different cyanobacteria but also by other bacteria species. They are known to show patchy spatial distribution in the water body due to wind drift, buoyancy and variable recruitment from sediments. One of the most common species is Microcystis, which is known for producing microcystins (MC). MC production and concentration within a bloom have been shown to vary in time and space during the blooming event. MCs are thought to be very stable and have been detected in previous studies bound to sediment. Compared to concentrations in blooms, much less is known of heterogeneity of Microcystis cell abundance and MCs in lake sediments. Our main task was to investigate the heterogeneity of MC distribution and microbial sediment communities in an eutrophic temperate lake. Therefore we investigated how (I) Microcystis abundance, (II) MC concentration and (III) bacterial community composition varies among sediments of different positions in a lake. We collected sediment samples at 12 positions from either mid-lake or near-shore positions from a shallow eutrophic lake with a long history of cyanobacterial blooms, including MC-producing Microcystis (Lake Rotorua, Kaikoura). Samples were taken from the first 0.5 cm of the sediment core. MC concentration was measured with liquid chromatography-mass spectrometry. A combination of high throughput sequencing targeting bacterial 16S rRNA and quantitative PCR was used to characterise microbial communities and to determine abundance of Microcystis cells as well as expression of genes involved in MC production. A range of physiochemical variables including temperature, pH and dissolved oxygen were also measured. Despite intense blooms in previous years, we did not detect any MCs in the sediment samples. High copy numbers of Microcystis were mainly detected in near-shore samples. Copy numbers of MC producing genes were only detected in traces. From these preliminary data we assume that due to the shallow water depth MCs might be less stable than in deeper lakes. UV radiation and wind mixing might be important factors influencing MC sediment concentration in this polymictic lake. Our data suggest that MC concentration and Microcystis abundance might differ significantly among lakes experiencing Microcystis blooms.

Disclosure of Interest: None Declared

HumanToxicology

O-028

Potential Biomarkers of Recent and Long-term Domoic Acid Exposure in Human Seafood Consumers

K. Lefebvre [1,*], B. Yakes [2], L. Castellon [3], E. Frame [4], P. Kendrick [5], S. Shum [6], N. Isoherranen [6], B. Ferriss [5], A. Robertson [7], D. Marcinek [8], L. Grattan [3]

[1]Research Biologist, NOAA Fisheries, Seattle, [2]FDA, College Park, [3]University of Maryland, Baltimore, [4]King County Laboratory, [5]NOAA Fisheries, [6]University of Washington, Seattle, [7]Dauphin Island Sea Lab, University of South Alabama, Dauphin Island, [8]Dept. of Radiology, University of Washington, Seattle, USA

Abstract: Domoic acid (DA) is a potent neurotoxin that is naturally produced by toxigenic *Pseudonitzschia*species during harmful algal blooms (HABs). In the Pacific Northwest, razor clams (RCs) are known to accumulate DA during HABs and retain the toxin for up to a year after a bloom ends. Consequently, coastal dwelling Tribal RC harvesters regularly consume RCs with detectible DA concentrations, albeit below the regulatory limit of 20 ppm. In the present study, consumption within the last seven days as well as long-term monthly consumption over years were calculated via surveys of Tribal harvesters. Domoic acid exposure doses were further extrapolated using RC consumption data and the average DA concentrations reported in RCs for corresponding time periods and harvest beaches by the Washington Department of Health (WDOH) biotoxin monitoring program. Additionally, urine and serum samples were collected from Tribal harvesters in conjunction with consumption surveys and analyzed for the presence of DA in urine and a DA-specific antibody in serum. Domoic acid concentrations in urine were quantified via ELISA and LC/MS/MS and were an indication of recent exposure, as DA is rapidly eliminated via urine. Domoic acid-specific antibody presence in serum was determined via a surface plasmon resonance (SPR) biosensor. Antibody presence in serum indicated long-term, repetitive exposure based on previous laboratory studies showing that multiple weeks of low level DA exposure were required before antibody production occurred. The relationships between DA levels in urine and antibody presence in serum to recent exposure and long-term exposure, respectively, will be discussed.

Disclosure of Interest: None Declared

HumanToxicology

O-029

HAB toxins and their effect on mammalian thioredoxin reductase

K. Rein [1,*], A. Tuladhar [1]
[1]Chemistry, Florida International University, Miami, USA

Abstract: *Karenia brevis*, the Florida red tide dinoflagellate produces a suite of neurotoxins known as the brevetoxins. The most abundant of the brevetoxins PbTx-2, was found to inhibit the mammalian thioredoxin- system, whereas the PbTx-3 has no effect on this system. The thioredoxin system is a major cellular reductant system present in the cell, whose role is to maintain cellular redox homeostasis. It does this in part, by regulating the activity of many other enzymes controlling cellular processes. This system also provides antioxidant defense, reducing destructive reactive oxygen species (ROS). The system is comprised of thioredoxin (Trx) which reduces target protein disulfide bridges by thiol-disulfide exchange and thioredoxin reductase (TrxR) which utilizes NADPH to reduce oxidized Trx, returning it to its active form. PbTx-2 inhibits the reduction of Trx by TrxR. On the other hand, PbTx-2 activates the reduction of small disulfides such as 5, 5'-dithio-bis-(2-nitrobenzoic acid) by TrxR. PbTx-2 has an α, β-unsaturated aldehyde moiety which functions as an efficient electrophile and selenocysteine conjugates are readily formed. It is proposed that the mechanism of inhibition of thioredoxin reduction is via the formation of a Michael adduct between selenocysteine and the α, β-unsaturated aldehyde moiety of PbTx-2. To our surprise, while PbTx-3 does not inhibit Trx reduction by TrxR, is does activate DTNB reduction. PbTx-3 does not have the α, β-unsaturated aldehyde moiety suggesting that reaction with the selenocysteine is essential for Trx reduction but not for DTNB reduction. It also suggests that small disulfides compete more effectively for TrxR reduction on the presence of brevetoxins. We have attributed the unusual activity of the brevetoxins toward TrxR to the functionality and the large size of the brevetoxin molecule. This suggested to us that other compounds which are similar in size and functionality would behave in a similar way towards TrxR. This led to the discovery that microcystins and nodularins also effect the activity of TrxR in a similar, but not identical fashion. The dysregulation of TrxR activity leads to an increase in ROS and oxidative stress in cells and represents another mechanism of toxicity for these two toxin classes. Competition assays with known TrxR inhibitors have helped to establish the binding sites of these toxins.

Disclosure of Interest: None Declared

HumanToxicology

O-030

Combined toxicity of mycotoxins and phycotoxins on fish and human cell line models

A. Hayashi [1,*], J. J. Dorantes-Aranda [1], J. P. Bowman [2], G. Hallegraeff [1]

[1]Institute for Marine and Antarctic Studies, University of Tasmania, [2]Tasmanian Institute of Agriculture, University of Tasmania, Hobart, Australia

Abstract: The incidence of extensive *Aspergillus sydowii* marine “fungal slicks” after the 2009 Australian dust storm raised concerns about ecosystem impacts and potential contamination of seafood with mixtures of mycotoxins and phycotoxins. The major *A. sydowii* metabolites sydowinin A & B, sydowinol and sydowic acid exhibited significant cytotoxicity to fish gill (RTgill-W1) cell lines with median lethal concentration (LC_{50}) values of 19.2 to 103.7 µM and also to human intestinal cell lines (HT-29) with LC_{50} values of 9.9 to 93.1 µM. Strong cytotoxicity on RTgill-W1 and HT-29 was also confirmed for other well-known mycotoxins, with increasing activity according to the series gliotoxin> alamethicin> patulin> sterigmatocystin.

Using the HT-29 cell line, the mycotoxins alamethicin, patulin and sydowinol exhibited moderate synergisms with the phycotoxin, okadaic acid (Chou & Talay combination index (CI) of 0.74 to 0.83 at the 90% effective dose (ED_{90})). Similar experiments with saxitoxin and domoic acid are in progress.

These findings highlight limitations of current regulatory approaches which do not regulate for mycotoxins in shellfish, and treat seafood toxins as if they occur as single compounds.

Disclosure of Interest: None Declared

HumanToxicology

O-031

Hepatotoxic and non-hepatotoxic cyanopeptides influence cytoskeleton organization in normal and tumor astrocytes *in vitro*

B. Sedmak [1,*], A. Bubik [2], R. Frangež [3], I. Gutiérrez Aguirre [4], C. M. Žužek [3], T. T. Lah [4]

[1]Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, [2]Environmental Protection College, Velenje, [3]Veterinary Faculty, University of Ljubljana, [4]National Institute of Biology, Ljubljana, Slovenia

Abstract: Apart from the known damages to the liver and kidneys, symptoms of neurotoxicity have been reported, which might be due to damaging effects of CCPs on astrocytes. These CCPs include the hepatotoxic (h)CCPs, such as several microcystins and nodularins, and the non-hCCPs, such as anabaenopeptins and planktopeptins. Representatives of both groups have cytotoxic and cytostatic effects on mammalian cells due to their inhibition of serine/ threonine phosphatases, and the consequent disruption of kinase–phosphatase homeostasis. However, responses of different cell types to CCPs depend on their specific modes of interaction. Hepatotoxic microcystins depend mainly on specific organic anion-transporting polypeptides for cell entry and activity, while non-hCCPs can interact with cells by membrane insertion and/or cell-membrane components. Here, we demonstrate that the non-hCPPs planktopeptin BL1125 and anabenopeptin-B affect cultured human astrocytes, with their almost immediate insertion into the lipid monolayer. These non-hCCPs have similar properties and activities as the more hydrophobic hepatotoxic microcystins, such as microcystin-LF and microcystin-LW. We conclude that the basic mechanism of CCP systemic toxicity is through impediment of normal intercellular and intracellular communication.

Disclosure of Interest: None Declared

HumanToxicology

O-032

Portimine toxicity on human neuronal nicotinic acetylcholine receptors and human cell lines

R. Araoz [1 2,*], M.-T. Ngyuen [1], P. Hess [3], D. Servent [1], M. Delepierre [4], V. Fessard [5]

[1]CEA/ DRF/ JOLIOT/ SIMOPRO/ Toxines Récepteur et Canaux Ioniques, CEA-Saclay, [2]Neuro-PSI, UMR9197, CNRS, Gif sur Yvette, [3]Laboratoire Phycotoxines, Centre Atlantique, IFREMER, Nantes, [4]Unité de Résonance Magnétique Nucléaire des Biomolécules, Département de Biologie Structurale et Chimie, Institut Pasteur, Paris, [5]Toxicology of contaminants, French Agency for Food, Environmental and Occupational Health & Safety, Fougères, France

Abstract: *Vulcanodinium rugosum* is an endemic dinoflagellate species of Ingril lagoon which is connected through the Rhône à Sète Channel with Thau, the most important shellfish farming lagoon in South France. *V. rugosum* blooms are associated with the world record accumulation of pinnatoxin-G (1.2 mg/ kg pinnatoxin-G) in mussel flesh samples collected in August 2010 at Ingril lagoon. The worldwide distributed *V. rugosum* is known to produce pinnatoxin-E, -F, -G and -H as well as portimine. Herein is described a multidisciplinary approach to unveil the toxicity mechanisms of portimine by electrophysiological studies and High Content Screening on human cell lines. Portimine ($C_{23}H_{31}NO_5$; $[M+H]^+$ *m/z* 402.22804) was purified to homogeneity from an ethanolic extract of *V. rugosum* IFR-VRU-01. The spirocyclic ether toxin containing a five-carbon imine ring was characterized and quantified by HR-NMR. Not unlike its cyclic imine congeners, electrophysiological analysis using *Xenupus laevis* oocytes expressing human neuronal nicotinic acetylcholine receptors showed that portimine is an antagonist of these neuronal receptors, albeit with a weaker potency as pinnatoxins or spirolides. The effect of portimine on cell viability, cell proliferation, cell mortality, genotoxicity, oxidative stress and inflammation were monitored by High Content Screening on human cell lines using the appropriate biological markers. Electrophysiology and High Content Screening are high-resolution technologies obviating the use of animal models that are used all along the preclinical drug discovery pipeline against human molecular target and/or metabolic pathways.

Acknowledgements. The authors acknowledge the funding support from the GDR-PHYCOTOX to the mini-project Vulcanodinium and to Interreg/ Atlantic Area for ALERTOX-NET EAPA_317/2016.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-033

Induction of domoic acid production: kinetics and types of grazers and diatom species

N. Lundholm[1,*], B. Krock[2], U. John[2], M. Pančić[3], S. Wohlrab[2], T. G. Nielsen[4], E. Selander[5], S. Hardardóttir[6]
[1]Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark, [2]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany, [3]University of Copenhagen, Natural History Museum of Denmark, Copenhagen, [4]National Institute of Aquatic Resources, Technical University of Denmark, Lyngby, Denmark, [5]Department of Marine Sciences, University of Gothenburg, Gothenburg, Sweden, [6]Natural History Museum of Denmark, University of Copenhagen, , Copenhagen, Denmark

Abstract: In costal ecosystems, copepods co-exist with toxin-producing phytoplankton. Grazers can induce toxin (domoic acid) production in diatoms, but this has only been studied in two species of *Pseudo-nitzschia*and induced by *Calanus*copepods. We explored induction by different grazers and found that all herbivorous copepods induced toxin production, whereas carnivorous copepods did not. The induced response is thus only elicited by copepods that pose a real threat to the responding cells, which supports that the induced toxin production in diatoms evolved as an inducible defense. Our results suggest that copepodamides, polar lipids known to induce production of paralytic shellfish toxins in dinoflagellates, are the chemical cues responsible for triggering the DA production. Subsequent to the high DA levels of the diet, copepods accumulated and retained high concentrations of DA. The depuration lasted 2-6 days and did not differ between two *Calanus*species. Long-term grazing experiments showed higher mortality rates of grazers fed toxic diatoms, supporting the idea that DA production is an induced defense mechanism.

Among the diatoms exposed to copepods, only two of six species of *Pseudo-nitzschia* and none of the *Nitzschia* or *Fragilariopsis* strains responded by producing DA, indicating that different diatom species have different strategies for coping with grazing pressure. Growth rate was negatively correlated to cellular domoic acid content indicating that an allocation cost is associated with the toxin production. Ten days after removal of the copepods, the cellular DA was reduced to 22-35% and further extrapolation suggests that it take the diatom cells up to 6 weeks to reach initial DA levels.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-034

Chemical diversity of prymnesins and do the structural changes in allelochemical toxins drive evolution?

S. B. Binzer [1], D. K. Svenssen [2], N. Daugbjerg [1], C. Alves-de-Souza [3 4], E. Pinto [5], P. J. Hansen [1], T. O. Larsen [2,*], E. Varga [2]
[1]Marine Biological Section, Department of Biology, University of Copenhagen, Helsingør, [2]Department of Biotechnology and Biomedicine, Technical University of Denmark (DTU), Kgs. Lyngby, Denmark, [3]Algal Resources Collection, MARBIONC at CREST Research Park, University of North Carolina Wilmington, Wilmington, USA, [4]Laboratório de Ficologia, Departamento de Botânica, Museu Nacional/Universidade Federal do Rio de Janeiro, Rio de Janeiro, [5]School of Pharmaceutical Sciences, University of Sao Paulo, São Paulo, Brazil

Abstract: *Prymnesium parvum* is a haptophyte microalga that forms blooms leading to devastating fish kills worldwide. In the 1990s, large ladder frame polyethers, prymnesin-1 and prymnesin-2, were proposed as causative agents and their structures elucidated. They are now considered to belong to A-type prymnesins. It was not until two decades later that another class of prymnesins (B-type) were isolated and characterised in our group in different isolates of *P. parvum* (Rasmussen *et al.* (2016), Journal of Natural Products, 79 (9): 2250-2256, DOI: 10.1021/acs.jnatprod.6b00345). They differ in the number of carbon-atoms in the backbone because a core 1,6 dioxadecalin ring system is replaced by a single bond. Furthermore, another group (C-type prymnesins) was tentatively annotated whose backbone structure is not solved yet.

Here we screened 26 strains of *P. parvum* with a wide geographical distribution for the production of prymnesins and could tentatively describe 51 different molecular features (9 A-type, 12 B-type and 30 C-type prymnesins). This showed that the diversity of prymnesins is larger than previously thought. Furthermore, all of the tested *P. parvum* strains produced prymnesins and although different analogous were observed within one strain, all of them belonged to the same prymnesin type (A, B or C). Biogeographical mapping of the prymnesin distribution indicated that each of the three toxin types had a global distribution.

Furthermore, we performed phylogenetic analyses based on internal transcribed spacer (ITS) sequences, both ITS-1 and ITS-2. A monophyletic origin of all types of prymnesins was revealed and clades could be defined by their type of toxic compound produced. This suggests that evolution of new species within the *P. parvum* species complex could be driven by changes in toxin type. This is the first time that such correlation has been documented for a harmful microalga. Chemotaxonomy and ITS-type classification may thus be used to further delimit the *P. parvum* species complex.

Concluding, we want to provide an overview of the complexity of prymnesin-toxins and their biosynthesis. Furthermore, a link to phylogenicity is drawn and it is proposed that the evolution of this microalga is driven by structural changes in the allelochemical toxins.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-035

Toxicity and toxin content of *Alexandrium catenella* natural populations in channels of Southern Chile.

P. Carbonell [1,*], G. Pizarro [2], V. Besoain [3], C. Zamora [2], E. Cascales [1], O. Espinoza-González [1], L. Guzmán [1]

[1]Centro de Estudios de Algas Nocivas (CREAN), Instituto de Fomento Pesquero, Puerto Montt, [2]Centro de Estudios de Algas Nocivas (CREAN), Instituto de Fomento Pesquero, Punta Arenas, [3]Centro Putemún, Instituto de Fomento Pesquero, Castro, Chile

Abstract: The estuarine channels Puyuhuapi, Yacaf and Moraleda (44°S-73°W) are tailored to study Paralytic Shellfish Toxins (PSTs) of *Alexandrium catenella* natural populations (toxin content and profiles) under different regimes of abiotic factors such as temperature, salinity and nutrients (phosphate, nitrite and nitrate). Our hypothesis is that the particular oceanographic conditions of the studied area produces different profiles in the populations of *A. catenella* triggering toxin gradients per cell. During a bloom in February 2018, we collected phytoplankton samples in different sectors of this channel system and oceanographic records. PSTs were detected by liquid chromatograph coupled to a fluorescence detector (LC-Fl). Results show that this species can produce and accumulate a wide range concentration (1-40 pg/cells). The toxin profiles were characterized by carbamates (C1–C2) and gonyautoxins (GTX1-GTX5), but decarbamoyl-gonyautoxins (dc-GTXs 2-3), saxitoxins (STX and neo-SXT) and decarbamoyl-saxitoxins (dc-SXT) were not detected. GTX1-4 dominated in areas with higher temperature ranges (11-19°C) and lower salinity (1-31) (i.e. Puyuhuapi channel), and GTX 2-3 in areas with lower temperature (8.85-14.59°C) and higher salinity (24.81-31.63) (i.e. Moraleda channel). Carbamate toxins concentrations (C2) were higher in Yacaf channel (0.01 - 3.54 ng/µL) with intermedia temperature (10-17°C) and salinities (16-31). The lowest nutrients concentrations (nitrite, nitrate and phosphate) were found in the Moraleda channel (1.27–3.78 µM). The toxin content showed an increasing tendency from Puyuhuapi to Moraleda channels, with values of 1-9 (Puyuhuapi), 7-19 (Yacaf) and 12-40 pg/cells (Moraleda). The highest cell toxins contents occurred in areas with lower salinity, higher temperature and lower phosphates concentrations (i.e. Moraleda channel).

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-036

The ichthyotoxic dinoflagellate *Cochlodinium polykrikoides* in Indonesia: resting cyst distribution and molecular identification.

M. D. Bayu Intan [1], H. Thoha [1], T. Muawanah [2], A. Rachman [1], O. R. Sianturi [1], T. Sidabutar [1], M. Iwataki [3], K. Takahashi [4], E. Masseret [5,*]

[1]Research Center for Oceanography, Indonesian Institute of Sciences (LIPI), Jakarta, [2]Main Center for Marine Aquaculture of Lampung (BBPBL), Pesawaran Lampung, Indonesia, [3]Asian Natural Environmental Science Center, The University of Tokyo, Tokyo, [4]Laboratory of Systematics and Evolution, Konan University, Kobe, Japan, [5]Marbec, University of Montpellier, Montpellier, France

Abstract: The increasing occurrence of the ichthyotoxic dinoflagellate *Cochlodinium polykrikoides*, considered as toxic invasive species according to DAISIE (Delivering Alien Invasive Species Inventories for Europe), is a significant and expanding threat to health and to fisheries and shellfish industries over the world. The Bay of Lampung, located southeast of the island of Sumatra, facing the Sunda Strait, hosts numerous economic activities such as finfish and shellfish aquaculture, pearl farming, fishing and harbor activities. In October 2012, the surface coastal waters off Bandar Lampung, the major city of the bay, became dark brown. The phytoplankton bloom progressively spread in the southern part of the bay, and in November 2012, mass mortalities occurred in many fish farms of the bay, resulting in significant economic losses. This phenomenon continued over the following months and periodically the following years. Based on the position of the sulcus and shape of chloroplasts, the causative species was identified as *C. polykrikoides* and clearly differentiated from the similar species *Cochlodinium fulvescens.* However, its vegetative cells were scarcely observed in water column monitoring. Thus, investigations were conducted to study the cyst beds distribution of this harmful species in Lampung bay and to determine the sub-clade/group of the *C. polykrikoides* isolated, in view of understanding its expansion. Field surveys indicated that the highest densities of *C. polykrikoides* cysts were recorded in the inner part of the bay, off Bandar Lampung and at Hurun Bay, two anthropogenized areas where blooms occurred periodically. From their ornaments, two cyst morphotypes were observed. Interestingly, molecular identification and phylogenetic analysis from cysts and vegetative cells showed the existence of two sub-clades/groups in this area. In addition to a regional “meteorogical pattern” favorable to the triggering of blooms, the anthropogenic activities, such as international harbor activities, fisheries and aquaculture, might play a role in *Cochlodinium polykrikoides* expansion and events, which now occur almost year-around.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-037

The dynamics of *Alexandrium pacificum* blooms in the Marlborough Sounds, New Zealand

L. MacKenzie [1,*], K. Smith [1]
[1]Cawthron Institute, Nelson, New Zealand

Abstract: Since a major bloom was first detected in 2011, detailed observations on the progression of seasonally recurrent *Alexandrium pacificum* blooms in Queen Charlotte Sound (QCS) have been made each summer. Data has been collected on physical, nutrient chemistry and biological factors that influence the timing, magnitude, duration and distribution of the bloom from year to year. Molecular tools (qPCR, meta barcoding) have been employed to track *A. pacificum* cyst distributions in sediments, cell numbers (using rRNA and STX genes) in the water column and successional processes in the plankton community. Physical water column dynamics are important in bloom initiation but it has become apparent that biological factors (e.g. inter-specific competition and possibly allelopathy) probably play a defining role in the trajectory of blooms by inducing bloom termination and encystment. There has been a progressive decline in bloom intensity and cyst distribution over the seven-year study period and observations have shown that incursion of the dinoflagellate into new areas and deposition of cysts does not necessarily lead to the establishment of new populations.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-038

Diversity and seasonal distribution of *Pseudo-nitzschia* from the Gulf of Trieste with insights in toxicity and potential introductions

T. Turk Dermastia [1,*], D. Stanković [1], J. Francé [1], M. Tušek Žnidarič [2], A. Ramšak [1], P. Mozetič [1]

[1]Marine Biology Station, National Institute of Biology, Piran, [2]Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia

Abstract: The diatoms from the *Pseudo-nitzschia* genus are heavily studied agents responsible for the amnesic shellfish poisoning via the production of domoic acid. These diatoms are important members of the coastal phytoplanktonic community of the Gulf of Trieste in the Northern Adriatic Sea. During the 18-month study, cultures were established from single cells taken monthly from the natural environment. Thirty-five cultures were identified with transmission electron microscopy (TEM) and by barcoding of the ITS and 28S regions of the ribosomal DNA. Based on the reconstructed phylogenies from publicly available sequences six species were identified. These are *P.calliantha, P.fraudulenta, P.multistriata, P.pungens, P. mannii* and *P.delicatissima*. There is also an agreement on the existence of at least one more species, which is probably *P.galaxiae*; however, we were unable to establish viable cultures of this species. Altogether, our institution now harbors 38 strains of *Pseudo-nitzschia,* which are awaiting further analysis. Most strains cultured in our institution belong to the *P.calliantha* clade. Preliminary tests on toxicity were attempted on six strains, providing results on one toxin producing strain of *P.multistriata,* even though the toxin levels in culture seemed to be very low (0,217 fg/cell). Our study also revealed some seasonal patterns of *Pseudo-nitzschia* distribution, especially when coupled with our long-term monitoring data on HABs. While some species tend to occur only in particular seasons (i.e *P.multistriata* from early autumn to late winter; *P.fraudulenta* and *P.delicatissima* mainly in spring), others such as *P. calliantha* seem to be present throughout the year. Reconstructed phylogenies based on the selected markers indicate that there seems to be no differences between strains occurring in different parts of the year, although intraspecific differences were not directly examined. Future work will focus on determining the *rbcL* marker sequences and establishing toxicity profiles of the cultured strains. In the long-term we hope to establish metabarcode profiles of environmental samples, to complement our long-term monitoring data.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-039

How harmful algae compete for limited nutrient resources. Lessons from the northern Adriatic Sea.

M. Pfannkuchen [1,*], M. Smodlaka Tanković [1], A. Baričević [1], I. Ivančić [1], N. Kužat [1], B. Gašparović [2], T. Novak [2], G. Kušpilić[3], D. Marić Pfannkuchen [1]

[1]Center for Marine Reseach, Ruder Boskovic Institute, Rovinj, [2]Division for Marine and Environmental Research, Ruder Boskovic Institute, Zagreb, [3]Institute of Oceanography and FIsheries, Split, Croatia

Abstract: Imbalanced nutrient supply is a general and common challenge to marine microalgae. The competition for the limiting resource quickly becomes a shaping pressure for microalgae community structures. Nitrogen and phosphorous are often key nutrients and limiting supply of phosphorous is hypothesized to be a global trend in marine systems. The northern Adriatic is a model system with respect to this trend and marine microalgae can be observed under a variety of steep ecological gradients. We observed the in situ “behavior” of microalgae with respect to competition for phosphorous, generally the limiting nutrient. Our results suggest a number of key strategies for the successful competition for this element: Increased phosphate uptake rates, specialization on organic phosphate sources at high or low concentrations by the use of alkaline phosphatases, delayed bloom initialization, phosphate pool formation, morphological adaptations and alternations of cellular nutrient requirements. This results in the characterization of a multitude of spatiotemporal niches across ecological gradients. We performed in vitro experiments on monoclonal algal cultures to quantify the performances of microalgal species in phosphate limited conditions. Here we present the relative performances of harmful algae with respect to the abovementioned mechanisms in the context of other common northern Adriatic microalgae and explain the spatio-temporal distribution of harmful algae from the genera (*Skeletonema, Chaetoceros, Pseudo-nitzschia, Prorocentrum and Ostreopsis*).

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-040

The role of phosphatase activities during a bloom of *Ostreopsis* cf. *ovata* in the northern Adriatic Sea

S. Accoroni [1,*], M. Pasella [1], T. Romagnoli [1], E. Razza [1], C. Totti [1], N. T. W. Ellwood [2]
[1]Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona, [2]Department of Science, Università Roma Tre, Rome, Italy

Abstract: Increased organic nutrient loads deriving from anthropogenic activities and natural processes frequently cause eutrophication of coastal waters. An increasing number of phototrophs have been shown to make use of organic nutrients. The potential utilization of dissolved organic phosphorus (DOP) by microbial-mats associated with the toxic dinoflagellate *Ostreopsis* cf. *ovata*, was investigated throughout a full cycle of a bloom occurred in 2015 in the Conero Riviera (N Adriatic Sea). Measurements of phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activities of the epiphytic mats (including cells and exopolymeric substances) and chemical-physical parameters were made from late summer to early autumn with a weekly frequency.

Analyses of ambient inorganic nutrient fractions revealed very low filterable reactive P (FRP) concentrations and DOP concentrations that were on average 85% of the total dissolved P. A rapid increase in PMEase and PDEase activities in the microbial community was recorded, coinciding with the onset of a proliferation of the *Ostreopsis* population. Chromogenic staining of samples showed that activity was closely associated with the *Ostreopsis* cells, located both extracellularly (cell surface and within the EPS) and intracellularly (ventral cytoplasm). The increase in both phosphatase activities indicates that *Ostreopsis*-mat community can utilize a wide range of DOP types. Tests in laboratory confirmed that *Ostreopsis* cf. *ovata* can utilize both phosphomonoester (D-Fructose 1,6-disphosphate, β-Glycerophosphate, α-D-Glucose 1-phosphate, Guanosine 5′-monophosphate and Phytic acid) and phosphodiester (DNA and RNA) sources to grow.

The experiments also demonstrated that PMEase and PDEase were strongly influenced by water temperature, with maximum values recorded at 30-35 °C.

Based on the present findings, *Ostreopsis* seems to have adaptations that allow it to thrive in P-limited environments where organic P is the main source of P, until water temperature is enough high to allow PMEase and PDEase activity.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-041

Autoecology studies of bloom forming algae from the Gulf of California, Mexico. A review

C. J. Band-Schmidt [1,*], J. J. Bustillos-Guzman [2], L. M. Duran-Riveroll [3], D. V. Ramirez-Rodriguez [4], M. Zumaya-Higuera [4], L. Fernandez-Herrera [4], A. Aquino-Cruz [5], A. C. Mendoza-Lara [4], D. J. Lopez-Cortes [2], F. E. Hernandez-Sandoval [2], T. Zenteno-Savin [2], I. Leyva-Valencia [6], E. J. Nuñez-Vazquez [2]

[1]IPN-CICIMAR, COFAA, [2]CIBNOR, La Paz, [3]CONACyT - Unidad Académica de Ecología y Biodiversidad Acuática, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Mexico City, [4]IPN-CICIMAR, La Paz, [5]Universidad Autónoma Metropolitana, Unidad Xochimilco. Laboratorio de Plancton y Bioenergética, Mexico City, [6]CONACyT - Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, La Paz, Mexico

Abstract: The Gulf of California, Mexico, is a semi-enclosed water body with approximately 3000 km of coastline, a high biodiversity and one of the most important fishery regions of the country. Although reports of harmful algal blooms (HABs) exist since the arrival of the Spanish conquerors in the 16th century, it was only after the 1979 bloom of the toxic dinoflagellate *Gymnodinium catenatum* that Mexican researchers brought major attention to these events. Since this event, a total of 94 species and intraspecific taxa of different groups of microalgae that can form HABs have been reported. Autoecology studies have been conducted in the dinoflagellate species *Alexandrium affine, Gymnodinium catenatum, Margalefidinium polykrikoides, Prorocentrum lima, P. koreanum, P. triestinum, P. mexicanum* and *Pyrodinium bahamense*; and also in species of Raphidophyceae: *Chattonella marina* var. *marina, C. marina* var. *ovata, C. subsalsa*, and *Fibrocapsa japonica*. Laboratory studies have contributed to confirm their identification to species level by combining detailed morphological and genetic sequences, by defining their growth characteristics and in some cases their life cycles; also the physiological characteristics under different environmental conditions, and the detailed description of the harmful effects on other organisms have been described. Laboratory studies on harmful phytoplankton allows us to have information on the physiology of the species that are present in this region and complement these results with the ecological studies of the zone. A review of these studies will be presented.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-042

Discovery of cyanotoxin diversity and ecology in Green Bay using a combination of molecular biology and mass spectrometry

T. Miller [1,*], S. Bartlett [2], E. Houghton [3], D. Dinsmore [4], G. Laliberte [4]

[1]Zilber School of Public Health, [2]School of Freshwater Sciences, University of Wisconsin - Milwaukee, Milwaukee, [3]Green Bay New Water, Green Bay, [4]Wisconsin Department of Natural Resources, Madison, USA

Abstract: Green Bay (GB), Lake Michigan located in the USA Laurentian Great Lakes is a eutrophic environment that has historically experienced large cyanobacterial blooms (cyanoHABs) due to extensive nutrient input from the agriculturally dominated Fox-Wolf watershed. As the world's largest freshwater estuary, GB is highly dynamic transitioning from eutrophic in the lower bay to mesotrophic and oligotrophic along a south-north gradient. While algal blooms and phytoplankton diversity have been well described relatively little is known about the occurrence of cyanobacterial toxins, and their bioactive metabolites (CBMs) along this gradient as well as ecophysiological dynamics of toxin production. Here we present the results of a multi-institutional effort to fully describe CBM dynamics in GB relative to phytoplankton diversity and nutrient distribution using a combination of molecular biology and mass spectrometry approaches. Sampling began with transects in 2014 and 2015 followed by weekly sampling at multiple locations in 2016 and 2017. Over two- dozen CBMs were targeted by liquid chromatography tandem mass spectrometry including microcystins, anatoxins, cylindrospermopsin, anabaenopeptins, cyanopeptolins, microginins, and saxitoxin. Seven different MC congeners, all three Apts, and two of three Cpts were detected in at least one sample. CBMs including MC, Apts, and Cpts were detected in the most northerly sampling sites indicating CBM production occurs in mesotrophic zones of GB. The expression of genes for MC synthesis and regulatory proteins are shown to largely fit with a previously published physiological model in some locations. MCs were highest in the lower southeastern corner of the bay and significantly correlated with chlorophyll, nutrients, and distance to the Fox River spatially. In addition, the MC congener profile transitioned to more rare congeners along the trophic gradient. In contrast, other CBMs did not follow the trophic gradient suggesting that cyanobacterial metabolite diversity is driven by other factors. This research is the first to fully describe cyanotoxin dynamics in GB.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-043

Seasonal gene expression and the ecophysiological implications of toxic *Microcystis aeruginosa* blooms in Lake Taihu, China

X. Tang [1,*], L. Krausfeldt [2], H. Paerl [3], G. Gao [1], B. Qin [1], S. Wilhelm [2]

[1]Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing, China, [2]University of Tennessee, Knoxville, [3]University of North Carolina at Chapel Hill, Morehead City, USA

Abstract: Increasingly harmful cyanobacterial blooms (CyanoHABs) represent a serious threat to freshwater resources. Despite increased research, there remains little functional information concerning the activity of the widespread and often dominant bloom-former, *Microcystis* spp., and its response to spatial and temporal changes in environmental conditions. To address this issue, we examined its bloom activity in Lake Taihu at 9 stations monthly between June and October in 2014. A metatranscriptomic survey, compared against the *M. aeruginosa* NIES 843 genome, revealed significant temporal gene expression patterns, falling into early- and later-bloom groups. About one third of genes observed in *M. aeruginosa* were differentially expressed between the two groups. Conductivity and nutrient availability appeared to be the most important environmental factors associated with the temporal shifts in cellular function. Compared with the early-bloom season (June and July), genes involved in N and P transport, energy metabolism, translation and amino acid biosynthesis were down-regulated during the late-bloom season (August to October). In parallel, many genes involved in regulatory functions, transposases, microcystin as well as extracellular polysaccharide production were up-regulated. This result, along with a high expression of buoyancy-related gas vacuole genes, demonstrate the eco-physiological shift that *Microcystis* spp. undertake as they turn from rapid growth in early summer to a bloom sustaining physiology in late summer and autumn.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-044

METABOLIC INTERACTIONS BETWEEN D. MAGNA AND MICROCYSTIN PRODUCING AND NON-PRODUCING M. AERUGINOSA PCC7806

G. Bojadzija [1,*], M. Bormans [1], E. Briand [2], L. Lawton [3], C. Edwards [3], C. Wiegand [1]

[1]UMR ECOBIO 6553 CNRS Université de Rennes 1, Rennes, [2]Laboratoire Phycotoxines / Unité DYNECO / Dept. ODE, IFREMER - Centre Atlantique, Nantes, France, [3]School of Pharmacy and Life Sciences, Sir Ian Wood Building Robert Gordon University, Aberdeen, United Kingdom

Abstract: Due to eutrophication, freshwater ecosystems frequently experience cyanobacterial blooms, many of which produce toxic and bioactive compounds affecting vertebrates and invertebrates. During their lifetime, zooplankton are able to develop tolerance as a physiological response to cyanobacterial metabolites, but only few of the mechanisms involved have been revealed so far. Detoxification mechanisms, oxidative stress responses and digestive enzymes contribute to the development of tolerance, however, this comes with energetic cost that in turn influences *Daphnia* life traits and may impair populations. At the same time, zooplankton graze on phytoplankton, including cyanobacteria. Although it has been suggested that *Daphnia* are able to reduce cyanobacterial dominance until a certain cyanobacterial density, it remains unclear whether *Daphnia* metabolites alone impact the physiological state and bioactive metabolites production of cyanobacteria. The focus of this study is investigating the mutual physiological reactions of the common and well-studied microcystin producing *Microcystis aeruginosa* PCC7806 and its non-producing mutant on *Daphnia magna*. We hypothesize that: a) the presence of *D. magna* will induce the production of cyanobacterial bioactive compounds, as well as affect growth and stress response in both MC producing and non-MC producing *M. aeruginosa* PCC7806 and b) The presence of MC producing and non-MC producing *M. aeruginosa* PCC7806 will affect physiological responses and life traits in *D. magna.* In order to test these hypotheses experiments are conducted in a purposely-designed co-culture chamber that allows the exchange of the metabolites without direct contact. Cyanobacterial growth, photosynthetic activity, production of ROS, as well as kinetics of cyanobacterial intracellular and extracellular secondary metabolites are monitored in the presence of *Daphnia.* In parallel, *Daphnia*'s physiological and life trait responses in the presence of cyanobacteria are recorded, including biotransformation, oxidative stress and energy allocation. First results confirmed the detrimental impact of cyanobacterial metabolites released into their culture medium on *D. magna*. Culture medium obtained after one and two weeks culture equivalent to 10^4 cells/mL, reduced feeding and survival. Vice versa, cyanobacteria reacted to spent medium from *D. magna* of both one and two weeks old cultures: with an increase of growth rates, to a larger extent for the toxic strain to the two weeks medium and with a decrease in growth rate to the one week medium suggesting a potential degradation over time of the metabolites produced by *Daphnia*. In addition, cyanobacterial metabolites tend to be excreted faster if they are exposed to *Daphnia* spent medium. Hence these preliminary results demonstrate physiological responses to these mutual interactions.

Disclosure of Interest: None Declared

Medical applications of algae, cyanobacteria and their toxins

O-045

Marine Toxins as Drug Leads

L. Botana [1,*], A. Alfonso [1], E. Alonso [1], M. C. Louzao [1], C. Vale [1], N. Vilariño [1], M. R. Vieytes [1]
[1]Department of Pharmacology, Fac. Veterinary, Lugo, Spain

Abstract: Marine toxins are a large group of compounds with very different and privileged chemical structures. Their mode of action covers many targets, but mechanistically they can be regarded as neurotoxic and non-neurotoxic compounds. Those with neuronal targets belong to several groups, each with a defined reference compound, namely saxitoxin, tetrodotoxin, brevetoxin, spirolide, ciguatoxin and domoic acid. Non-neurotoxic toxins are yessotoxin, azaspiracid, pectenotoxin, palytoxin or maitotoxin, among others, each with partially understood mechanisms of action. Both neurotoxic and non-neurotoxic compounds are considered as harmful substances, however due to their interesting structures and mode of action marine toxins are attractive molecules to develop new drugs or as pharmacological tools. This presentation will discuss about the target for each toxin group, and their potential application in the therapeutic field, especially in cancer, Alzheimer disease and inflammation and its related pathologies.

Disclosure of Interest: None Declared

Medical applications of algae, cyanobacteria and their toxins

O-046

Gambierol and analogs reverse paralysis caused by d-tubocurarine and botulinum type-A neurotoxin at vertebrate neuromuscular junctions

J. Molgó [1 2,*], S. Schlumberger [2], M. Sasaki [3], H. Fuwa [3], M. C. Louzao [4], L. M. Botana [4], D. Servent [1], E. Benoit [1 2]

[1]Institut des sciences du vivant Frédéric Joliot, Service d'Ingénierie Moléculaire des Protéines, Université Paris-Saclay, CEA, [2]Institut des Neurosciences Paris-Saclay, UMR 9197 CNRS / Université Paris-Sud, CNRS, Gif sur Yvette, France, [3]Graduate School of Life Sciences, Tohoku University, Sendai, Japan, [4]Departamento de Farmacología, Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo, Spain

Abstract: Gambierol is a marine polycyclic ether toxin, first isolated with ciguatoxins by Satake, Murata, and Yasumoto (1993) from cultured *Gambierdiscus toxicus* dinoflagellates, collected in French Polynesia. The chemical synthesis of gambierol and truncated analogues has been achieved successfully allowing detailed analyses of their mode of action. Interestingly, gambierol and analogs inhibit voltage-gated K^+ (Kv) channels. It is well known that Kv channels play important roles in modulating acetylcholine (ACh) release from motor nerve terminals of vertebrate neuromuscular junctions. Therefore, the aim of our study was to investigate the action of synthetic gambierol and analogues on K^+ currents in nerve cells and nerve terminals, using electrophysiological techniques. In addition, gambierol was studied on neuromuscular junctions in which muscle nicotinic ACh receptors have been blocked with d-tubocurarine (postsynaptic block), or in junctions in which quantal ACh release has been greatly reduced (presynaptic block) by botulinum neurotoxin type-A (BoNT/A). This neurotoxin induces a selective proteolytic cleavage of SNAP-25 an important nerve terminal protein for the neurotransmitter release process. Our results showed that gambierol and analogues inhibited Kv channels in neuronal cells in a concentration-dependent manner. Also, nanomolar concentrations of gambierol prolonged the duration of the presynaptic action potential in motor nerve terminals, as revealed by focal current recording. This indicates that Kv channels in nerve terminals were also blocked by the polyether. The loading of the calcium-sensitive dye Fluo-3/AM to frog motor nerve terminals and the use of spectrometric imaging techniques showed that gambierol increased the transient calcium fluorescence signals in response to 1-10 Hz nerve-stimulation, indicating that blockade of Kv channels enhanced calcium entry into nerve terminals. At the neuromuscular junction, such actions of gambierol are essential for increasing nerve-evoked quantal ACh release and can explain the observed reversal of the neuromuscular block produced by d-tubocurarine, a competitive inhibitor of the muscle nicotinic ACh receptor. Interestingly, gambierol also increased the quantal content of endplate potentials in BoNT/A-intoxicated mouse neuromuscular junctions. These results indicate that blockade of Kv channels and enhanced calcium entry into terminals by gambierol increased the probability of quantal ACh release, supporting the view that SNAP-25 cleaved by BoNT/A can enter into the protein-complex required for calcium-dependent neurotransmitter release. In conclusion, our results suggest that gambierol and analogues can have potential medical application in neuromuscular pathologies in which it is necessary to antagonize pre- or post-synaptic neuromuscular blockade, or both.

Disclosure of Interest: None Declared

Medical applications of algae, cyanobacteria and their toxins

O-047

Microalgae-derived phycotoxins: medical applications

N. Lagos [1,*]

[1]Laboratorio Bioquímica de Membrana, Programa de Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile, Santiago, Chile

Abstract: A drug is defined as an agent intended for use in the diagnosis, mitigation, treatment, cure, or prevention of disease in humans or animals. It is difficulty to conceive our civilization devoid of these beneficial agents. Human disease and the instinct to survive have, through the ages, led to their discovery. Throughout history, knowledge of drugs and medical applications have permanently meant power.

The first definition of a “Red Tides” was given by Okamura 1916 in Japan. He named “Akashio” (red tide in Japanese) and he referred to the water colour change due to outbreaks of microscopic plankton which can cause the death of fish or faunas. The expression “red tide” became common in Japan during the latest half of 1960s, but this description “red tide” is only visual, right now these phenomena are known more accurately as Harmful Algae Blooms (HAB), since they produce serious negative impacts in marine environments. The occurrences of harmful microalgae represent a significant threat to human health and Paralytic Shellfish Poison (PSP) is the most dangerous threat due to its high mortality rate in mammals.

The toxicity of PSP toxins is due to the reversible binding to a site receptor on the voltage-gated sodium channel on excitable cells, in the nanoMolar range, inhibiting channel opening and blocking neuronal transmission. The Nav channels play a key role in neurotransmission at both neuronal synapses and neuromuscular junctions, for that reason, when these PSP toxins are locally infiltrated, two clinical actions are manifested simultaneous: (i) the control of pain, and (ii) the control of muscle hyperactivity.

In all therapeutic procedures, PSP toxins have been shown to be effective and innocuous, they display a remarkable muscle relaxant and an amazing local pain killer effect. Both properties occur at the same time and are manifested instantaneously. Considering instantaneously, relaxation and anesthesic effects can be measured minutes post-injection. The long-lasting local anesthesic effect of Neosaxitoxin, the most potent of all PSP toxins, is even better in combination with bupivacaine or epinephrine. The last one being the best Neosaxitoxin potentiation drug for the enhancement of the long lasting anesthesic effect. On the other hand, considering the immobilization of healing tissues as a fundamental therapeutic principle, the treatment with PSP toxins may be found to be applicable in other pathologies in which muscle hypertonicity results in stiff or/and awkward movements. The data published in those clinical trials will be analysed and discussed in this Panel.

Disclosure of Interest: None Declared

Medical applications of algae, cyanobacteria and their toxins

O-048

Beneficial activities from cyanobacteria towards obesity, diabetes and cancer – screening and mechanism of action

R. Urbatzka [1,*], M. Costa [2], M. Ligia Sousa [1], T. Ribeiro [1], F. Rosa [1], R. Hernandez Butista [3], F. Eirikson [2 4], M. Thornsteindottir [2], S. Ussar [3], V. Vasconcelos [1 5]

[1]CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, Matosinhos, Portugal, [2]University of Iceland, Reykjavik, Iceland, [3]Helmholtz Center, Institute for Diabetes and Obesity, Munich, Germany, [4]ArcticMass, Reykjavik, Iceland, [5]FCUP, Faculty of Science, University of Porto, Porto, Portugal

Abstract: Cyanobacteria are a prolific source of novel bioactive compounds. A wide range of bioactivities were observed including for treatment of human diseases. The most famous example of a compound derived from cyanobacteria is brentuximab vedotin that is a FDA-approved anticancer drug for the clinical treatment of soft carcinoma (http://marinepharmacology.midwestern.edu/clinPipeline.htm). An urgent demand for new bioactive compounds for human diseases is present, and marine cyanobacteria promise to be an excellent source for natural-derived molecules and novel nutraceuticals. The aim of this research is i) to discover bioactive strains of cyanobacteria for obesity, obesity related co-morbidities (fatty liver disease, diabetes) and cancer, ii) to isolate responsible compounds and iii) to characterize their modes of actions.

Initial screening revealed several cyanobacterial strains with interesting bioactivity for obesity (UCP-1 and PPARy in brown adipocytes), hepatic steatosis (lipid reduction in fatty acid fed HepG2 cells) and diabetes (2-NBDG glucose uptake in HepG2 cells) in cell-based screening assays and in physiologically relevant whole small animal screenings (zebrafish larvae Nile red fat metabolism assay). Toxicity was analyzed in complementary assays (MTT, SRB) to select fractions with activity, but without toxicity. Metabolite profiling with UPLC-QTOF-MS was applied to characterize active fractions for known and unknown compounds. In anticancer screening, bio-assay guided fractionation revealed differences between traditional 2D cell culture and 3D culture of cancer spheroids, presumably due to different compounds active in the different assays. Previously, portoamides were isolated from our group at CIIMAR with cytotoxic activity in cancer cells. Here, we present the mechanism of action in colon cancer cells analyzed by quantitative proteomics, high content analyses, qPCR and functional assays. Cytotoxic activity of portoamides is associated with disturbance of energy metabolism, and alterations in mitochondrial structure and function.

This work was supported by the European ERA-NET Marine Biotechnology project CYANOBESITY (ERA-MBT/0001/2015), financed by national funds through FCT (Fundacio para a Ciencia e Tecnologia, Portugal).

Disclosure of Interest: None Declared

Medical applications of algae, cyanobacteria and their toxins

O-049

***In vitro* and *in vivo* anticancer activity of stressed *Dunaliella* sp. aqueous extract against breast cancer**

F. Elleuch [1 2,*], P. BARIL [2], S. ABDELKAFI [3], I. FENDRI [1], C. PICHON [2]

[1]Doctor in biology, Laboratoire de Biotechnologie Végétale Appliquée à l'Amélioration des Cultures, Faculty of Sciences of Sfax, University of Sfax, Sfax 3000, Sfax, Tunisia, [2]Doctor in biology, Center for Molecular Biophysics (CBM), CNRS UPR 4301, Orléans, France, [3]Doctor in biology, Unité de Biotechnologie des Algues, Biological Engineering Department, National School of Engineers of Sfax, University of Sfax, Sfax 3038, Sfax, Tunisia

Abstract: Today, natural compounds constitute are admitted as alternative or adjuvants for conventional medicines to treat different types of diseases including cancer. Marine biodiversity represents an important source of active molecules. The aim of this work is to investigate the anticancer activity of *Dunaliella* sp. isolated from the Tunisian coastal zone. *Dunaliella* is a green microalga known for its ability to produce high levels of carotenoids under well-defined growing conditions. The originality of our study concerns the combination of stress factors to improve carotenoids accumulation in the isolate species. The assessment of the biological activity of *Dunaliella* performed on the triple Negative Breast Cancer (TNBC) model. We produced solvent and aqueous extracts of *Dunaliella* grown under non-stressed -control- conditions (DSC) or stressed conditions (DSS). 4T1 murine breast cancer cells were used to assess their biological activity. Results indicate that the aqueous extract of DSS was the most cytotoxic. As shown by confocal microscopy, the extract accumulates in the perinuclear region of the cells upon uptake. TUNEL assay and agarose gel analysis of genomic DNA revealed a DNA fragmentation. The deactivation and the cleavage of PARP involved in maintaining the DNA integrity, supports this effect. Moreover, Caspase 3 and 9 activation suggest the involvement of apoptosis intrinsic pathway. *In vivo* tumorigenicity experiments performed on 4T1 cells orthotopically implanted in mice revealed that DSS extract was also potent in vivo. Intratumoral injection of DSS low dose (5mg/kg) induced a significant inhibition of tumour growth.

To conclude, *Dunaliella* sp. grown under stressed conditions accumulate active molecules in aqueous extract exhibits *in vitro* and *in vivo* biological activity through the initiation of apoptosome complex activation. We plan to make a liposomal formulation to enhance the efficacy of the anti-tumour activity and to assess if it could be used as an adjuvant of classical drug as paclitaxel.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-050

Vertical migrations of *Dinophysis acuta* and *D. acuminata*: the problems of being in the right place at the right time

B. Reguera [1,*], P. Díaz [2], L. Escalera [3], S. González-Gil [4], G. Pizarro [5], L. Velo-Suárez [6], T. Wyatt [7]

[1]Oceanographic Centre of Vigo, IEO, Vigo, Spain, [2]Centro i~mar, Universidad de Los Lagos, Puerto Montt, Chile, [3]Stazione Zoologica Anton Dohrn, Napoli, Italy, [4]IEO, Madrid, Spain, [5]IFOP, Punta Arenas, Chile, [6]Inserm UMR 1078, Faculté de Médecine, Brest, France, [7]Borreiros, Gondomar, Pontevedra, Spain

Abstract: *Dinophysis acuminata* and *Dinophysis acuta* are the main agents of Diarrhetic Shellfish Poisoning (DSP) events in Western Europe. Predictive models to forecast their blooms require a sound knowledge of their nutritional sources and behavior.

We review evidence from the Galician Rías Bajas and elsewhere which indicates that co-occuring *Dinophysis acuminata* and *D*. *acuta* have quite distinct vertical migration patterns. Vertical migrations of the vegetative phase of motile phytoplankton have a variety of tactical values: a) to seek conditions which enhance the cell division rate, upwards for light and in stratified waters downwards for nutrients; for *Dinophysis* species which require *Mesodinium*, there is the additional complication that the prey organisms have their own migration schedules; b) to reduce losses due to advection (horizontal dispersal); c) to counteract turbulence (vertical dispersal) and maintain preferred depths in the water column; retention allows population to reach densities necessary for life history needs like aggregation and syngamy; c) to reduce losses due to grazers. Clearly these varying requirements are unlikely to be simultaneously compatible; each cell receives a different spectrum of relevant stimuli, resulting in a variety of migrational responses even within the members of a single population. Here we examine distributions in the water column, from daily to seasonal, of the two species in the Galician Rías and in other contrasting systems with a comparative approach. We conclude that the parameters which determine the amplitude and timing of vertical migrations change constantly on all time scales, from hours to the duration of the seasonal cycle of the vegetative phase, and that these parameters must be specific to species and site.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-051

THE TURBULENT LIFE OF *DINOPHYSIS*

M. García Portela [1,*], B. Reguera Ramírez [1], M. Ribera d'Alcalà [2], F. Rodríguez Hernández [1], M. Montresor [2]
[1]Departamento de Microalgas Nocivas, Instituto Español de Oceanografía, Vigo, Spain, [2]Department of Integrative Marine Ecology, Stazione Zoologica Anton Dhorn, Naples, Italy

Abstract: Dinoflagellates species of *Dinophysis,* in particular *D. acuminata* and *D. acuta*, produce lipophilic toxins and jeopardize shellfisheries in Western Europe. In northwestern Iberia, *D. acuminata* has a long (spring-summer) growing season and populations start growing as soon as a mild stratification develops in the upwelling season. In contrast, *D. acuta* blooms in late summer when stratification, combined with moderate upwelling, is maximal. Project DINOMA aimed to compare growth conditions of both species to describe their niches and interpret their spatio-temporal distributions. Based on field observations, our starting hypothesis was that *D. acuta* is more sensitive to turbulence than *D. acuminata*.

We tested the response of well-fed cells of *Dinophysis* to three different turbulence levels –low (LT), 10^{-6} m^2 s^{-1}; medium (MT), 10^{-5} m^2 s^{-1} and high (HT), 10^{-3} m^2 s^{-1}–generated by *Turbogen*, a sophisticated, computer-controlled system. These levels can be found in the Galician Rías during the upwelling season. Patches of *Dinophysis* in the bottom of the containers were observed in all treatments and controls (with no turbulence). Cells of both species exposed to LT and MT showed similar growth to the controls although *D. acuminata* exposed to MT grew faster and reached an earlier plateau phase followed by a sharp decline not observed in the control. Marked differences between species were found in the response to HT: *D. acuminata* showed a moderate growth after an initial lag phase, whereas *D. acuta* declined and remained stationary. Results from this study confirmed our initial hypothesis and the importance of turbulence to shape the species-specific spatio-temporal distribution of *Dinophysis.*

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-052

Dynamics and primary causes of *Aureococcus anophagefferens* brown tide occurrence in the Qinhuangdao coastal waters of China

Q.-C. Zhang [1 2,*], R.-C. Yu [1 2 3], M.-J. Zhou [1], F.-Z. Kong [1 2], L.-M. Qiu [4 5], Z.-F. Chen [1 3]

[1]CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, [2]Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao, [3]University of Chinese Academy of Sciences, Beijing, [4]CAS Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, [5]Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China

Abstract: Since 2009, *Aureococcus anophagefferens* brown tides have consecutively occurred along the Qinhuangdao coastal waters in the Bohai Sea (BS). Dynamics of brown tides and major causes of their occurrences is still not well understood. An accurate quantitative PCR method with a broad detection range (from 1.5 cells l^{-1} to 6.0×10^8 cells l^{-1}) was developed based on *A. anophagefferens* 28S rRNA gene, and the established method was applied to study on the dynamics of *A. anophagefferens* brown tides in the Qinhuangdao coastal waters. There was a thermocline in this area during the brown tides, and *A. anophagefferens* cells mainly appeared in the cold high-salt water mass under the thermocline in the initiation of brown tides. The cold high-salt water mass originated from a depression basin west of BS central bank. With the thermocline disappeared in the neritic waters along the Qinhuangdao coastline, *A. anophagefferens* cells accessed and grew in the mixed water, and then formed brown tides with the cell densities more than 1.0×10^8 cells l^{-1}. High concentrations of DON and DOP companied with low concentrations of DIN and DIP attributed in the Qinhuangdao coastal waters during the emergence of brown tides in 2013 and 2014. Moreover, the salinity of sea water has increased in the BS, especially in the west coastal area, due to the drying-up of the Yellow River and some other factors. *A. anophagefferens* is a Pelagophyte species favoring high-salt environmental conditions containing high concentrations of dissolved organic substances. Therefore, the hydrological and nutrient characteristics were the benefit of the growth of *A. anophagefferens* and the occurrences of brown tide along the Qinhuangdao coastal area.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-053

Intraspecific trait variation and trade-offs within and across populations of a toxic dinoflagellate

K. Brandenburg [1,*], S. Wohlrab [2], U. John [2], A. Kremp [3], J. Jerney [3], B. Krock [4], D. Van de Waal [1]

[1]Aquatic Ecology, Netherlands Institute of Ecology, Wageningen, Netherlands, [2]Ecological chemistry, Alfred Wegener Institute, Bremerhaven, Germany, [3]Leibnitz Institute for Baltic Sea Research Warnemuende, Roshtock, Germany, [4]Alfred Wegener Institute, Bremerhaven, Germany

Abstract: A high intraspecific variation in functional traits promotes the success of a species, allowing clone sorting and selection under changing environmental conditions. The toxic dinoflagellate *Alexandrium* is known for its considerable genetic and phenotypic variation, which may facilitate the formation of dense blooms. To test the extent of intraspecific variation and potential complementarity of traits, we quantified allometric, competitive, and defensive traits in 15 strains of *Alexandrium ostenfeldii* derived from two populations. Our results demonstrate substantial variation in all analyzed traits both across and within populations, which was in some cases even comparable to reported interspecific variation across phytoplankton species. We also found distinct trade-offs, most notably between maximum nitrogen uptake rate and affinity, and partly between defensive and competitive traits. Furthermore, we observed differences in trait variation between the genetically similar populations, suggesting evolutionary feedbacks on trait expression. A high trait variation may ultimately buffer fitness losses and promote species' resilience to environmental change.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-054

Morphological, molecular and ecological insights on *Kryptoperidinium foliaceum* strains from two Mediterranean lagoons (Sardinia, Italy)

C. T. Satta[1 2,*], S. Pulina[1], A. Reñé[3], T. Caddeo[1], N. Fois[2], N. Sechi[1], B. M. Padedda[1], A. Lugliè[1]

[1]Dip. Architettura e Design, University of Sassari, [2]Agris-Sardegna, Sassari, Italy, [3]Dept. Biologia Marina i Oceanografia, Institut de Ciències del Mar, CMIMA (CSIC), Barcelona, Spain

Abstract: *Kryptoperidinium foliaceum* (Stein) Lindemann is a harmful dinoflagellate categorized as high biomass bloom forming species. Its blooms have been associated with massive water discolorations, but also with fish mortality events. The species is widely distributed with a great tolerance range to salinity, being reported from almost freshwater to transitional ecosystems (i.e. coastal lagoons, marshes and estuaries), but also from hypersaline tidal creeks. Despite its wide distribution, information on the morphology, genetic and ecology of *K. foliaceum* is scarce. Recurrent blooms of *K. foliaceum* have been detected in Calich Lagoon (North West coast of Sardinia, Italy) in the last decade. The species was also seldom observed in Santa Giusta Lagoon (central west coast of Sardinia, Italy), where bloom events have not been registered in recent years. The aim of the present study was to investigate morphological, molecular and ecological features of *K. foliaceum* from the two lagoons. Firstly, the long-term ecological data from the field (2008-2015 for Calich, and 1990-2002 and 2010-2018 for Santa Giusta) were analyzed to characterize the two lagoons and the temporal dynamic of the species. Therefore, two cultured *K. foliaceum* strains from Calich and Santa Giusta lagoons were characterized for morphology and genetic. In addition, the growth rates of the two strains were determined at three different temperatures (11°C, 20°C and 30°C) and six salinities (5, 10, 20, 30, 40, 50). Field data showed the constant presence of *K. foliaceum* in Calich along the series, with highest cell densities mainly observed in spring (maximum of 4×10^6 cell L^{-1} in April 2015). On the contrary, the species was sporadically observed in Santa Giusta in the last decade, with cell densities usually rather low ($<10^4$ cell L^{-1}) and the maximum observed in July 2013 (6×10^5 cell L^{-1}). Vegetative cells from both strains were almost oval in shape and extremely flattened dorsoventrally, sharing the same plate formula 3', 2a, 7'', Xc,?s, 5''', 2'''' and the same plate arrangement (e.g. very large 1'). The two strains also showed identical LSU rDNA and ITS sequences. Morphological and molecular results are in agreement with information available for other 'European' strains of *K. foliaceum*. The highest growth rates were detected at 20°C for both strains, and at different salinity depending on the strain. The results of this study combining laboratory and field data add new information on the scarcely investigated ecology of *K. foliaceum*. Further analyses will help elucidate the different behaviour of the species in the considered lagoons.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-055

Diversity, optimal conditions and tolerance of planktonic cyanobacteria to different environmental conditions in Chile, South America

V. P. Almanza [1,*] on behalf of 1, A. Wilmotte [2] on behalf of 2, H. D. Laughinghouse [3] on behalf of 3, P. Pedreros [1] on behalf of 1, R. Urrutia [1] on behalf of 1

[1]Chriam Center, University of Concepcion, Concepción, Chile, [2]Centre for Protein Engineering, University of Liège, Liège, Belgium, [3]University of Florida / IFAS, Fort Lauderdale, USA

Abstract: Phytoplankton samples from 23 Chilean freshwater bodies (lotic and lentic) monitored by the General Water Directorate and by the EULA Center were examined to identify the diversity, optimal conditions and tolerance of planktonic cyanobacteria to different conditions. We selected ecosystems between 34 - 41° S latitude with different environmental conditions (trophic status) and human intervention in the watershed (land usage). Cyanobacteria and overall phytoplankton were identified and their abundance and biovolume were determined using the Utermöhl method. In seven lakes or rivers with visible blooms, samples were identified using a polyphasic approach, in which morphological, ecological and molecular markers (rRNA loci) were integrated.

The optima and tolerance of each cyanobacterial genus at different conditions was calculated by weighted average regression (WA). The relationship among the genera and the environmental characteristics (water temperature, pH, transparency, dissolved oxygen, nitrogen and total phosphorus) were evaluated by pRDA.

The results showed that cyanobacteria were found in all lakes, although they were rare (less than 5% abundance) at the southern limit. In total, 15 genera were identified morphologically, 6 Chroococcales (*Aphanocapsa*, *Aphanothece*, *Gomphosphaeria*, *Limnococcus*, *Merismospedia*, *Microcystis*), 1 Spirulinales (*Spirulina*), 3 Synechococcales (*Coelomoron*, *Snowella*, *Pseudanabaena*), 4 Nostocales (*Anabaenopsis*, *Aphanizomenon*, *Dolichospermum*, *Nodularia*) and 1 Oscillatoriales (*Oscillatoria*). The same genotype (16S rRNA) of *Microcystis aeruginosa* was distributed in the eutrophic ecosystems between 34 and 37°S and formed dense blooms. Blooms of picocyanobacteria (e.g. *Cyanobium*) and other filamentous cyanobacteria, such as *Anabaenopsis elenkinii* and *Aphanizomenon ovalisporum*,were also observed.

In general, cyanobacteria correlated positively with eutrophic ecosystems located at lower latitudes (34 - 37°S), with a total P concentration (min - max: 0.3 - 1.9 mg L^{-1}), and where the river basin was urbanized or used for monoculture tree plantations. The low nutrient concentration limited cyanobacterial abundance in oligotrophic systems. The comparison between ecosystems of different latitudes can be an indicator of the long-term status of cyanobacteria (species diversity and abundance) in the context of global climate change. In the Southern Hemisphere, climate change projections indicate warming, together with reduced precipitation, and a higher incidence of extreme events (periods of rain and drought). If the nutrient conditions increase, genera such as *Microcystis*, *Aphanocapsa*, *Aphanothece*, *Aphanizomenon* could be favored, together with a higher abundances of *Dolichospermum*.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-056

Impacts of CO2 and nitrogen availability on the eco-physiology of harmful cyanobacteria

D. Van de Waal [1,*]

[1]Aquatic Ecology, Netherlands Institute of Ecology, Wageningen, Netherlands

Abstract: Elevated pCO_2 may fuel phytoplankton photosynthesis and thereby promote their growth. Particularly CO_2 fixation in cyanobacteria may be facilitated as they possess a RubisCO (Ribulose-1,5-bisphosphate Carboxylase/Oxygenase) with among the lowest affinities for CO_2. To compensate for this low affinity, they developed carbon concentrating mechanisms (CCMs), which are cellular mechanisms to enhance CO_2 concentrations in the vicinity of RubisCO. Various freshwater cyanobacteria species are toxic and can proliferate under eutrophic conditions, forming dense harmful blooms. Among the most common toxins produced are microcystins. Consisting of seven amino acids, these are nitrogen-rich compounds of which many variants exist with distinct toxicities as result of two variable amino acid positions. The development of a cyanobacterial bloom results in the depletion of resources, including CO_2, light and nitrogen. During bloom development, cells thus experience large shifts in resource availabilities with possible consequences for CO_2 fixation, nitrogen acquisition, carbon:nutrient stoichiometry and toxin synthesis. In this talk, I will explore the extent to which elevated pCO_2 may alter both carbon and nitrogen assimilation, and how this may affect the toxicity of freshwater cyanobacteria.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-057

Impact of salinity variation on growth, physiology and domoic acid production of two strains of *Pseudo-nitzschia australis*

N. Ayache [1,*], F. Hervé [2], V. Martin-Jézéquel [3], Z. Amzil [2], A. Caruana [2]
[1]PhD student, [2]IFREMER, Nantes, Nantes, [3]CNRS UMR 7266, LIENSs, La Rochelle, France

Abstract: Several coastal countries including France have experienced an increasing and major problem related to harmful algal blooms of the toxic diatom *Pseudo-nitzschia*. These toxic blooms are repeatedly present in estuarine and coastal waters widely subject to extreme fluctuations in salinity and often used as shellfish growing and harvesting areas. In this study, we document for the first time the *Pseudo-nitzschia australis* viability, growth, photosynthetic capacity and domoic acid (DA) production under different salinity conditions that could cover natural and anthropogenic environmental changes. Therefore, two strains of *P. australis* (IFR-PAU-16.1 and IFR-PAU-16.2) were subjected to five different salinity conditions (10, 20, 30, 40 and 35 as control) in 2 modes: (i) sudden changes of salinity and (ii) acclimation (cells acclimated for several weeks before salinity changes). First, following the sudden change of salinity, the two strains of *Pseudo-nitzschia australis* survived over a restricted salinity range of 30 to 35, with no impact on cell physiology with maximum growth, photosynthetic capacity and DA production. However, the osmotic stress carried out at salinities 20 and 10 resulted in cell lysis and instantaneous DA leakage in the medium. Interestingly, in acclimation mode, cells were able to acclimate at salinity 20, growing slowly and accumulating high amounts of particulate DA. While the other cultures grown at salinities 30, 35 and 40 showed higher growth rates with a progressive release of DA in the medium. In terms of growth and toxin production, a large tolerance and intra-specific variability have been observed, including the strain IFR-PAU-16.1 that seem to bear higher salinities than the strain IFR-PAU-16.2 with an optimal salinity at 35 instead of 30 for IFR-PAU-16.2. Since most of the shellfish harvesting areas along the French coast are located in areas where the salinity vary between 30 and 35, these areas are prone to potential highly toxic blooms of *Pseudo-nitzschia* and massive toxin release in the environment with salinity variation due to meteorological events.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-058

Nitrogen uptake of the benthic toxic dinoflagellates *Ostreopsis fattorussoi* of the Eastern Mediterranean Sea

L. Açaf [1 2,*], C. Jauzein [3], M.-P. Gémin [4], T. Blasco [2], S. Marro [2], M. Abboud-Abi Saab [1], Z. Amzil [4], R. Lemée [2]

[1]National Council for Scientific Research, National Center for Marine Sciences, BP 534, Batroun, Lebanon, [2]Sorbonne Université, CNRS, Laboratoire d'Océanographie de Villefranche, 06234 Villefranche-sur-Mer, [3]Ifremer, Centre de Brest, DYNECO PELAGOS, F-29280 Plouzané, France, [4]Ifremer, Centre de Nantes, DYNECO PHYC, Rue de l'île d'Yeu, Nantes 44311, France

Abstract: Nitrogen availability could be a limiting factor of microalgae growth. This study characterized N uptake abilities of two Eastern Mediterranean strains (MMCV57 and MCCV58) of the toxic benthic dinoflagellate *Ostreopsis fattorussoi,* using the ^{15}N labeling technique and EA-IRMS analysis on the particular fraction. Three potential N-sources were investigated: inorganic NH_4^+ and NO_3^- and organic N-urea within the concentration gradient 0 to10 µmol N L^{-1}. Toxin profile analysis was also performed for the two strains using LC-MS/MS analysis. Results revealed mixotrophy in both strains. The strain MCCV58 showed the following order of preference for N-sources: $NH_4^+ > NO_3^- >$ N-urea, whereas MCCV57 had similar uptake abilities for NH_4^+ and NO_3^-. Uptake abilities of the two strains were similar for NO_3^- and N-urea, whereas MCCV58 was almost twice as competitive in NH_4^+ uptake the along the concentration gradient. In addition, toxin profile analysis revealed up to 4.28 pg eqPLTX $cell^{-1}$ for the strain MCCV58, whereas no toxins could be detected for strain MCCV57. A combination of variability in N-uptake abilities between the different strains of *O. fattorussoi* as well as in toxin profile led us to suggest that NH_4^+ availability, particularly in the summer, could selectively favor the development of a toxic *vs.* a non-toxic strains of *O. fattorussoi* in Lebanese coastal waters.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-059

The role of nutrient-related genes in understanding the saxitoxin production of *Alexandrium minutum*

K. S. Hii [1,*], N. F. Kon [2], P. T. Lim [1], C. P. Leaw [1]

[1]Institute of Ocean and Earth sciences, University of Malaya, Bachok, [2]Sarawak Biodiversity Centre, Kuching, Malaysia

Abstract: *Alexandrium minutum* is a saxitoxins-producring marine dinoflagellate that responsible for paralytic shellfish poisoning (PSP). It is widely distributed in the tropical and temperate waters with various hydrographic regimes. The physiological responses of this species to various macronutrients have been studied intensively, but little is known about the regulating mechanism of nutrient uptakes and their roles in saxitoxin production. The effect of varying levels of nitrogen and phosphorous nutrition on the transcriptional responses of a tropical ribotype of A. minutum was investigated in a laboratory setting. The nutrient-related genes, ammonium transporter (*AmAmt1*), nitrate transporter (*AmNrt2*), phosphate transporter (*AmPiPT1*), nitrate reductase (*AmNas*), glutamine synthetase (*AmGSIII*) and carbarmoyl phosphate synthase (*AmCPSIII*) were assembled from Sequence Read Archive (SRA) data set, and later used in the differential gene expression analyzed by qPCR. The results showed that, *AmAmt1* was suppressed in excess ammonium-grown culture; conversely *AmNrt2* and *AmNas* were induced. Gene expression of *AmAmt1*, *AmNrt2*, *AmNas*, *AmGSIII*, *AmCPSII* and *AmPiPT1* was highly induced under P-deficient condition, suggesting that the cells are scavenging to take up nutrients in the P-stress condition. Gene expression of *AmCPSII* was well correlated with the toxin cell quota, suggesting the gene might involve in arginine metabolism and simultaneously increased the toxin production. Nonetheless, *AmGSIII* expression might play a role in the stress adaption towards high toxic levels of ammonium ambient by producing toxins as a counteracting response. The results of this study have provided better insights into the eco-physiology of *A. minutum* in relation to the toxin production and its adaptive strategies in unfavorable environments.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

O-060

A multi-traits approach reveals physiologic impacts of Cu on *Alexandrium minutum* and their exudates

M. Long [1 2,*], A. Holland [3], C. Lambert [2], P. Soudant [2], H. Planquette [2], G. Sarthou [2], H. Hégaret [2], D. F. Jolley [1]

[1]School of Chemistry, University of Wollongong, Wollongong, Australia, [2]Laboratoire des Sciences de l'Environnement Marin (LEMAR), UMR 6539 CNRS UBO IRD IFREMER, Institut Universitaire Européen de la Mer, Plouzané, France, [3]School of Life Science, Department of Ecology, Environment and Evolution, Murray Darling Freshwater Research Centre, La Trobe University, Albury/Wodonga Campus, Australia

Abstract: Harmful algal blooms (HABs) of the dinoflagellate *Alexandrium* occur in nutrient-rich coastal ecosystems that are also subject to other anthropogenic contaminants, such as copper (Cu). However, the effects of contaminants on the physiology and toxicity/allelopathy of *Alexandrium* are poorly described. Here, we investigated the physiological responses of *A. minutum* to a pulse of dissolved Cu (0.18 µM) through a phenomic approach by measuring several physiological parameters. Cu concentrations were followed by SF-ICP-MS measurements during the 16 days of exposure. Flow-cytometry allowed enumeration of bacteria and microalgae as well as measurement of esterase activity, reactive oxygen species, lipid reserves and chlorophyll autofluorescence from microalgae. Effects on photosynthesis (photosystem II and non-photochemical quenching) were assessed by PAM fluorometry and allelopathic exudates were characterized with a bioassay and by fluorescence spectroscopy.

Cu exposure induced a partial encystment of *A. minutum.* In presence of Cu, growth was inhibited by 60% within the first 8 days and cells saved their lipid reserves compared to controls. In the meantime, primary metabolism and intracellular reactive oxygen species were temporarily increased at 8 days in presence of Cu. Photosynthesis was also affected by Cu exposure for the whole duration of the experiment (16 days). After 8 days, the presence of Cu increased the toxicity of the exudates against a diatom by either increasing the release of allelochemicals or inducing a mixture effect. Over 16 days, Cu induced an increase in dissolved organic carbon (DOC) in the exposure solution with significant increases in the proportion of fulvic-like and tryptophan-like components exuded over 16 days.

After 16 days, *A. minutum* was still in exponential growth phase and no longer seemed stressed according to the different physiologic parameters. The multi-traits approach allowed a general overview of main physiological functions affected by Cu and highlighted defense mechanisms against Cu stress. We hypothesized that concomitant increase in cysts (increase of the biological surface area able to bind Cu), changes in exudates and bacterial community may have helped *A. minutum* to cope with Cu stress by decreasing its bioavailability.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

O-061

Accumulation and biotransformation of algal toxins in the marine food web: case studies from the Portuguese coast

P. Reis Costa [1,*]
[1]IPMA – Portuguese Institute of the Sea and Atmosphere, Lisbon, Portugal

Abstract: Dynamics of accumulation, transformation, and elimination of algal toxins in biota and its transference along the food chain, from tiny zooplankton species to top predators, such as marine mammals and seabirds, opens the window to a myriad of toxins derivatives, toxic effects and metabolic pathways. Domoic acid (DA) that is a small water soluble molecule easily released from most shellfish species and only found in planktivorous fish, such as sardines, during *Pseudo-nitzschia* blooms, has been continuously found in great concentrations in the digestive gland and branchial hearts of the common octopus (*Octopus vulgaris*) and cuttlefish (*Sepia officinalis*). Field observations indicate ontogenic differences on the accumulation of DA. Higher levels are found in young immature female O. vulgaris. The recent development of more sensitive analytical detection methods allowed detecting DA in the brain of cephalopods, which are organisms with complex central nervous system where glutamatergic transmission is involved in vertebrate-like long-term potentiation (LTP). The uptake and elimination kinetics of PSP toxins, as well as their biotransformation modulation and genotoxicity, have been studied in mussels, octopus and finfish. Ester metabolites of okadaic acid (OA) and *dinophysistoxins* 1 & 2 (DTX 1, 2) are known to be produced by shellfish exposed to *Dynophysis* or *Prorocentrum* dinoflagellates. These esterified forms vary in abundance between shellfish species, are less toxic (to humans) than parent compounds and are easily eliminated. Therefore shellfish species with high ability for OA and DTX1&2 esterification, such as cockles, are generally not intensively closed to harvesting as is mussels that typically contain high proportions of free DSP toxins. A revision of the ecosystem-wide implications of algal toxins kinetics, the species-specific metabolism, and trophic transfer is presented.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

O-062

Dynamic variation of paralytic shellfish toxins and metabolites during the exposure of scallops and mussels to *Alexandrium pacificum*

A. Li [1 2,*], J. Qiu [1], L. Ding [1], P. McCarron [3], D. Beach [3]
[1]College of Environmental Science and Engineering, Ocean University of China, [2]Key Laboratory of Marine Environment and Ecology, Ministry of Education, Qingdao, China, [3]Measurement Science and Standards, National Research Council Canada, Halifax, Canada

Abstract: New *C*-11 hydroxyl metabolites of paralytic shellfish toxins (PSTs) have been reported in diverse species of shellfish. To explore further information on these new metabolites, as well as the potential formation of phase Ⅱ metabolites and acyl esters of PSTs, shellfish were fed with the PST-producing dinoflagellate *Alexandrium pacificum* (formerly *A. tamarense*) ATHK in laboratory. Scallops (*Chlamys farreri*) were fed for 9 days and mussels (*Mytilus galloprovincialis*) for 5 days plus an additional 5 days of depuration, with representative samples taken throughout the experimental period. The strain of *A. pacificum* ATHK mainly produced C1-4, GTX1-6. Additionally, trace amounts of metabolites (M1, M7 and M9) were also detected in the extract of ATHK. During the accumulation and elimination periods, several common PSTs (C1-4, GTX1-6 and NEO) and metabolites including M1, M3, M5, M7, M9, M2 and M8 were detected in the hepatopancreas of scallops and mussels. The mole percentage of M-toxins was significantly increased in shellfish after exposure, which demonstrates that these new derivatives can still be considered shellfish metabolites of PSTs. The relative molar ratio of metabolites to precursor molecules was used as a parameter to roughly reflect the metabolic conversion rate. Conversion rates of C1/2 and GTX2/3 were higher than that of C3/4 and GTX1/4, in both experimental shellfishes. The first metabolic products M1/3 were observed in bivalves here, which are bio-transformed from C1/2. However, the conversion of GTX2/3 to M2 was more quickly than the other metabolic pathways in mussels and scallops, respectively. In general, the metabolic rates of PSTs in mussels were faster than that in scallops, which is consistent with the species-specific for the conversion between some common components of PSTs. No acyl esters or conjugation products of PSTs with glucuronic acid, glutathione, cysteine and taurine were discovered using the liquid chromatography with high resolution tandem mass spectrometry. Additionally, only GTX1/4 and GTX2/3 were detected in the kidney of scallops, which hinted that PSTs are mainly metabolized in bivalves through the hepatic metabolism pathway. This work improves the understanding of PST metabolism during toxin accumulation and depuration processes in the commercially harvested shellfishes.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

O-063

Saxitoxin Uptake, Assimilation, Depuration, and Analogue Changes of the Southeast Asian Green-Lipped Mussel (*Perna viridis*) toxin

J. K. Q. Andres [1,*], A. Yniguez [1], J. M. Maister [1], A. Turner [2], J. Mendoza [1], R. Azanza [1]

[1]College of Science, University of the Philippines Marine Science Institute, Quezon City, Philippines, [2]Food Safety Group, Center for Environment, Fisheries and Aquaculture Science, Weymouth, United Kingdom

Abstract: This study focused on the uptake, depuration, assimilation, and analogue changes of toxins from the Southeast Asian green-lipped mussel *Perna viridis* fed with the toxic dinoflagellate *Alexandrium minutum*. Mussels were acclimatised in seawater aquarium with aerators at 27 °C and 32 ppt temperature and salinity levels, respectively. Mussels in acclimation tanks were held for 4 days whilst fed with cultures of non-toxin producing *Isochrysis galbana* twice a day. After this period, they were starved for 24 hours prior to the grazing experiment. The experiment consisted of the treatment of shellfish fed with *A. minutum* (Treatment), and controls with *A. minutum* (Control 1), and one with shellfish (Control 2). Sampling was performed every six hours for 24 hours and daily thereafter up to six days. Five individual mussels were taken from Treatment 1 and Control 2 during each sampling period. Mussels were divided into three parts for toxin analysis: 1) Gills + Mantle + Gonads; 2) Foot + Adductor Muscle; and, 3) Digestive gland. The analogues used for this study were Saxitoxin (STX), Neo-saxitoxin (NSTX), Gonyautoxin 1,4 (GTX1,4), and Gonyautoxin 2,3 (GTX2,3). Uptake and depuration cycles appeared to occur several times, with the first uptake during the start up to 18 hours followed by depuration on the 24th hour. Uptake was observed again during the 48-hour period with depuration on the 72-hour period, and the last observed uptake was from the 96-hour to 120-hour with depuration at 144-hour. This cycling may be due to the release of toxins to the surrounding water from the dead shellfish in the tanks. The dominant toxin signature for the water of *A. minutum* in Control 1 is GTX1,4. Interestingly, the dominant toxin signature for the water in the Treatment is GTX2,3, suggesting possible analogue changes with the toxin profile of *A. minutum*. Uptake of toxin was observed up to 18 hours followed by depuration on the 24-hour sampling. In terms of variation of overall toxicity between parts, the mantle had the highest microgram STX equivalent over 100g of shellfish meat during the 0-24 hrs. Toxin burden began to shift towards the digestive gland area after the 48 hour period and became the part with the highest toxin burden up to the end of the study period. As for the muscle organ group, toxin distribution was higher after the 48-hour period but contributed less compared to the mantle and digestive gland. In terms of the toxin signature for each part, GTX2,3 is the dominant toxin type for both digestive gland and muscles. As for the mantle, GTX1,4 was observed to be dominant from the start up to the 72-hour period shifting to GTX2,3 later.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

O-064

Multiple climate drivers affect physiological health and toxin load of the tropical filter-feeding clam *Meretrix meretrix*

L. Turner [1], J. Havenhand [2], C. A. Alsterberg [2], A. Turner [3], S. Girisha [4], A. Rai [4], M. Venugopal [4], I. Karunasagar [5], A. Godhe [2,*]

[1]Plymouth University, Plymouth, United Kingdom, [2]University of Gothenburg, Goteborg, Sweden, [3]Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Weymouth, United Kingdom, [4]Karnataka Veterinary Animal and Fisheries Sciences University, [5]Nitte University, Mangalore, India

Abstract: Research on the effects of climate change on the marine environment continues to accelerate, yet we still know little about the effects of multiple climate drivers in more complex, ecologically relevant settings – especially in sub-tropical and tropical systems. In tropical marine ecosystems, climate change has been reported to increase the prevalence of toxic microalgal species, leading to bioaccumulation of toxins by filter feeders, with resultant negative impacts on metabolic and immunological performance. Here we manipulated multiple climate drivers (warming, freshening, and acidification), and the availability of toxic microalgae, to determine their impact on the physiological health and toxin load of the tropical filter-feeding clam, *Meretrix meretrix*. We found that physiological responses of the clam to projected marine climates were modified by indirect effects mediated through the food chain. Specifically, we found that: i) warming and/or freshening increased the toxin load of tropical clams; ii) this effect was amplified when bivalves were fed with a toxin-producing dinoflagellate; and iii) exposure to acidification in combination with warming and freshening removed this toxin load. Our results show the importance of indirect effects of climate drivers on a key tropical food species, and have important implications for shellfish production and food safety in tropical regions.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

O-065

Drivers of Ciguatoxin Prevalence in Herbivorous Reef Fish from the Caribbean

J. Gwinn [1 2,*], W. Patterson [3], T. Smith [4], A. Robertson [1 2]

[1]Dept. Marine Sciences, University of South Alabama, Mobile, AL, [2]Dauphin Island Sea Lab, Dauphin Island, AL, [3]Dept. Fisheries and Aquaculture, University of Florida, Gainesville, FL, [4]Center for Marine and Environmental Sciences, University of the Virgin Islands, St. Thomas, VI, USA

Abstract: The prevalence of *Gambierdiscus* and incidence of ciguatera fish poisoning (CFP) are increasing in geographical extent and frequency in the Greater Caribbean Region as a result of several factors, including warming sea surface temperatures. It is important to identify the pathways of toxicity in these ecosystems to improve prediction and mitigation of human exposures to ciguatoxins (CTX). The transfer of toxic algal metabolites to herbivorous fish represents one of the primary entry points into marine food webs and is a critical link in the fate of CTX. To understand the factors relevant to these processes, we examined herbivore toxicity and life history (i.e., size, age, diet) in a CFP hyper-endemic region. A total of 363 individuals representing three reef associated herbivores from St. Thomas, US Virgin Islands were studied including: 168 ocean surgeonfish (*Acanthurus bahianus*), 143 redband parrotfish (*Sparisoma aurofrenatum*), and 52 stoplight parrotfish (*Sparisoma viride*). Fish were collected quarterly each year from 2009-2016 from four long-term monitoring sites (both nearshore and offshore). Approximately 50% of each species showed CTX activity using the ouabain-veratridine dependent mouse neuroblastoma assay. Quantified extracts had toxicities ranging from 0.004-0.018 ppb C-CTX-1 equivalents, which are below the current guidance levels for human consumption (0.1 ppb C-CTX-1 equivalents), but ecologically relevant at low trophic levels in marine food webs. Though not correlated with fish size, herbivore toxicity did show seasonal and spatial trends which concurred with field algal toxicity reported in our previous work. This illustrated the potential value of surveying herbivores as sentinels to detect toxin pulses in these ecosystems. Stable isotope analyses (SIA; C, N, and S) of representative primary producers and 121 randomly selected fish were conducted to identify dietary differences and life history traits (size and age) that may relate to toxicity. An overlap in trophic position was observed between *A. bahianus* and *S. aurofrenatum* (82% relative to *S. aurofrenatum*), and a clear separation of *S. viride*, which had less than 10% similarity to the other species tested. Stable isotope data also revealed an ontogenetic diet shift in *S. aurofrenatum* which may be an important factor in CTX movement through these food-webs. Herbivore diet composition can be challenging to evaluate with traditional methodologies, so these studies highlight the value of SIA to identify dietary differences in fish and relevant substrate primary producers that may harbor *Gambierdiscus spp*. This work demonstrates that trophic position alone may not provide effective predictive capacity for risk in these fish but emphasizes the need to consider factors like substrate selectivity, grazing rates, and seasonal availability of resources for effective prediction and management of CFP.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-066

Revealing the Hybrid Zone and Genetic Polymorphisms within *Pseudo-nitzschia pungens* the Cosmopolitan Diatom Species

J. H. Kim [1 2,*], B. S. Park [2], J.-H. Kim [2], P. Ajani [3], S. Murray [3], H. C. Lim [4], S. T. Teng [5], P. T. Lim [6], M.-S. Han [2]

[1]Korea Institute of Ocean Science and Technology, Geoje, [2]Life Science, Hanyang University, Seoul, Republic of Korea, [3]University of Technology Sydney, Sydney, Australia, [4]Tunku Abdul Rahman University College, Johor, [5]Universiti Malaysia Sarawak, Kota Samarahan, [6]Bachok Marine Research Station, Kelantan, Malaysia

Abstract: Genetic sub-populations (clades) of cosmopolitan marine diatom *Pseudo-nitzschia pungens* might have distinct habitats, and their hybrid zone is suspected in the West Pacific area, however, it is still unrevealed because of technical difficulties and lack of evidences in nature. We employed the 137 number of genetic information of *P. pungens* and its sampling data originated from the West Pacific area, and used field application of qPCR assay for intra-specific level of *P. pungens*. Only two genotypes, clade I and III, were identified in the West Pacific area. Clade I was distributed from 39 to 32.3 °N, and clade III were from 1.4 to 34.4 °N. The estimated habitat temperature for the clade I and clade III ranges were 8.1 - 26.9 °C and 24.2 - 31.2 °C, respectively. The latitudal distributions and habitat temperature ranges of each clade were significantly different, however, the results of qPCR assay revealed their hybrid has being created in the southern Korean coasts. In laboratory condition, the sexual-reproduction was successfully occurred, and offspring cells were produced between parental clade I and III strains. The offspring strains showed recombinant sequences types, and the high ratios (38.6 and 65.1%) of single nucleotide polymorphism in its ITS2 region. The hybrid zone and genetic polymorphism phenomenon should contribute to maintain the genetic diversity and to produce the new genotypes within this species. Furthermore, the diverse hybrid types might provide adaptive strategies to various natural environments and may even be the source of new recombinant species in the future.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-067

Evaluation of DNA extraction kits for assessing harmful algal community from fish farm sediments

C. C. Gaonkar[1,*], T. Nishimura[1], H. Funaki[1], K. Hagino[1], T. Yamatogi[2], S. Okunishi[3], H. Maeda[3], K. Nagasaki[4], M. Adachi
[1]Laboratory of Aquatic Environmental Sciences (LAQUES), Kochi University, Nankoku, [2]Nagasaki Prefectural Institute of Fisheries, Nagasaki, [3]Faculty of Fisheries, Kagoshima University, Kagoshima, [4]TEAM AQUA-VIRUS, Nagasaki Laboratory, Kochi University, Nankoku, Japan

Abstract: Biodiversity characterization has been extensively used for monitoring and assessing environmental health, especially in terms of red tide causing species. Benthic communities have been assessed via the traditional approaches, using visible morphological traits, which are tedious and entails limitations. Environmental DNA (*e*DNA) metabarcoding coupled with high-throughput sequencing (HTS) offers an alternative to rapid biodiversity survey on a broader scale and cost-effective manner. However, DNA must be efficiently extracted from the sediment samples to achieve accurate and reproducible DNA sequencing results. DNA extraction from the sediment samples is challenging, because of the complexity of the sediment type; low biomasses; composition of resting spores and cysts; DNA extraction methods. The present study aims to compare the DNA extraction efficiency using the commercially available DNA isolation kits, in order to recover reliable qualitative and quantitative DNA that can be used for metabarcoding analysis. The best suitable protocol was determined on the basis of cell lysis efficiency, DNA yield, and qPCR effectiveness. We evaluated a few of the commonly used convenient DNA extraction kits i.e., DNeasy PowerSoil Kit (Qiagen), FastDNA SPIN Kit for Soil (MP Biomedicals) and ISOIL (Nippon Gene Co., Ltd). DNA was extracted employing several sediment samples spiked with known concentrations of red-tide causing *Alexandium catenella* cysts. DNA yield was estimated using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). DNA recovery efficiency was calculated by adding exogenous plasmid DNA to both samples of spiked and un-spiked sediments followed by qPCR. Obtained results indicated that FastDNA Kit had higher DNA recovery, but PowerSoil Kit exhibited advantage on the DNA extraction efficiency from the spiked *A. catanella* cysts sediments. In general, DNeasy PowerSoil kit could provide high-quality *e*DNA, especially from algal cysts for direct downstream analysis, as in qPCR and microbial community profiling. Furthermore, this subsurface sediment DNA was used for metabarcoding of microbial community profiling, with special emphasis on the red tide forming species from the fish farm sediments. HTS amplicons for metabarcoding were generated using universal primers for the V8-V9 regions of the 18S rDNA. Molecular analysis provided information on the biodiversity, applicable to most taxa and ecosystems, expanding the opportunity to use a single extraction procedure for HTS analysis, and offers a possibility to implement this technique to standardize monitoring surveys.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-068

The role of lateral gene transfer for bioactive tetrapeptides (aeruginosins) among the bloom-forming cyanobacteria *Planktothrix*

E. Entfellner [1,*], K. Baumann [1], K. Moosbrugger [1], C. Edwards [2], R. Kurmayer [1]

[1]Research Department for Limnology, University of Innsbruck, Mondsee, Austria, [2]Robert Gordon University, Aberdeen, United Kingdom

Abstract: Aside from toxins, a number of bioactive peptide families such as the aeruginosins are produced by the genera *Planktothrix* and *Microcystis*. The diversity of the aeruginosin (*aer*) biosynthesis genes and the structural variation in aeruginosin molecules is high. Based on a larger number of isolated *Planktothrix* strains from different regions and climatic zones, the genetic variability in the non-ribosomal biosynthesis pathway (NRPS) was assessed and functional consequences for aeruginosin synthesis were analyzed. Molecules of this peptide family are synthesized by NRPS in combination with polyketide synthase (PKS), using amino acids or acetyl-coenzyme A as substrates. Substantial modifications occur through chlorination, sulfation and glycosylation.

Out of 124 *Planktothrix* strains, 25 widely distributed strains (occurring in two different lineages) carried *aer* genes, which were possibly derived from *Microcystis* by lateral gene transfer (LGT), and inserted in frame. The resulting chlorinated aeruginosins, i.e. $[M+H]^+$ 717 and $[M+H]^+$ 689, showed high structural similarity to the aeruginosins 89A/B and aeruginosin 98A elucidated from *Microcystis*. The occurrence of the *aerJ* gene, encoding a halogenase, and the occurrence of chlorinated aeruginosins showed a positive correlation, but chlorinated aeruginosins were also found in *aerJ*-deficient strains and vice versa.

The bioactivity of three purified chlorinated aeruginosins was tested in a standard toxicity assay (*Thamnocephalus platyurus*) and compared with that of microcystins and other bioactive peptides. Even though the observed toxicity was lower than that of [Dhb, Asp]-MC-RR, it was in the lower micromolar range and comparable to other frequently occurring cyclic peptides such as anabaenopeptin B and F. In summary, the evolution of the aeruginosin pathway is shaped by LGT events in a continuous manner and one of the most variable synthesis pathways within this genus.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-069

Pseudo-nitzschia biogeography combining qPCR analysis and oceanographic models to investigate long term trends of ASP toxicity.

R. Salas [1,*], A. Lefran [2], B. Siemering [3], C. Cusack [3], J. Silke [1]

[1]Marine Environment and Food Safety, Marine Institute, Oranmore, Ireland, [2]Biology, Institut Universitaire Européen de la Mer, Brest, France, [3]Oceanography, Marine Institute, Oranmore, Ireland

Abstract: *In situ* HAB data revealed a strong variability in the intensity and toxicity of *Pseudo-nitzschia* blooms between 2005-17 in Killary Harbour and Bantry Bay, the most important production areas for rope grown mussels in Ireland. A multidisciplinary approach was used to determine possible relationships between ASP toxicity, *Pseudo-nitzschia* biodiversity and variations in environmental conditions. To study these complex interactions a combination of different methods is used, including qPCR analysis, statistical analysis and a Lagrangian particle model.

The biotoxin and phytoplankton results over a 12 year period suggest a very strong relationship of ASP toxic episodes in Bantry Bay in spring (March-April) compared to Killary Harbour, where toxic events occur in summer (June-July). In both cases, Real-Time PCR analysis shows that *Pseudo-nitzschia australis* is the species involved in these toxic events. qPCR data shows that *P. australis* dominates the water column in spring and is succeeded by *P. pungens* in Bantry Bay. In Killary Harbour, blooms of *Pseudo-nitzschia* spp. are recorded later in the year (May-June) Although the causative organism is *P. australis,* ASP events in Killary Harbour never quite match the higher toxin concentrations found in Bantry Bay's shellfish above the EC regulatory closure limit (20 µg/g).

The particle tracking model shows a high level of variability in advective transport of cells within bays and along the coastline, suggesting that even small environmental changes in spring could have dramatic effects on bloom transport and development.

A second *Pseudo-nitzschia* spp. bloom occurs in late summer-autumn in both Killary Harbour and Bantry Bay. These blooms do not result in shellfish toxicity over the period studied, indicating that another non-toxin producing species predominates the *Pseudo-nitzschia* spp. assemblage at this time.

Comparison of the relative abundance of *Pseudo-nitzschia spp.* in water samples against other diatoms and dinoflagellates suggest that ASP toxic episodes are not explained by large monospecific blooms of these toxic species alone. In Bantry Bay, ASP events can occur when *Pseudo-nitzschia* spp. are present at relatively low cell abundances and represent only 20-25% of the total microplankton community during the spring bloom.

However, statistical models, such as Generalised Linear Models and Principal Component Analysis suggest a relationship between water temperature and currents as the more relevant environmental drivers of ASP toxicity in farmed blue mussels in Bantry Bay..

In conclusion, species biodiversity and environmental drivers can help to explain observed variability in past ASP toxic events. Studies like this help to understand the causes of toxic phytoplankton events and are invaluable to the improvement of short term prediction and HAB risk response management.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-070

Environmental entrainment of the endogenous circannual clock of *Alexandrium catenella* cysts from the Gulf of Maine

A. D. Fischer [1,*], J. Lewis [2], M. Brosnahan [3], D. Kulis [3], D. Anderson [3]

[1]Ocean Sciences, University of California, Santa Cruz, Santa Cruz, USA, [2]Department of Life Sciences, University of Westminster, London, United Kingdom, [3]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, USA

Abstract: Many dinoflagellate cysts experience dormancy, a reversible state that prevents germination during unfavorable periods. Mature *Alexandrium catenella* cysts from deep "seedbed" areas of the Gulf of Maine (GOM) cycle between dormancy and quiescence under control of an endogenous circannual clock. This rhythmicity ensures that dormancy is broken prior to the onset of favorable bloom conditions within the euphotic zone, even with dampened seasonal signals at depth. A curious aspect of the endogenous circannual rhythm documented in GOM cysts is that the period is less than a full year, only 11 months. In situ, such a short cycling period would pose a substantial ecological disadvantage to older year classes. An explanation is offered by the recent discovery for *A. catenella* cysts from Nauset Marsh System (Massachusetts, USA), that cold exposure shortens the duration of dormancy and that cysts must surpass a quantifiable chilling requirement to break dormancy. This poses the question: is the endogenous circannual clock in GOM cysts modulated by environmental temperature? Mature, dormant cysts from the GOM were stored at the temperature of the rhythmicity experiments (2ºC), the average temperature of GOM bottom waters (6ºC), and the upper limit of GOM bottom waters (11ºC) for almost two years. Dormancy status was determined at regular intervals using a germination assay. Like the Nauset cysts, dormancy was shorter in colder treatments, and interestingly had similar physiological parameters for quantifying chilling. Extraordinarily, the period of the dormancy cycle for the cysts stored at 6ºC, the average bottom water temperature of the GOM, was a full year. These results demonstrate that the endogenous clock is not as rigid as originally thought, and instead is entrained to the bottom water temperatures of the GOM. The presence of this chilling response in two *A. catenella* populations from distinct habitats, a shallow estuary and deep coastal ocean, demonstrates that this physiology may be common to other dinoflagellates.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-071

The anchoring effect– long-term dormancy and genetic population structure

J. Sefbom [1,*], L. Sundqvist [1], A. Godhe [1], P. R. Jonsson [1]
[1]Department of Marine Sciences, University of Gothenburg, Gothenburg, Sweden

Abstract: Understanding the genetic structure of populations is key to revealing past and present demographic and evolutionary processes in a species. In the past decade contradicting patterns of gene flow have been reported for a number of microalgal species. Where high genetic differentiation has been observed, this challenges the previous view of cosmopolitan distribution of such species. In some cases populations have displayed high genetic differentiation, even at small spatial scales, despite apparent high dispersal. Here we provide a conceptual framework using a simple model to illustrate that long-term resting stages may play an important role in facilitating diversity and population genetic differentiation in the presence of gene flow. Numerous species of microalgae have a life-history strategy that includes a long-term resting stage, which can accumulate in the sediments and serve as a refuge during adverse conditions. With a simple model we show that resting stages have an anchoring effect on populations that consequently influences microevolutionary processes, e.g. patterns of gene flow and potential for adaptation. Furthermore, we collected empirical data obtained from a systematic literature review which supports our model. When comparing genetic diversity (number of alleles) and genetic differentiation (Fst) between microalgae species with- and without resting stages we could indeed observe a significant difference in population genetic patterns. With this work we propose that seed banks in aquatic microalgae play an important role in the adaptive potential and population dynamics in species with long-term resting stages.

Disclosure of Interest: None Declared

Mitigation of HABs

O-072

Prevention, control and mitigation of cyanobacterial blooms in lakes and reservoirs

P. M. Visser[1,*], J. Huisman[1], J. Fastner[2], M. Bormans[3], B. Ibelings[4]

[1]Freshwater and Marine Ecology, University of Amsterdam, Amsterdam, Netherlands, [2]Federal Environment Agency, Berlin, Germany, [3]CNRS/Universite´ de Rennes, Rennes, France, [4]University of Geneva, Geneva, Switzerland

Abstract: In this overview presentation, successful techniques for prevention, control and mitigation of harmful cyanobacterial blooms will be discussed. Prevention of blooms requires that external and internal sources of nutrients are managed to levels where development of cyanobacterial blooms is restricted. A system analysis of the lake, in particular its morphometry, water and nutrient balance is needed. If internal loading is substantial, hypolimnetic aeration, dredging or sediment capping can reduce the release of phosphorus from the sediment. Nevertheless, changes in chlorophyll and abundance of cyanobacteria in lakes where phosphorus in the inflow had been reduced show that it may take years before the phytoplankton responds, depending largely on the extent of the decrease in the nutrient load that was achieved. Control of blooms, in which their presence is reduced to a level where they no longer pose a risk through additional measures such as biomanipulation or artificial mixing, demands understanding of the key ecological traits of the dominant cyanobacteria taxa, and adequate design and execution of the management methods of choice. These elements are important for choosing effective management interventions and predicting their outcome. Mitigation of blooms reduces the risks and harmful effects of blooms if they cannot be prevented or sufficiently controlled, methods such as harvesting of surface scums or application of cyanocides may be used in those cases where water quality improvement is urgent. Cyanocides like hydrogen peroxide can be used to reduce the cyanobacterial biomass since cyanobacteria typically show a higher sensitivity to hydrogen peroxide than other groups of aquatic biota and this control agent does not leave any harmful residuals due to its breakdown into oxygen and water. The choice of the method for a specific lake is dependent on its lake morphology, water and nutrient balance, dominant cyanobacterial species, and economical aspects.

Disclosure of Interest: None Declared

Mitigation of HABs

O-073

Toxicity of the cyanocide hydrogen peroxide to zooplankton and macrofauna

E. Weenink [1,*], M. van Herk [1], M. Leon-Grooters [1], S. Kuijt [1], C. van Teulingen [1], M. Kraak [1], J. Huisman [1], P. Visser [1]
[1]Freshwater and Marine Ecology, University of Amsterdam, Amsterdam, Netherlands

Abstract: A new method to quickly and effectively suppress harmful cyanobacterial blooms in freshwater systems is the application of the cyanocide hydrogen peroxide (H_2O_2) at low concentrations ranging between 2 and 5 mg/l. Since 2009, H_2O_2 has been applied in ca. 20 lakes in The Netherlands. In general, H_2O_2 breaks down into water and oxygen in one or two days after application.

To minimize the impact on ecosystem structure and functioning, adverse effects of H_2O_2 to non-target species such as zooplankton and macrofauna should be avoided. Some species show a high sensitivity to H_2O_2, while others are less sensitive and are not affected at the applied H_2O_2 concentrations. The aim of the project was to assess H_2O_2 toxicity to non-target species in controlled laboratory experiments and during actual lake treatments.

Most traditional toxicity tests expose the animals for 24 or 48 hours to a toxicant, while this does not represent the conditions in a lake during a H_2O_2 treatment, where the H_2O_2 concentrations are often decreasing to zero within 24 hours. Therefore, we also tested the H_2O_2 toxicity after only a few hours of exposure. This greatly decreased the sensitivity of species. We tested the survival of different zooplankton and macrofauna species during lake treatments with H_2O_2 using an enclosure in the lake as a control.

Our research showed that the effect-concentrations for some target and non-target species overlap. As a consequence, only a narrow operational concentration range is available for an effective treatment of cyanobacteria, while safeguarding non-target species. However, other non-target species are less sensitive than target species, providing a larger available concentration range.

Disclosure of Interest: None Declared

Mitigation of HABs

O-074

Harmful algal blooms suppression by a conventional water circulation system

B. Lee [1 2,*], H.-C. Oh [1], J.-H. Ahn [1], Y. Kim [1], H. Kang [1 2], S.-K. Kim [1 2]

[1]Korea Institute of Civil Engineering and Building Technology, [2]University of Science and Technology, Goyang, Republic of Korea

Abstract: Harmful algal blooms (HABs) occur when light, warm water temperature, nutrients, and stagnant water are present. An artificial water circulation technology can be applied even to nutrient-rich water to prevent HABs. A new conventional water circulation technology was developed to circulate stagnant water and supply oxygen to hypolimnion for algal blooms suppression. This study presents the results from a computational fluid dynamic (CFD) analysis of the conventional water circulation system and field research data.

The key features of the system is an impeller generates currents and a flat round booster (attached to the end of the impeller) that shifts the vertical flow to horizontal. As a CFD analysis model, ANSYS CFX was used to understand the operational characteristics of the system and to predict the effect radius of the impeller with a diameter of 500 mm which applied in the field study. Shingal reservoir in Yongin city in South Korea was selected as a study area. The reservoir experiences HABs every year due to the eutrophication. To evaluate the performance of the system, it was deployed in the reservoir and water temperature and dissolved oxygen (DO) concentration of the surface and hypolimnion were measured. The same parameters were measured at a control point (B) which is 200 m away from the deployment point (A). The field study was conducted 7 times from September to October in 2016.

From the CFD analysis results, the water discharge from the top and side of the impeller was observed. The velocity of the flow was changing until it reaches 20 m of the effect radius, but a uniform velocity is achieved after 20 m. Results from analyzing the effective radius of the free surface as well as beneath the free surface, water layer, after 16 minutes of the operation are 85 m and 26 m, respectively. The surface temperature in Shingal reservoir was up to 30 ℃ due to the hot weather. The average and maximum temperature differences between the surface and hypolimnion were 1.6℃ and 3.6℃ in A and 2.6℃ 4.7℃ in B, respectively. This indicates the operation of the system promotes water circulation. The values of DO concentrations showed the similar patterns. The average surface DO concentrations were 9.8 mg/L in both points, higher than the values of hypolimnion which were 8.2 mg/L in A and 7.5 mg/L in B. This difference demonstrates the influence of the system.

The influence of the system was demonstrated in both CFD analysis and the field study and it is believed that the system can be applied to suppress HABs. However, to collect more convincing and detailed data, the water temperature and chlorophyll-a values are being monitored in real time in Shingal reservoir as the system operates. It is expected that meaningful outcome will be achieved, regarding an effective suppression of HABs and an improvement of water quality of the reservoir.

Disclosure of Interest: None Declared

Mitigation of HABs

O-075

Biocontrol essays of Microcystis sp dominated in harmful algal blooms by medicinal plant extracts

L. Tebaa [1,*], M. Douma [1], Z. Tazart [1], K. Mouhri [1], M. Loudiki [1]

[1]Laboratory of Biology and Biotechnology of Microorganisms,, Laboratory of Biology and Biotechnology of Microorganisms, Faculty of Sciences Semlalia, University Cadi Ayyad, Marrakech, Morocco, Marrakech, Morocco

Abstract: Water pollution caused by harmful cyanobacteria blooms (cyanoHABs) is a problem worldwide, increasing with eutrophication and climate changes. *Microcystis aeruginosa* is one of the representative toxic species of bloom-forming cyanobacterium. The use of medicinal plants to control nuisance algae by allelopathic inhibition will be a promising and ecofriendly approach.

In this study, two Moroccan medicinal plants: *Thymus marocanus; Salvia officinalis* were selected for their potential inhibitory effect. The antialgal activity of leaf aqueous extracts of these plants on toxic algae *Microcystis aeruginosa* was assessed. An experiment was designed using aqueous treatments (1%, 0.75%, 0.5%, 0.25% and 0. 1%). The *Microcystis* growth, cell and colonial morphology changes and photosynthetic pigments (chlorophyll a and carotenoids) on exposure to the aqueous extracts were explored. Also, some phytochemical parameters in the extracts were analyzed to reveal the potential allelochemical compounds.

The results showed that *Thymus marocanus and Salvia officinalis* aqueous extracts inhibited the *Microcystis* growth in a concentration-dependent way. After 8 days of treatment, the highest inhibition rates reached were 94,05% and 81,32% for *Thymus marocanus* and *Salvia officinalis* extracts respectively. For the two plants chlorophyll a and carotenoid concentrations in cultures decreased especially in the 1% treatment group.

Our results illustrated that *Thymus marocanus and Salvia officinalis* aqueous extracts can be able to control *Microcystis* blooms and these may be recommended as a remedy for water bodies contaminated by cyanobacterial harmful blooms.

Disclosure of Interest: None Declared

Mitigation of HABs

O-076

Algal Taste & Odor compounds: Advanced methods for detection and control

T. Kaloudis [1,*], C. Avagianos [1], T. T. Triantis [2], M. Antonopoulou [2], C. Christophoridis [2], A. Hiskia [2]

[1]Water Quality Control, EYDAP SA, [2]Institute of Nanoscience & Nanotechnology, NCSR Demokritos, Athens, Greece

Abstract: Toxins are not the only hazards associated with HABs. A plethora of (semi)-volatile algal metabolites and degradation products including terpenoids, aldehydes, ketones, sulfurous compounds and amines are common sources tastes & odors (T&O) such as “earthy”, “musty”, “fishy” and “swampy”. Algal T&O have serious negative impacts to drinking water supplies (acceptability of water by consumers), aquacultures (losses of production) and tourism near water bodies where HABs occur. More than 200 algal T&O compounds have been identified so far, the most frequently reported and studied being geosmin and 2-methylisoborneol.

Combined sensory panel techniques and advanced analytical methods can be used to detect and identify algal T&O. Flavour Profile Analysis (FPA) is a powerful sensory method applied by trained panels that describes sensory attributes;it can be combined with the water Taste and Odor Wheel (TOW) that organizes the relationships between specific tastes and odours. Detection, identification and quantitation of water T&O is an analytical challenge, since very low detection limits have to be achieved (e.g. low or sub ng/L levels). Advances in methods for determination of wide range of algal T&O are presented, such as Purge and Trap (P&T), Solid Phase Extraction (SPE), Head-Space Solid Phase Microextraction (HS-SPME), Stir-Bar Sorptive Extraction (SBSE) and Closed-Loop Stripping Analysis (CLSA), coupled to GC-MS techniques, including GC-Olfactometry and GC-HRMS. Development and optimization of a fast and sensitive automated HS-SPME - GC-MS/(MS) method for multi-class algal T&O screening of freshwater cyanobacteria strains is presented as a case study.

Treatment of water for removal of algal T&O ischallenging, because of the need to achieve very low residual concentrations. Emerging Advanced Oxidation Processes (AOPs) such as UV/H_2O_2, UV/O_3/H_2O_2, UV/Cl_2 and heterogeneous TiO_2-based photocatalysis, which are commonly based on the effects of reactive oxygen species (ROS) including the potent hydroxyl radical (OH^*) are currently studied with the aim to improve efficiency and effectiveness of algal T&O removal from water. An overview of AOPs for degradation of algal T&O in water is presented, as well as work done by our group to assess the basic mechanisms and performance of AOPs in water treatment for algal T&O.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

O-077

Persistent Domoic Acid in the California Current Food Web: An Emerging Threat?

R. Kudela [1,*], K. Hayashi [1], A. Boyer [1]

[1]Ocean Sciences, University of California, Santa Cruz, Santa Cruz, USA

Abstract: *Pseudo-nitzschia* blooms along the U.S. West Coast have been documented since 1991, with good evidence for trophic transfer of domoic acid to birds at least since the 1950s. Until recently the management response for both humans and wildlife has focused on acute toxicity associated with episodic bloom events, with short-term closures of fish and shellfish, under the assumption that domoic acid, a water-soluble toxin, will depurate rapidly from contaminated organisms. However, the 2015 “mega-bloom” that extended from Southern California to coastal Alaska demonstrated the potential for widespread accumulation of domoic acid in multiple trophic levels. Re-examination of data from before, during, and after that event suggests that domoic acid is ubiquitous in the food web both during these massive bloom events and during more “normal” periods. Here we document the presence and persistence of domoic acid in multiple organisms including shellfish, fish, squid, and marine mammals, as well as particulate and dissolved domoic acid in estuarine water, seawater, desalinated water, the benthos, and shallow benthic systems. With increasing evidence for chronic exposure leading to impairment in both humans and marine animal populations, we suggest that the existing paradigms need to be reexamined, and that chronic exposure to domoic acid is a common feature of estuarine and marine foodwebs. In particular, Market Squid (Loligo opalescens) appears to be a key source of chronic toxin exposure for multiple higher trophic levels, including humans, while dissolved levels of toxin may pose a serious threat for aquaria and human consumption via desalinated water. Data showing both chronic and accute exposure to domoic acid will be presented in the context of increasing risk related to long-term trends associated with basin-scale oscillations and climate change.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

O-078

Universal detection of the neurotoxin β-N-methylamino-L-alanine in marine mollusks in China

A. Li[1 2], Y. Hu[1,*], J. Song[1], S. Wang[1]

[1]College of Environmental Science and Engineering, Ocean University of China, [2]Key Laboratory of Marine Environment and Ecology, Ministry of Education, Qingdao, China

Abstract: The neurotoxin *β*-*N*-methylamino-*L*-alanine (BMAA) has been supposed as an environmental factor triggering neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS) and Alzheimer's disease (AD). In this study, diverse species of marine mollusks were periodically and continuously collected from the coasts of Dalian, Rongcheng, and Zhoushan cities in China, in April, August, and November 2017. All samples were analyzed for BMAA and its isomers 2,4-diaminobutyric acid (DAB), *β*-amino-*N*-methylalanine (BAMA), and N-2(aminoethyl)glycine (AEG) using the hydrophilic interaction liquid chromatography with tandem quadrupole mass spectrometer (HILIC-MS/MS) method. Some samples were checked again using the liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) analysis of pre-column AQC-derivatization. Results showed that diverse species of mollusks contained BMAA, and BMAA with high contents was especially detected in gastropods such as *Neverita didyma*, *Neptunea arthritica cumingii*, *Volutharpa ampullacea perryi*, and *Laguncula pulchella*. In particular, the highest content of BMAA was present in the gastropod *Neverita didyma* in different seasons. BMAA was also detected in most of bivalves such as *Solen strictus*, *Saxidomus purpurata*, *Atrina pectinate*, and *Callista chinensis*. BAMA was also identified in some samples using LC-MS/MS analysis with pre-column derivatization. Although no regular pattern of seasonal variation was clearly found, the bioaccumulation of BMAA along food chains was still hinted in Chinese marine ecosystem in this study. DAB was universally detected in most of the mollusk samples with no species-specific or regional differences. But no AEG was detected in any mollusk samples tested here. Results indicated that the contamination of BMAA is popular in mollusks in Chinese marine ecosystem and the environmental risk to human health should be paid attention to.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

O-079

Food web transfer of biotoxins as part of a suite of multiple contaminants: a case study of brevetoxins and metals

S. Pouil [1 2], R. Clausing [1,*], M. Metian [1], P. Bustamante [2], Y. Dechraoui Bottein [1]
[1]IAEA Environment Laboratories, International Atomic Energy Agency, Monaco, Monaco, [2]Littoral Environnement et Sociétés, Université de La Rochelle, La Rochelle, France

Abstract: Algal toxins in the environment are known to accumulate up the food chain via dietary pathways, often with little apparent effect on the health of the accumulating organism. Several laboratory studies on bivalves, however, have found that exposure to toxic dinoflagellates may alter important processes such as clearance rates or oxygen consumption. Neurotoxins produced by some of these algae have also been shown to act on ion channels in fish. These behavioral or physiological effects may, in turn, alter species responses to concurrent stressors in the environment, such as variable temperatures or salinity, or the presence of other contaminants (e.g. metals) often co-occurring with biotoxins in coastal waters. Experimental food chains involving toxic dinoflagellates provide a model to assess interactions of toxin exposure with other environmental factors or contaminants in marine organisms. In this example, we examined the trophic transfer of brevetoxins (PbTxs) in a *Karenia brevis*-*Mytilus edulis* (blue mussel)-*Scophthalmus maximus* (turbot) food chain with simultaneous exposure to metals (combinations of radioisotopes ^{65}Zn, ^{54}Mn, ^{109}Cd and ^{57}Co). This model allowed us to examine accumulation-depuration processes of PbTxs along the food web as well as how accumulated PbTxs affected metal uptake and assimilation. We found that after 4 days feeding with 980 ± 20 cells *Karenia brevis* mL^{-1}, mussels had accumulated 0.49 ± 0.22 µg PbTx eq g^{-1} wet wt in their soft tissues. Despite high toxin retention (78% remaining after 23 d depuration during metals exposure), PbTx exposure did not affect the assimilation of metals in mussels, excepting for small but significant increases in rates of ^{109}Cd uptake. Turbot appeared to depurate dietary PbTxs more efficiently than mussels, as fish fed once with PbTx-containing mussel tissue contained similar concentrations of toxin in their viscera to those fed 15 times over 3 weeks. Similarly to mussels, assimilation of metals (^{65}Zn and ^{54}Mn) in turbot showed no influence of PbTx exposure. These results indicate that, despite the effects of PbTxs on ion-transport processes, dietary PbTx exposure may not strongly alter metal kinetics in bivalves or fish. Yet, PbTx doses and accumulated concentrations were low compared to what can be found in nature; moreover, the diverse group of algal toxins have numerous modes of action. This laboratory model provides a useful means for further investigation of interactions between biotoxins and contaminants along the food web.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

O-080

Kinetics of microcystin accumulation and detoxification by the freshwater bivalves *Anodonta anatina* and *Dreissena polymorpha*

A. Lepoutre [1,*], A. Geffard [1], E. Lance [1]
[1]Université de Reims Champagne-Ardenne, Reims, France

Abstract: Cyanobacterial blooms often occur in fresh waters. A follow-up of cyanobacterial densities and of cyanotoxins concentrations, through weekly water samplings, is often the practice for the management of reservoirs used as sources of drinking water or for recreation. There is still a need to develop integrative samplers to biomonitor cyanotoxins. However, passive sampling devices are susceptible to clogging, do not provide quantitative data and can only be used for a limited amount of time. Therefore, we study the use of freshwater bivalves *Anodonta anatina* and *Dreissena polymorpha* as biosensors of the hepatotoxins microcystins (MCs) through laboratory experiments and active caging in areas undergoing cyanobacterial blooms. Bivalves are filter-feeders known to ingest cyanobacteria and accumulate MCs under a free form in cells that can be eliminated via glutathione pathways but also under a protein-bound form that can represent more than 90% of total MCs in tissues after a depuration period.

The purpose of this study was to evaluate which mussel species and which MC fraction (free or total) in tissues may faithfully represent the contamination of water by MC-producing cyanobacteria. For this, both mussels were exposed to three densities of *Planktothrix agardhii* producing an equivalent of 1, 10 and 100 µg MCs/L for 3 weeks, followed by 3 weeks of depuration. Control tanks without mussels contained the same cyanobacterial densities as exposed tanks. The daily intake of MCs by mussels was evaluated through the numeration of cyanobacterial cells in controls and in exposed tanks. At days 1, 3, 7, 14 and 21 of exposure and depuration periods, the free and total MC contents in mussel tissues were evaluated. We compared, for all exposure concentrations: i) the kinetics of free MCs to the kinetics of total MCs in tissues of both species, ii) both species for their abilities to reflect the amount of MCs to which they have been exposed to, via the evaluation of bioaccumulation factors. Both mussels were also caged *in situ* during two campaigns in summer 2016 and 2017 in three stations, presenting contrasting level of cyanobacterial proliferations, of the lake «Ailette» (France), and monthly (from July to November 2016) or weekly (in August 2017) sampled.

Results showed that both species accumulate free and total MCs, including when exposed to only 1 µg/L, from the first day to the end of exposure periods, with higher elimination of free MCs during the depuration compared to the total MCs. A. *anatina* accumulates far more MCs per unit of dry weight (from 25.2 to 48440.7 times) than *D. polymorpha,* but its accumulation is reduced at the highest exposure concentration. For both mussels, the free fraction represented less than 10% of the total MCs, but their kinetics were closer to the environmental changes in MC concentrations in the medium.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

O-081

MICROBIAL DEGRADATION OF MICROCYSTINS IN CENTRAL EUROPEAN WATERBODIES

D. Dziga [1 2,*], A. Maksylewicz [1], M. Maroszek [1], J. Meriluoto [2 3], Z. Svircev [3]

[1]Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland, [2]Biochemistry, Faculty of Science and Engineering, Åbo Akademi University, Turku, Finland, [3]Laboratory for Paleoenvironmental Reconstruction, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

Abstract: Biodegradation of cyanobacterial toxins has been observed in several freshwater environments but the mechanisms of such processes have been reported only occasionally. One well-documented pathway of enzymatic degradation of microcystins (MC) is based on the *mlr* gene cluster which has been detected in several species from distinct branches of the bacterial kingdom.

Recent screening of several water bodies in Poland resulted in the elucidation of a new pathway of MC biodegradation which involves the formation of at least 3 semiproducts. A continuation of these experiments was performed in Serbian waterbodies which are frequently dominated by toxic cyanobacteria. Among twelve studied freshwaters (lakes, fish ponds, channels) MC biodegradation was observed in all investigated samples but the kinetics of the process varied. PCR for *mlrA* gene (which encodes for MlrA capable of MC linearization) was positive in one sample from Starcevo reservoir. However, the *mlrA* gene was not confirmed in the sample collected one year later and the linear MC resulting from MlrA activity was not observed during MC degradation experiment. On the other hand, biodegradation assays with different MC variants allowed identification of new products of MC decomposition, two of which are cyclic molecules.

The results confirmed our previous hypothesis that the microbial degradation of microcystins is frequent in cyanobacteria-blooming Central European waterbodies but the process differs from that known for bacteria possessing an *mlr* cluster. The successful isolation of new MC biodegraders followed by characterisation of biochemical and genetic aspects of MC biodegradation is important as it would broaden our knowledge about biotic factors influencing the ambient concentrations of cyanotoxins.

Acknowledgements: This work was supported by National Science Centre, Poland [grant number UMO-2013/11/B/NZ9/00114] and Faculty of Biochemistry, Biophysics and Biotechnology of Jagiellonian University, a partner of the Leading National Research Center (KNOW) supported by the Ministry of Science and Higher Education.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

O-082

Is the density of potentially toxic phytoplankton a reliable indicator for the presence of toxins in Dutch marine bivalves?

E. Faassen [1,*], T. Bovee [1], M. Klijnstra [1], M. Alewijn [1], A. Gerssen [1]
[1]RIKILT, Wageningen, Netherlands

Abstract: In the Netherlands, potentially toxic phytoplankton species and the occurrence of toxins in commercially harvested bivalves are frequently monitored in the production areas. In case the density of potentially toxic phytoplankton exceeds a certain limit, products may only be put on the marked if the marine toxin concentrations are shown to be below the regulatory limits. This approach ensures a high level of consumer safety, but in practice it frequently leads to situations where the phytoplankton norms are exceeded, while the toxin concentrations in the products are below the regulatory limit. Therefore, Dutch phytoplankton and toxin data from the period 2012-2017 were evaluated to see whether the density of potentially toxic phytoplankton species is a reliable indicator for the occurrence of marine toxins in bivalves.

In the evaluated period, the phytoplankton limits were exceeded on 130 occasions, this was caused by high densities of the potentially toxic phytoplankton species *Dinophysis acuminata*, *Prorocentrum lima*, *Pseudo-Nitzschia spp*, *Alexandrium* spp. and *A. ostenfeldii*. During the same period, the paralytic, amnesic and diarrheic shellfish toxin concentrations were below the toxin norms for bivalves. Yessotoxins and azaspiracids, toxins for which no phytoplankton limits are set in the Netherlands, were not detected in the evaluated period. Only the tetrodotoxin concentration, of which the producing organism is unknown in the Dutch areas and which has only been monitored from October 2015 on, exceeded the national regulatory limit on ten occasions.

In accordance with earlier Dutch monitoring data and with the situation in neighbouring countries, no general relation could be established between the presence of potentially toxic phytoplankton and the occurrence of (low concentrations) of toxins in bivalve molluscs. Only in one case, in a relatively isolated lagoon, a correlation was observed between the *D. acuminata* density and diarrheic shellfish poisons in mussels. We therefore conclude that for the Dutch situation, the density of potentially toxic phytoplankton species is not the best indicator for the occurrence of marine toxins in bivalves.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-083

Amphidomataceae in the Labrador Sea and western Greenland waters

U. Tillmann [1,*], S. Wietkamp [1], K. Toebe [1], B. Krock [1], H. Gu [2]

[1]Alfred Wegener Institute, Bremerhaven, Germany, [2]Third Institute of Oceanography, Xiamen, China

Abstract: Azaspiracids (AZA) are the most recently discovered group of lipophilic marine biotoxins of microalgal origin associated with human incidents of shellfish poisoning. Over the last couple of years, four out of 27 described species of Amphidomataceae have been identified as primary source of AZA. Diversity and global biogeography of species of Amphidomataceae, however, still are poorly known. In summer 2017 we sampled the central Labrador Sea and the western Greenland coast from Gothaab Fjord (64° N) to 75° N for the presence of Amphidomataceae and AZA. In the central Labrador Sea, light microscopy revealed the presence of small *Azadinium*-like cells at fairly high densities of 9,200 cells L^{-1}. Single cell isolation from that station yielded 14 clonal strains representing four different species, *Azadinium obesum*, *Az. trinitatum*, *Az. dexteroporum*, and a new species which is currently described taxonomically. For all cultured strains, no known AZA at measureable amounts were detected. From all stations along the cruise, filtered seawater samples were negative for AZA. Specific qPCR assays for DNA samples, targeting ribosomal genes of *Azadinium* and *Amphidoma*, revealed positive signals from the majority of stations along the Greenland west coast indicating a not yet recorded widespread occurrence, albeit at low densities, of Amphidomataceen species in that area. Solid phase adsorption toxin tracking (SPATT) samplers were long-term deployed during the expedition in a continuous water sampling system (FerryBox) and interestingly revealed the presence of AZA-1 and AZA-2. These results highlight the presence of AZA toxigenic species in the area and the potential risk of toxic Amphidomataceae blooms in arctic coastal waters.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-084

The response of harmful algae to environmental and community changes along the French coast

S. Karasiewicz [1,*], A. Chapelle [1], C. Bacher [1], D. Soudant [2]

[1]ODE/DYNECO, IFREMER - Centre de Bretagne, Plouzané, [2]ODE/VIGIES, IFREMER - Centre Atlantique, Nantes, France

Abstract: Distribution, frequency and intensity of key phytoplanktonic harmful species are impacted by environmental conditions. These later are expected to change. Recently in the Brest bay, *Alexandrium minutum* was responsible for several harmful algal blooms (HABs) associated with toxin production causing paralytic shellfish poisoning (PSP). Beside, *Lepidodinium chlorophorum* is a dinoflagellate which locally causes green waters and hypoxia along the Bay of Biscay. Previous studies revealed that *L. chlorophorum* success was related to the failure of *Karenia mikimotoi*, by competitive exclusion. It follows that phytoplankton composition has to be taken into account as environmental conditions. Herein, the study aims to assess the combined effect of changes in habitat conditions and community structure on the occurrence of HAB species, on a spatial-temporal scale. For the investigation we use the revisited Hutchinson's niche concept, the subniche theory to, 1) differentiate the species response to HAB and non-HAB environmental conditions and 2) to quantify and observe the biotic and abiotic constraint on the harmful species under the two HAB and non-HAB environmental condition. The French National Phytoplankton and Phycotoxin Monitoring Network (REPHY) dataset was used. The data was acquired from 21 stations from the Seine Bay down to the Bay of Arcachon over the period of 1998 to 2016. The Outlying Mean Index (OMI) analysis was used to observe the species community affinity to the environment. The Within Outlying Mean indexes (WitOMI) were calculated under environmental conditions promoting and inhibiting known HAB species. In addition, the BV-Step analysis was performed to expose species assemblages characterizing the phytoplankton community in each habitat condition. 43 taxa groups had significant niche and were mostly distributed along nutrient and salinity gradients. *L. chlorophorum*'s niche was defined by a stratified water condition with relatively low nutrient concentration and temperature conditions. Common phytoplankton genera such, as *Skeletonema* and *Thalassosira* are found in both HAB and no HAB condition due to their respective broad environmental tolerance. As expected the two species occurred in both HAB and non HAB conditions. On the other hand, the genera *Paralia* and *Gymnodinium* were unveiled as being distinctive species under HAB environmental conditions. In opposition, the genera *Cryptomonadales* and *Dactyliosolen* are characteristic of characteristic of HAB condition. The different nutrient conditions select the species assemblage structuring the community, which in turns, bloom succession and competitive exclusion determine the bloom of *Lepidodinium chlorophorum*. The quantification of the biotic and abiotic contribution onto *Lepidodinium chlorophorum* could be further revealed by statistical niche overlap calculation.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-085

MODELING HARMFUL ALGAL BLOOMS IN INTERSPECIFIC COMPETITION

S. Nzeneri [1,*], A. Chapelle [1], M. Sourisseau [1], M. Plus [1]
[1]DYNECO, IFREMER, Brest, France

Abstract: The proliferation of Harmful Algal Blooms (HABs) is today, a subject of study with strong societal demands and research has been intense because the frequency of observed events has rapidly increased in coastal waters. ***Alexandrium minutum*** is one of the toxic species that have the ability to threaten public health, aquaculture and tourism. In France, it was observed in 1988 in the region Bretagne and has continued to proliferate ever since. High levels of PSP toxicity have been detected in the estuaries of Morlaix, Penze, Rance and more recently, in the Bay of Brest. Its presence in the Bay led to a ban on aquacultural activities and restrictions on the sale and consumption of sea food. Following this incident, the project '*Alex-Breizh*' was launched with the objectives to understand - why ***Alexandrium minutum*** dominates the microphytoplankton community during a short period, what parameters control its dynamics and how to place them in order of hierarchy. Two approaches were adopted in this study. The first was analyzing field data (nutrients and abundance of species) obtained from the study site since 2009 to 2016. The second approach was the use of a 0D numerical model to integrate the existing biotic and abiotic factors driving ***Alexandrium minutum*** bloom. It evaluates the ability of a species to grow with respect to environmental factors such as light, temperature and nutrients (PO_4, NH_4, NO_3 and Si), while competing with other species. This modeling technique is based on physiological traits that take phosphorus and nitrogen cell quotas into account. Simulation was performed with 50 species which were uniformly selected with a defined cell size ranging from 1 to 100µm, an optimal temperature of 10 to 20°C and an irradiance of 8 to 100W/m^2 and consisted of silicate and non-silicate phenotypes. Results of the model show similar succession in all simulated years where the bloom is marked by micro-, followed by nano- and then pico- phytoplankton. We noticed an increase in simulated diversity during the spring and autumn but less during the summer due to exclusive competition. We studied the inter-annual variability by comparing a year with ***Alexandrium minutum*** bloom (2012) and a year without the bloom (2016). Year 2012 presented a rainy spring and summer while 2016 was a dry year. It also showed a higher abundance of phytoplankton, specific richness and Shannon indice than 2016. We equally tested scenarios of nutrient reduction and observed that a 50% nitrogen reduction has no effect on any of the phenotypes whereas such a reduction in phosphorus slightly reduced the abundance of species. In general, maximum growth and abundance were observed between June and August with high inter-annual variability. Our model equally showed that growth is limited by temperature and light during cold periods but limited by phosphate in warm periods. Finally, the model was validated using the field data.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-086

Influence of nutrient pulses on the bloom persistence of Lingulodinium polyedra

A. K. K. Purz [1,*], D. A. Caron [2], S. D. Moorthi [1]

[1]Planktology, University of Oldenburg, Oldenburg, Germany, [2]USC Dornsife, University of Southern California, Los Angeles, USA

Abstract: Bloom dynamics of HABs in upwelling areas are the result of a variety of environmental factors. The Californian eastern boundary current system represents a coastal upwelling-influenced system where a variety of different HAB species occur. One species that is often abundant in the region during summer and autumn is the red tide dinoflagellate *Lingulodinium polyedra*. *L. polyedra* can form dense blooms, leading to rapid oxygen depletion, especially in closed embayments. *L. polyedra* blooms only irregularly along the Southern Californian Coast, raising the question which factors drive bloom formation and persistence of this species.

Studies aiming to explain bloom formation of *L. polyedra* have mainly focused on the physiology and ecology of this species in unialgal cultures, or its interaction with few selected species, but not with natural high-complexity communities. In this study, we investigated population dynamics and bloom persistence during an autumn bloom of the dinoflagellate *L. polyedra* along the southern Californian coast and its relationship to community structure and the timing of nutrient additions. We conducted two experiments with natural phytoplankton communities, taken from two *L. polyedra* blooms at two dates. Dissolved nutrients were added either as a pulse at the beginning of each experiment or after a ‘starvation’ phase to mimic the natural nutrient variability occurring in upwelling regions.

We found an overall increase of phytoplankton biomass in all treatments with the input of nutrients. Independent of the timing of the nutrient pulse, *L. polyedra* decreased after an initial increase in one community, whereas it decreased in the other community from the beginning on. Although the communities had different dynamics, the relative concentration of *L. polyedra* dropped rapidly in both communities. Under laboratory conditions, diatoms dominated the community within a few days. This effect of decreasing *L. polyedra* and increasing diatoms was independent of the initial *L. polyedra* abundance and from the timing of the nutrient pulse, even if initial *L. polyedra* abundance contributed more than 70% to the overall community biomass.

Our results suggest that nutrient pulses, at least as a single factor, are not the drivers for *L. polyedra* bloom persistence. We speculate that nutrient input is a benefit to this bloom-forming dinoflagellate only in combination with other factors, such as light and temperature. Also the form of the nutrient, such as urea as a nitrogen source, might play a role in enabling *L. polyedra* to compete with other species, especially with fast growing diatoms.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-087

Decadal and bi-decadal distribution of harmful species in the Magellan region: South-North or North-South dispersion?

G. Pizarro [1,*], C. Alarcón [1], H. Pacheco [1], C. Toro [1], P. Salgado [1], L. Guzmán [2]
[1]Environment, IFOP, Punta Arenas, [2]IFOP, Puerto Montt, Chile

Abstract: In 1972, *Alexandrium catenella* was the first toxin-producing microalgae, Paralytic Shellfish Toxin (PST), detected in Chile, Strait of Magellan (53° S). Since then, this species has been observed along the estuarine fjords system (55° – 41° S) and since 2016 it has extended its distribution beyond the fjords up to 39° S through the Pacific Ocean. Microalgae producing Lipophilic Toxins (LT) seem to have expanded from north to south, after being observed for the first time in the Los Lagos (42° S) and Aysén (46° S) regions, in 1970 and 1993, respectively. An increase in the frequency of positive mouse bioassays to LT within the Aysén and Magellan regions, seems to support this macro-scale trend. To confirm this hypothesis, we analyzed the relative abundance (RA) time series available for the Magellan region, since 1996 for *A. catenella* and *Dinophysis acuta,* and since 2006 for *D. acuminata* and *Protoceratium reticulatum*.The analyses showed that the three LT-producing microalgae have increased their geographic percentage coverage (RA >0) since 2012. For the period 2006-2011, *D. acuminata* increased from 14-57% to 73-89%, and *P. reticulatum* from 0-5% to 18-28%; *D. acuta* for the period 1996-2011, increased from 1-13% to 6-25% since 2012 up to present. Interestingly, *A. catenella* also increased its coverage percentage from 11-40% period 1996-2009, to 43-67% since 2010. The results suggest that have occurred a north-south (42° – 55° S) expansion of lipophilic producing microalgae as well as an increase in the geographical coverage in the Magellan region.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-088

Toxic dinoflagellates in the Mediterranean ecosystems facing global change, LAGUNOTOX project.

M. Laabir [1,*], E. Masseret [1], O. Kéfi-Daly Yahia [2], H. Zmerli Triki [2], H. Ben Gharbia [2], B. Rijal Leblad [3], F. ElMadani [4], H. Frihi [5], A. Draredja [5], Z. Amzil [6], P. Hess [6], N. Chomerat [7], E. Abadie [1]

[1]MARBEC, Université de Montpellier, CNRS, Ifremer, IRD, Montpellier, France, [2]Research Group on Oceanography and Plankton Ecology, Tunisian National Institute of Agronomy (INAT), 43 Avenue Charles Nicolle, Tunis 1082, IRESA-Carthage University Tunisia, Tunis, Tunisia, [3]Toxic Microalgae Laboratory, Institut National de Recherche Halieutique , Tangier, [4]Toxic Microalgae Laboratory, Institut National de Recherche Halieutique , Nador, Morocco, [5]Sciences de la Mer, Laboratoire Bioressources Marines. Faculté des Sciences. Université Badji Mokhtar , Annaba, Algeria, [6]Laboratoire Phycotoxines, Institut Français de Recherche pour l'Exploitation de la Mer, Centre Atlantique, Nantes, [7]Laboratoire Environnement Ressource de Bretagne Occidentale (LER-BO), Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), Station de Biologie Marine, Place de la Croix, Concarneau, France

Abstract: Phytoplankton blooms involving toxic dinoflagellate species are expanding worldwide. Anthropization, global warming and globalization are thought to contribute to the extension of their dispersal areas in many coastal sites located both north and south of the Mediterranean. Coastal lagoons due to their specific ecology, are the first concerned. Numbers of these ecosystems hold important shellfish production areas, where the appearance and maintenance of harmful microalgae lead to serious economic problems. The lagoons are particularly affected by recurrent proliferations of toxic algae which synthesize endo and/or exotoxins that affect human health (ocular or respiratory distress, neurological and gastric disorders) and can sometimes lead to death. The main objectives of LAGUNOTOX project is to study the diversity of toxins and toxic dinoflagellates in the main French Mediterranean lagoons (Thau, Leucate, Ingril...) and in three important lagoons in the Southern Mediterranean (Nador in Morocco, Bizerte in Tunisia and Mellah in Algeria). The Bay of Mdiq and Oued Laou estuary in Western Mediterranean of Morocco were also investigated. These ecosystems are contrasting in their degree of anthropization and environmental characteristics of the water column including temperature, nutrients and pollutants. They are concerned by the recurrence of the development of HABs species including *Alexandrium catenella, Alexandrium pseudogonyaulax, Ostreopsis cf. Ovata, Dinophysis sp. and Gymnodinium catenatum* related to serious threats to ecosystem components. We surveyed *in situ* (population dynamics investigations) the main toxic dinoflagellates species responsible for already observed poisoning episodes in these ecosystems and also some emerging toxic dinoflagellates which could cause harmful events in the coming years/decades. Diversity of toxins was assessed using *in situ* passive sampling. The main toxic species colonizing the corresponding ecosystems were fully characterized using molecular techniques. HAB species isolated from lagoons of both sides of the Mediterranean are cultured and enriched an algal collection named ALGOMED (Toxic Mediterranean Algae) set up in Montpellier (MARBEC) and extended to Tunisia (INAT) and Morocco (INRH Tangier). LAGUNOTOX project succeeded to identify formerly a number of established and emerging toxic dinoflagellate species and to characterize the produced toxins using HPLC MS/MS and brought lacking data on their ecology and ecophysiology. Such data will be useful to stakeholders and scientists to prevent /manage/mitigate the development of toxic species in these vulnerable Mediterranean ecosystems.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-089

Tracing the deposition region of massive floating green algae in the Yellow Sea

H.-X. Geng [1,*], R.-C. Yu [1], M.-J. Zhou [1]

[1]CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China

Abstract: Since 2007, large-scale green tides formed by green alga *Ulva prolifera* have been recorded consecutively in the southern Yellow Sea for 11 years. The green tides in the Yellow Sea, which are different from green-tide events in other regions around the world, have huge biomass of green algae about several million tonnes, and impact an extensive area up to 30−50 thousand square kilometers. The rapid growth, long-distance transport, and decomposition of green algae during the green tides will change the distribution, concentration, and composition of nutrients, and probably affect the structure of the phytoplankton community, or even lead to derived ecological disasters in a specific region. However, there is still little knowledge on the deposition region of massive floating green algae in the southern Yellow Sea, which limits the understandings on the ecological effects of green tides. To track the deposition region of green algae, 28-isofucosterol was selected as a biomarker of green algae in genus *Ulva*. The methods for the extraction and determination of sterols in algae and sediments, including 28-isofucosterol, were established by using gas chromatography–mass spectrometry (GC−MS). Based on a series of simulation experiments, the specificity and stability of 28-isofucosterol were examined, and it was found that 28-isofucosterol in sediment was positively correlated with the amount of decomposed *U. prolifera*, and it remained stable for at least one month in sediment. The content of 28-isofucosterol in surface sediment samples collected from the Yellow Sea and Bohai Sea was then analyzed, and the potential sedimentation region of floating green algae was primarily identified in the sea area (122–124°E, 36–36.5°N) southeast to Shandong Peninsula. The findings will help to further understand the ecological consequences of green tides and promote our knowledge on the ecosystem changes and their driving forces.

Disclosure of Interest: None Declared

Mitigation of HABs

O-090

Impact of eutrophication on adsorption efficiency during water treatment: reactivity of organic matter & Microcystin

H. Thuret Benoist [1,*], J. Afouda [1], V. Pallier [1], G. Feuillade [1]
[1]PEIRENE, 87000 Limoges, France

Abstract: Eutrophication is a natural process describes as ponds' enrichment by nutrients conducting progressively to their filling. Excessive inputs of nitrogen and phosphorus by human activities accelerate the phenomenon and climate changes magnify the symptoms. In eutrophic ponds, the primary producers (mainly phytoplankton) are no longer limited by nutrients and can proliferate. Their proliferations affect drinking water treatment processes by increasing Organic Matter (OM) quantity ($DOC_{oligotrophic\ pond}$=2ppm–$DOC_{eutrophic\ pond}$=10ppm (Thurman, 1985)) and modifying its characteristics. Algal OM (AOM) produced by phytoplankton is less hydrophobic and aromatic than Natural OM (NOM) ($SUVA_{winter\ 2012}$=42L/cm/gC-$SUVA_{summer\ 2012}$=28L/cm/gC (Leloup *et al.,* 2013)). Besides, in function of cyanobacteria species, non-negligible toxins content can be quantified in water.

To respect the European guidelines, DOC and toxins in drinking water must be lower than 2ppm and 1µgMC-LR.L^{-1} respectively. Coagulation-flocculation process is efficient to remove OM and intracellular toxins (80%) unlike extracellular (<10%) and presents the advantage not to damage cells. (Sheng *et al.,* 2003; Kemp & John, 2006). Adsorption on powdered activated carbon (PAC) is efficient on extracellular toxins removal (85%) but with high PAC doses (>20ppm) and the efficiency is reduced by OM competition (Maghsoudi *et al.,* 2015).

In this study, the adsorption of NOM and MC-LR on PAC was investigated on synthetic and natural waters spiked or not with AOM and MC-LR. Experiments were also conducted on culture of *M. aeruginosa* grown under 2 temperatures (23-15°C). The objectives were to determine the AOM reactivity according to its hydrophobic characteristics, qualify the competition between AOM and MC-LR and evaluate the adsorption efficiency on PAC according to the trophic statue of the resource. The maximum adsorption capacity ($\Gamma\infty$) and the affinity (1/n) between PAC and NOM and MC-LR were estimated by applying Langmuir and Freundlich linearization models.

On March 2018, exclusively algae (Chl-a=77µg.L^{-1}) were present in the water resource from Pigeard, whose characteristics were: DOC=4.4±0.1ppm, SUVA=33L/cm/gC, %HPI=43%. After 240min and with PAC at 150ppm, 55% of $UV_{254}$and 40% of DOC were removed and the more aromatic and hydrophobic molecules (HPO) were better adsorbed. Indeed, the %HPO decreased by 33% and $SUVA_{index}$ by 20%. When Pigeard's sample was spiked with transphilic fraction (DOC_{final}=11ppm), the DOC removal decreased ($\%_{removed}$DOC=24%) unlike UV_{254} ($\%_{removed}UV_{254}$=52%). These results confirmed the better affinity of PAC for aromatic and HPO. For Pigeard's sample, $\Gamma\infty$=448mg COD.g^{-1}PAC and 1/n=0.90 were calculated. Experiments conducted on synthetic water with extracted fraction of various hydrophobicity will allow confirming these results and understanding OM competition with toxins

Disclosure of Interest: None Declared

Mitigation of HABs

O-091

Plant vs cyanobacteria. Searching for a plant capable of preventing Cyano-HABs and eliminating cyanotoxins in water

A. J. Kaminski [1,*], E. Chrapusta [1], K. Duchnik [1]

[1]Department of Plant Physiology and Development, Faculty of Biochemistry Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

Abstract: Cyanobacterial harmful algal blooms (Cyano-HABs) and cyanotoxins, a group of secondary metabolites synthesized by these microorganisms, have become a great worldwide concern due to their high toxicity and widespread presence in aquatic systems. Some of the cyanotoxins like microcystin-LR, cylindrospermopsin (CYN), and anatoxin-a (ANTX-a) are determined more often than other toxic compounds.

The aim of this research is to determine the physiological response of common freshwater plant *Lemna trisulca* (L.) to various concentrations of CYN and to verify the future use potential of this macrophyte as a cheap, effective and common agent for prevention of Cyano-HABs, and elimination of cyanotoxins from the water.

Axenic cultures of *L. trisulca* were cultivated in Erlenmeyer flasks in the BG11 medium in a phytotron at 20±1°C with 80% humidity and 50 µmol·m^{-2}·s^{-1} PAR under a 12h photoperiod. To the appropriate flasks were added 0.0; 0.1; 0.5; 1.0; 2.0 or 5.0 µg CYN·mL^{-1} (5 replicates). At fixed time intervals depending on the subject of research, protein concentration (spectrophotometrically), photosynthesis and respiration processes (polarographically), damage of cell membranes (conductometrically), biomass accumulation (weight method), quantitative ion release (ion-exchange chromatographically) have been measured.

Obtained results showed that CYN is more toxic to *L. trisulca* than previously analyzed ANTX-a (on average 5 times), but in the most frequently documented concentrations in a natural water environment (<1 µg CYN·mL^{-1}) this macrophyte seems to be resistant to its toxic effect. During the plant cultivation in media with the CYN concentrations equal to 0.1 and 0.5 µg·mL^{-1} determined the stimulation of biomass accumulation, minimal cell membrane damage, a slight release of some analyzed ions (NO_3^-, Na^+, NH_4^+, and Ca^{2+}) and the initial increase in the intensity of photosynthesis. Treatment of the plants with higher toxin concentrations resulted in the cells membranes damage (up to 15%), significant release of all determined ions (up to 33 and 12 times higher compared to the control for NO_3^- and K^+, respectively), and a decrease in fresh weight and intensity of photosynthesis process. In all the analyzed concentrations, the total protein and pigment contents were enhanced up to 11% and 6%, respectively.

The relatively high resistance of *L. trisulca* to the most frequently found CYN concentrations in water, as well as previously described its effective ability to accumulate nutrients and to degrade ANTX-a in the entire depth of the water reservoir make it possible to be used in the future as an anti-Cyano-HABs and anti-cyanotoxins agent.

This work was funded by National Science Centre, Poland (grant no.2016/23/B/NZ9/01041). Ariel Kaminski in 2016/2017 was supported by the Foundation for Polish Science (FNP, START program).

Disclosure of Interest: None Declared

Mitigation of HABs

O-092

Advanced Oxidation Treatment of Cyanotoxins: Elucidation of Degradation Pathways

A. Hiskia [1,*], T. T. Triantis [1], T. Kaloudis [2], C. Christophoridis [1]
[1]Institute of Nanoscience and Nanotechnology, NCSR Demokritos, [2]Water Quality Control, EYDAP SA, Athens, Greece

Abstract: Cyanobacteria blooms pose an environmental hazard because of the release of water soluble toxic compounds, called cyanotoxins (CTs). Health effects of CTs have resulted in the need of using efficient treatment methods for their removal in water supplies.

Advanced Oxidation Processes (AOPs) such as UV/H_2O_2, photocatalysis, radiolysis and UV/Cl_2 have emerged as important and increasingly viable alternatives of water treatment. AOPs generate Reactive Oxygen Species (ROS) and sometimes other radicals as well, which react with a wide range of organic compounds. Recently, we have shown that AOPs have been effective for treatment of new emerging pollutants such as CTs. Our main objectives were to study the degradation process, define the reactive species involved and elucidate the reaction mechanisms.

Upon treatment of microcystin-LR (MC-LR) with UV/H_2O_2, the formation of hydroxyl radicals ($OH^\bullet$) is responsible for isomerization, hydroxylation, hydroxyl addition or oxidative cleavage of double bonds. In photocatalytic based AOPs destruction of MC-LR and cylindospermopsin (CYN) was effectively achieved under UV-A and solar irradiation. Photocatalytic degradation proceeds to complete toxin mineralization giving CO_2, NH_4^+ and NO_3^- for MC-LR and CO_2, NO_2^-, NO_3^-, SO_4^{2-} and NH_4^+ for CYN as final products, respectively. A large number of intermediates were identified using liquid chromatography tandem mass spectrometry (LC-MS/MS) and complete degradation pathways are proposed, leading to the conclusion that $OH^\bullet$ attack is the main mechanism followed. Under visible light irradiation (VIS) only photo-sensitized catalysts (e.g. Kronos vlp-7000) were able to degrade CTs. Reaction pathways of MC-LR and CYN were defined through identification and monitoring of intermediates for VIS in comparison with UV-A photocatalytic treatment. The effects of scavengers and probes on the degradation process under VIS, as well as the differences in product distributions under VIS and UV-A, suggested that the main species in VIS photocatalysis is superoxide (O_2^-), with $OH^\bullet$ and singlet oxygen ($^1O_2$) playing minor roles in the degradation.

Disclosure of Interest: None Declared

Mitigation of HABs

O-093

Interaction between *Microcystis*, microcystin and lytic phage Ma-LMM01

N. H. Sukarji [1,*], S. H. Te [1], W. Li [1], Y.-H. K. Gin [2]

[1]National University of Singapore Environmental Research Institute, [2]Civil and Environmental Engineering, National University of Singapore, Singapore, Singapore

Abstract: *Microcystis* is a notorious bloom cyanobacteria that produces the hepatotoxin, microcystin. Many studies have attempted to predict and also to control these toxic blooms, with some studies suggesting the use of cyanophages to do so. Hence, this study created a model using STELLA software (version 10) to predict and simulate the interaction between *Microcystis* growth and buoyancy, microcystin production and *Microcystis*–infecting lytic phage MA-LMM01. The model was calibrated using data of a *Microcystis* bloom in a Southeast Asian reservoir till satisfactory (Average R^2 = 0.851). Afterwards, simulations done revealed that Ma-LMM01 inoculation (of 10^5 to 10^9 phages/mL) during the bloom are capable of controlling the bloom and toxin production by reducing up to 95% and 80% of the overall *Microcystis* and microcystin concentrations respectively. However, its success was dependent on the timing and quantity of Ma-LMM01 inoculation due to the infectivity of the phage being sensitive to environmental conditions. These results imply that phages could be used to control bloom and toxin occurrence, but more research is needed to find optimal conditions for phage inoculations. Future works could also experiment on phage inoculation in reservoirs to test the results of the simulation.

Disclosure of Interest: None Declared

Mitigation of HABs

O-094

www.HABreports.org

Early warning of Harmful Algae and Their Biotoxins for the Aquaculture Industry

K. Davidson [1,*], C. Whyte [1], S. Gontarek [1], D. Aleynik [1]
[1]Scottish Association for Marine Science, Oban, United Kingdom

Abstract: Since 2014 SAMS has provided the aquaculture industry in the Scottish Shetland Islands with weekly Harmful Algal Bloom/Biotoxin risk assessment bulletins. These bulletins contain a number of metrics (phytoplankton counts, biotoxin concentrations, environmental information) that are used by industry to plan their husbandry and harvesting practices in order to minimise the health and economic impacts of HABs.

A web portal (www.HABreports.org) is used to deliver the bulletins and also to provide the Scotland wide aquaculture industry with map based risk assessment tools to further support their business operations.

The HABreports system allows users to view and interrogate spatial and temporal data sets of HAB abundance and shellfish biotoxin concentrations.

Recently we have incorporated automatic calculation of the Food Standards Scotland Toxin Traffic Light Guidance index into our risk assessments. This is designed to allow food business operators to implement risk management processes on the basis of current and recent trends in toxin/HAB data.

The HABreports system also allows for unstructured grid mathematical model based simulation of harmful bloom development and progression. We next intend to further develop this component of the system to include more realistic biology in our models.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

O-096

THE APPLICATION OF THE PHYTOXIGENE CYANODTEC QPCR ASSAYS AS A SCREENING TOOL MONITORING INLAND WATER SYSTEMS FOR HARMFUL ALGAL BLOOMS

M. Van Asten [1,*]

[1]Phytoxigene, Inc., Belrose, Australia

Abstract: There is good understanding of the genetic basis for toxin production in Cyanobacteria. The utilization of these toxin biosynthetic pathway genes in screening tests to identify the presence or absence of toxin producing organisms, has for many years been proposed as a useful tool for screening, prediction and management of HABS.

From May 2016 the Ohio Environmental Protection Agency implemented an integrated HAB monitoring strategy for their public water systems. The CyanoDTec multi-plex qPCR assay CyanoDTec was utilised to screen for the presence and level of toxin genes responsible for the production of microcystin,/nodularin (mcyE), cylindrospermopsin (CryA) and saxitoxin (SxtA). The assay also identified and quantified total cyanobacteria using a 16s rRNA gene.

Water samples were collected biweekly for all 119 public water systems (PWSs) and analyzed using the CyanoDTec assay along with a microcystin ELISA test. ELISA analysis for saxitoxins and cylindrospermopsin was also conducted if sxtA or cyrA genes were detected. McyE was detected in source waters for 57 PWSs and six inland lakes and microcystins were detected at 45 PWSs and five inland lakes. 2% of PWS samples and 15% of inland lake samples had microcystins detections without corresponding mcyE detections. At several sites, detection of mcyE preceded microcystins detections by 1-4 weeks. sxtA was detected at 33 PWSs and 14 inland lakes, and saxitoxins were detected at 15 PWSs and 10 inland lakes. At one PWS, mcyE, sxtA, and cyrA were all detected, demonstrating multi-plex assay functionality. Results from 2016/7 demonstrated the utility of the assay and showed it out-performed cyanobacteria cell counts as a predictor for inland lake cyanotoxin production.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

O-097

Advanced characterisation of cyanobacterial bloom using spectral fluorescence at an extended wavelength

S. Imran Khan [1,*], A. Zamyadi [2], N. Rao [3], R. Stuetz [4], R. Henderson [2]
[1]PhD candidate, [2]Assistant Professor, [3]Post doc, [4]Professor, University of New South Wales, Sydney, Australia

Abstract: The frequency and intensity of toxic cyanobacterial blooms have increased in surface water sources worldwide due to effects of global climate change. Effective management strategies depend on the early warning of bloom development and accurately monitoring cyanobacterial cells, toxins and taste and odour (T&O) compounds. On-line monitoring of cyanobacterial blooms using in-situ fluorescence probes provides information on cell biomass via the analysis of fluorescent cell pigments, i.e. chlorophyll-a and phycocyanin. However, fluorescent pigment measurement alone is not able to identify the specific species, stage of the bloom, or organic matter released, such as toxin/taste and odours. Due to recent developments in fluorescence, it is now possible to monitor the fluorescence of algal-derived organic matter (AOM) which could give further real time information on bloom health. Overall, the aim of this study is to make recommendations for potential modifications to existing fluorometer optic specifications and development of an online protocol which could lead to the creation of 'next generation fluorescence probes'. A major component of this research was to apply advanced characterisation using 3D fluorescent excitation emission matrix (EEM) spectroscopy across a wide range of wavelengths encapsulating both AOM and cell pigment fluorescence. Six cyanobacterial species were monitored along their growth phases to investigate the presence of species specific spectral fingerprints. A six component (C1-C6) PARAFAC model was developed for the analysed data. Varying patterns of peak signals originating from the AOM indicated that potential species specific markers could be ascertained. For example, C3 (amino-acid like) fluorescence dominated in the AOM originating from *C. vulgaris*, while that of *M. aeruginosa* was dominated by C2 (humic-like) fluorescence. Among the identified PARAFAC components fluorescence response of C2 and C3 was found to be significant across the exponential and stationary phases and were positively related with total AOM released as DOC. With fluorescence probes on the market that measure fluorescence in these key AOM regions, these peaks could be monitored simultaneously with pigment fluorescence to give information on the dominant species, phase of the bloom and OM character.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

O-098

Characterization of oceanic Noctiluca blooms not associated with hypoxia in the Northeastern Arabian

V. Trainer [1,*], A. Lotliker [2], S. Baliarsingh [2], M. Wells [3], C. Wilson [4]

[1]NOAA Northwest Fisheries Science Center, Seattle, Washington, USA, [2]Indian Centre for Ocean Information Services, Hyderabad, India, [3]University of Maine, Orono, Maine, [4]NOAA Southwest Fisheries Science Center, Monterey, California, USA

Abstract: Intense blooms of the heterotrophic dinoflagellate, green *Noctiluca scintillans,* have been reported annually in the Northern Arabian Sea since the early 2000s. Although not known to produce organic toxins, these blooms are still categorized as a harmful due to their association with massive fish mortalities. Recent work has attributed these blooms to the vertical expansion of the oxygen minimum zone, driven by cultural eutrophication from major coastal cities in western India. As diatoms are preferred prey of green *Noctiluca scintillans,* more frequent blooms of this mixotroph will likely impact the productivity of important fisheries in the region. The present study uses a satellite algorithm to determine the distribution of both diatom and green *Noctiluca* blooms in the Northeastern Arabian Sea from 2009 to 2016. The results from shipboard microscopy of phytoplankton community composition were used to validate the satellite estimates. The satellite algorithm showed 76% accuracy for detection of green *Noctiluca* and 92% for diatoms. Shipboard measurements and data from biogeochemical-Argo floats were used to assess the relationship between oxygen concentrations and green *Noctiluca* blooms in the Northeastern Arabian Sea. Regardless of the presence of a *Noctiluca* bloom, the dissolved oxygen in the photic zone was always >70% saturated, with an average oxygen saturation >90%. The variability in the relative abundance of diatoms and green *Noctiluca* is not correlated with changes in oxygen concentration. These findings provide no evidence that cultural eutrophication has contributed to the decadal scale shifts in plankton composition in the Northeastern Arabian Sea oceanic waters. Conversely, the climatic warming of surface waters would have intensified stratification, thereby reducing net nutrient flux to the photic zone and decreasing silicate to nitrate ratios (Si:N); both factors that could increase the competitive advantage of the mixotroph, green *Noctiluca*, over diatoms. If so, the decadal-scale trajectory of phytoplankton community composition in the Northeastern Arabian Sea may be a harbinger of future climate-driven change in other productive oceanic systems.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

O-099

Microfluidic devices to evaluate phototaxis, chemotaxis, and gravitaxis for motile micro-algae

K. Ozasa [1,*], J. Won [2], S. Song [2], M. Maeda [1]
[1]RIKEN, Saitama, Japan, [2]Hanyang University, Seoul, Republic of Korea

Abstract: The cell distribution of motile microalgae is affected by various external stimuli, such as strong light, environmental chemical substances, and gravity/buoyancy. When a stimulus is preferable (unprofitable), the cells move toward (escape from) the source of stimulus as positive (negative) taxis. The analysis of the response of a target cell is rather simple and easy for a single stimulus, but difficult for multiple simultaneous competing stimuli, such as optical and chemical stimuli.

We developed a microfluidic system to evaluate the behavior of motile microalgae cells under two simultaneous competing optical and chemical stimuli, and demonstrated the quantitative observation for *Euglena gracilis*. The cells were confined in a quasi-2D microchamber of 2.5 mm in diameter, made with porous and transparent soft plastic of polydimethylsiloxane (PDMS). Two microchannels were running aside of the microchamber to supply chemical substances to the microchamber by diffusion through porous PDMS [1]. Light illumination was performed using LC projection for spatial intensity gradient or LED for in-plane irradiation [2]. The movements of the cells were monitored with a video camera and analyzed real time.

When a strong blue light with an intensity gradient of 0.5–18 mW/cm^2 was illuminated on the microchamber, the *E. gracilis* cells were accumulated in areas of weak blue light by photophobic responses. As keeping the blue light illumination, 100% CO_2 gas was supplied to one of the two microchannel closer to the cell accumulated area to form a CO_2 gradient (0%–100%) in the microchamber. The CO_2 gradient evoked negative chemotaxis of *E. gracilis* cells to the counter direction to photophobic responses. Then the cells avoided higher CO_2 areas rather than strong blue light, indicating that CO_2 chemotaxis is dominant over blue light photophobicity. Some cells accumulated in niche areas, where CO_2 concentration was <25% and at the same time blue light intensity was rather weak. Over all, the observed results that competing chemotactic and photophobic or phototactic stimuli evoked superimposed responses suggest that the two independent tactic signaling pathway are merged into one flagellar control mechanism. Using the same system, we also clarified how *E. gracilis* cells behave in response to simultaneous photophobic response and negative gravitaxis competing each other, and found that the photophobic response overcame the gravitaxis.

[1] K. Ozasa et al., Gas/liquid sensing via chemotaxis of Euglena cells confined in an isolated micro-aquarium, LabChip, 13 (2013) 4033-4039.

[2] K. Ozasa et al., Transient freezing behavior in photophobic responses of Euglena gracilis investigated in a microfluidic device, Plant Cell Physiol., 55 (2014) 1704-1712.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

O-100

Colorimetric and electrochemical DNA-based tools for the rapid detection and quantification of toxic marine microalgae

A. Toldrà [1,*], K. B. Andree [1], M. Fernández-Tejedor [1], J. Diogène [1], I. Katakis [2], C. O'Sullivan [2], M. Campàs [1]
[1]IRTA, Sant Carles de la Ràpita, [2]Departament d'Enginyeria Química, Universitat Rovira i Virgili, Tarragona, Spain

Abstract: Benthic toxic microalgae of the genera *Ostreopsis*, *Gambierdiscus* and *Fukuyoa* have become a serious concern due to their direct impact on the marine environment and human health. Current microalgae identification is performed via optical microscopy, which is time-consuming and not able to differentiate among species of the same genus. Therefore, rapid, species-specific and *in-situ* detection tools are highly needed to provide early warnings.

In this work, we report the development of two innovative DNA-based assays: a colorimetric assay for *O. ovata* and *O. siamensis* and an electrochemical assay for *O. ovata*. Both assays exploited isothermal recombinase polymerase amplification (RPA) using species-specific primers designed to bind within the ribosomal DNA. These primers were designed to render an amplicon with single stranded DNA tails for the subsequent colorimetric and electrochemical detection of this RPA amplicon via a sandwich hybridization assay. For the colorimetric assay, thiolated capture probes were immobilized on maleimide-modified microtitre plates, whereas for the electrochemical assay the thiolated capture probe was immobilized on maleimide-modified magnetic beads, which were subsequently captured on a magnetized electrode. Following hybridization of the RPA amplicon, colorimetric and electrochemical detection was achieved via addition of an HRP-conjugated reporter probe. Both systems showed high specificity and calibration curves were successfully constructed using genomic DNA, achieving a limit of detection of ~10 pg/µL. Both assays were applied to the analysis of *Ostreopsis* spp. present in coastal water and epiphyton samples from different sites of the Catalan coast. These samples contained *O. ovata* and *O. siamensis* and quantifications obtained by the two assays were in agreement with qPCR analysis.

Similarly, a DNA-based assay using RPA followed by lateral flow readout is currently being developed for the simultaneous identification of *Gambierdiscus* and/or *Fukuyoa* genera, *Fukuyoa* genus, *G. australes* and *G. excentricus*.

The developed DNA-based tools show strong potential for application in the monitoring and management of harmful algal blooms.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

O-101

Exploring benefits of combined microscopy & next-generation sequencing to identify cyanobacteria water treatment critical points

A. Zamaydi [1,*] and Arash Zamyadi, Caitlin Romanis, Toby Mills, Brett Neilan, Florence Choo, Lucila A Coral, Deb Gale, Gayle Newcombe, Nick Crosbie, Richard Stuetz1 Rita K Henderson, Farhad Jalili, Sarah Dorner, Michele Prevost
[1]Montreal Polytechnique, University of Montreal, Montreal, Canada

Abstract: A wide range of cyanobacterial species and their harmful metabolites are increasingly detected in water bodies worldwide, exacerbated by climate change and human activities. The resulting bloom conditions represent significant challenges to production of safe drinking water and cost effective water reuse, therefore their removal is a priority to ensure public safety. While current microscopic taxonomy identification methods provide valuable information about cell numbers during treatment, these methods are incapable of providing information about the fate of cells during treatment. The objectives of this study were to (1) identify the critical control points for breakthrough and accumulation of cells by investigating the fate of cells during treatment processes using a combination of taxonomy, cell integrity and next-generation sequencing, and (2) assess the impact of pre-treatment processes on breakthrough prevention at critical control points, and the benefits of cell integrity and next-generation sequencing analysis for improved management purposes. This paper presents the results of an unprecedented cyanobacterial monitoring program conducted in four Australian and three Canadian full scale water treatment plants. Cyanobacterial cell integrity and accumulation during operation process were assessed for the first time using next generation of gene sequencing methods. Next-generation sequencing analysis led to detection of cyanobacterial and melainabacteria orders in water samples that were not identified by microscopy. 80 ± 5% of cells were completely lysed post pre-oxidation (for both ozone and potassium permanganate). However unlike pre-ozonation, the remaining cells were undamaged cells with the potential to accumulate and grow within the plants post-$KMnO_4$ treatment, particularly in clarifier sludge. To effectively monitor water quality, this study presents a synergistic approach coupling new and traditional analytical methods and demonstrates the importance of identifying critical points for managing accumulation of cyanobacteria within plants.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-102

Modeling ciguatoxin flux into multiple vectors in the coral reef benthic food web

M. Parsons [1,*], M. Richlen [2], A. Robertson [3], T. Smith [4], D. Anderson [2]

[1]Coastal Watershed Institute, Florida Gulf Coast University, Fort Myers, FL, [2]Biology, Woods Hole Oceanographic Institution, Woods Hole, MA, [3]Marine Sciences, Dauphin Island Sea Lab, Dauphin Island, AL, [4]Center for Marine and Environmental Studies, University of the Virgin Islands, St. Thomas, USVI, USA

Abstract: Although ciguatera fish poisoning is the most prevalent form of phycotoxin-borne seafood poisoning globally, fisheries and health agencies have limited capabilities to predict or broadly monitor these incidents. In this study, we present refinements to a model that simulates the growth of five species of the epiphyte dinoflagellate, *Gambierdiscus*, known to occur in the Greater Caribbean Region, and six macrophytes known to host *Gambierdiscus* populations (*Dictyota*, *Halimeda*, *Laurencia*, *Lobophora*, *Thalassia*, and turf algae). The *Gambierdiscus* species vary significantly in their toxin content, as determined in culture studies. Responses seasonal changes in wave action, temperature and light intensity at eight locations in the Florida Keys and St. Thomas (U.S. Virgin Islands) were simulated, and the cell densities and their toxin contents were used to estimate the overall toxin load of *Gambierdiscus* populations on each macrophyte. Seasonal changes in the grazing rates of herbivorous fishes were then simulated as a function of temperature and substrate preference, resulting in an integrated model capable of estimating toxin ingestion rates by reef grazers over time. While this model is a work in progress, it represents an important step forward in promoting seafood safety world-wide.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-103

Toxicity, chemistry, and implications of *Gamberdiscus silvae*: A ciguatoxin superbug in the Greater Caribbean Region

A. Robertson[1,*], M. L. Richlen[2], D. Erdner[3], T. B. Smith[4], D. M. Anderson[5], J. Liefer[6], Y. Xu[7], P. McCarron[8], C. Miles[8], M. L. Parsons[9]

[1]Marine Sciences, Dauphin Island Sea Lab & the University of South Alabama, Dauphin Island, [2]Woods Hole Oceanographic Institute, Woods Hole, [3]University of Texas Marine Sciences Institute, Port Aransas, USA, [4]University of the Virgin Islands, St. Thomas, Virgin Islands, U.S, [5]Biology, Woods Hole Oceanographic Institute, Woods Hole, USA, [6]Mount Allison University, Sackville, Canada, [7]Guangxi Teachers Education University, Nanning, China, [8]National Research Council, Measurement Science and Standards, Halifax, Canada, [9]Florida Gulf Coast University, Fort Meyers, USA

Abstract: Recent taxonomic and toxicological studies, including our own, have shown that multiple *Gambierdiscus* species co-occur and have demonstrated differences in toxicity, supporting a direct link between community dynamics and toxicity; however, critical information on the primary vectors of ciguatoxin production and flux in the food web has been lacking. One of the major outcomes of this study was a more complete characterization of diversity and toxicity of *Gambierdiscus* species and strains in the Greater Caribbean Region, particularly local diversity present at our study sites in the Florida Keys and St. Thomas, USVI. Over 600 *Gambierdiscus* isolates were established in culture and identified using molecular methods. These studies revealed that *Gambierdiscus* communities at both locations were diverse, yet similar in composition and structure, comprising at least five described *Gambierdiscus* species and one ribotype (*G. belizeanus*, *G. caribaeus*, *G. carolinianus*, *G. carpenteri*, *G. silvae,* and *Gambierdiscus sp.* Ribotype 2), as well as several undescribed ribotypes. Of these species*, G. caribaeus* and *G. carolinianus* comprised the largest proportion of isolates identified in both regions. Subsequent mouse neuroblastoma assay (N2A) and mass spectrometry analyses of a subset of these cultures revealed considerable differences in toxin-producing capabilities and metabolite profiles among these, with just two species identified as substantial toxin-producers. One species, *Gambierdiscus silvae* had toxin cell quotas that were 1000 times higher than other species examined. Toxicity assessment of 37 strains of *G. silvae* isolated from field samples collected between 2010-2017 from long term monitoring sites in the Virgin Islands, had toxin cell quotas in the range 2.1–4.8 pg C-CTX-1 equivalents. These findings suggest that the presence of CTX in fish may be traced to just one or two algal species, and that these species may comprise just a minor proportion of the *Gambierdiscus* community. The presence of 1-2 super-strains amidst a diverse community has important implications for monitoring and management of ciguatera, which for algae has focused on genus level abundance estimates to establish risk.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-105

Ciguatera risk assessment in a hot spot in south central Cuba

L. Díaz Asencio [1,*], M. Vandersea [2], D. Chamero Lago [1], N. Chomerat [3], R. Clausing [4], J. Hernández Albernas [5], M. Gómez Batista [1], L. Rañada [6], W. Litaker [2], P. Tester [7], C. M. Alonso Hernández [1], M. Y. Dechraoui Bottein [4]

[1]Centro de Estudios Ambientales de Cienfuegos, Cienfuegos, Cuba, [2]National Ocean Service, NOAA, Beaufort, USA, [3]Ifremer, Nantes, France, [4]IAEA Environment Laboratories, Monte Carlo, Monaco, [5]Gaviota, Cayo Santa Maria, Cuba, [6]Philippine Nuclear Research Institute, Quezon City, Philippines, [7]Ocean Tester, Beaufort, USA

Abstract: In Cuba, ciguatera is the most significant non-bacterial seafood borne illness in terms of occurrence and impacts. Prevention is currently conducted through fish market regulation by imposing bans of selected species over certain weights. This study assessed ciguatoxin occurrence and transfer within a marine food web in an area of southern Cuba locally known to be prone to ciguatera. Epiphytic dinoflagellate communities were sampled using artificial substrates and macroalgae. Cell abundances of *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* genera were enumerated microscopically. In addition, qPCR assays were used to unambiguously identify *Gambierdiscus* species and to estimate cell abundances of field samples and of cultured isolates. *G. caribaeus*, *G. belizeanus*, *G. carpenteri*, *G. Carolinianus* and *F. ruetzleri* were identified in the field samples. A total of 60 fish and invertebrate specimens were collected for ciguatoxin analyses. Ciguatoxin concentrations were measured using an optimized radioligand receptor binding assay (RBA) and toxin analyses were further corroborated using the N2A bioassay. Sixty % of the samples presented significant levels of toxicity ranging from 2 to 8 ng of P-CTX-3C equivalent g^{-1} of tissue. A culture of *G. belizeanus* expressing low toxicity levels was successfully established. This study revealed a high species diversity of *Gambierdiscus* and a prevalence of fish with ciguatoxin concentrations above the human consumption save level of 1 ng g^{-1}, confirming the risk of ciguatera in this area. The relationship between toxicity and fish species, weight, size, trophic level, and the presence of *Gambierdiscus* species will provide insights into CTX food web transfer, essential information to develop a model for ciguatera risk assessment.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-106

ARE MACROALGAE A RELIABLE METHOD FOR THE QUANTIFICATION OF BHAB?

J. F. Zabala [1,*], F. Tuya Cortes [2], A. Amorim [3], E. Soler-Onis [1]

[1]Banco Español de Algas. FPCT de la Universidad de Las Palmas de Gran Canaria, Telde, [2]Departamento de Biología, Facultad de Ciencias del Mar, Campus Tafira, Las Palmas, Spain, [3]MARE-Centro de Ciências do Mar e do Ambiente, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

Abstract: In the last decades, blooms of benthic harmful dinoflagellates (BHABs) have become a major concern because of their expansion to areas outside their known endemic distribution. One of the constraints to the understanding of the dynamics of BHABs has been the lack of a standardized sampling method for the estimation of cell abundances. The most common sampling method consists on the collection of macroalgae. The cells are then counted and results expressed as cells per gram wet weight. Macroalgae have the advantage of being present in many different marine habitats, where they can be collected in a simple way. However, they have a variable distribution in time and space as well as different thalli morphologies which affects their surface to weight ratio. This makes difficult the comparison of benthic dinoflagellate abundances among different studies. More recently, to overcome these limitations, several studies have suggested the use of artificial substrates as for instance the fiberglass screens proposed by Tester *et al*. (2014).

In this study, several experiments were carried out to compare the estimation of BHAB species abundances using macroalgae and artificial substrates (fiberglass screens). Samples were collected across environmental gradients, from El Hierro, Tenerife, Gran Canaria and Fuerteventura (Canary Archipelago) and São Vicente (Cape Verde), between March 2017 and April 2018.

Artificial substrates, settled for 24h, were successfully colonized by the same genera observed on the surrounding natural substrates (*Gambierdiscus*, *Ostreopsis*, *Coolia*, *Prorocentrum* and *Amphidinium*). However, linear regression analyses on cell abundances obtained by the artificial and macroalgae substrate methods indicated a weak relationship between the two. Sample variability was, in most cases, much higher for the natural substrates (Coefficient of Variation ~ 100%) than for the fiberglass screens (Coefficient of Variation < 50%). The results obtained from our data show that the variability is greater when different macroalgae are used for the quantification of benthic dinoflagellates. The election of one single macroalgal species would be more convenient for the best comparison among different sampling sites. However, the high variability in the abundances obtained in different macroalgae which makes difficult the election of a single ideal species.

The use of fiberglass screens for sampling BHABs has advantages relative to natural substrate methods: it ensures statistically robust results, may be used in contrasting habitats independent of local flora, and allows comparison among different studies. These results add to previous studies, and contribute towards the long awaited standardization of BHAB sampling methods. In this way, it provides a reliable quantification method for its use in different studies and BHABs monitoring programs worldwide.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-107

Diversity of Epibenthic Dinoflagellates from Brazilian Waters

S. M. Nascimento [1,*], R. Neves [1], C. Silva [1], C. Ferreira [2], G. de'Carli [1], B. Diniz [1], T. Cockell [1], Á. Moraes [1], T. Santiago [1], R. Silva [1], G. Borsato [1], V. Chiapetta [3], F. Oliveira [3], F. Salgueiro [3]

[1]Departamento de Ecologia e Recursos Marinhos, Universidade Federal do Estado do Rio de Janeiro, [2]Departamento de Biologia Marinha, Universidade Federal Fluminense, [3]Departamento de Botânica, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: Harmful events associated with benthic microalgae (BHABs) have been reported more frequently over the last decade. Outbreaks of benthic dinoflagellates are of particular concern because many species are able to produce potent biotoxins that can cause ecological impacts and may constitute a human health threat. Of particular concern are the outbreaks of *Gambierdiscus*, the source of toxins related to ciguatera fish poisoning (CFP) and *Ostreopsis*. Other benthic dinoflagellate species of the genera *Prorocentrum* and *Coolia* can produce a diverse array of toxins. In Brazil studies on benthic dinoflagellates started in the last decade. The country has a coastline that expands from 03º 50' N to 33º 45' S mostly on the tropical climate and with diverse coastal environments with suitable substrates for benthic microalgae to colonize, such as sandy beaches, mangroves, algae and coral reefs, mud flats, bays, coastal lagoons and rocky shores. In this study the diversity of epibenthic dinoflagellates of the genera *Gambierdiscus*, *Ostreopsis*, *Prorocentrum* and *Coolia* from Brazilian waters will be presented. Macroalgae samples were collected from ten sampling stations along the Brazilian coastline and three oceanic islands (St Paul´s Rocks, Trindade and Fernando de Noronha) located between latitudes 3°50' S and 22°58' S. Stations along the Rio de Janeiro coastline were visited monthly during three years while stations along the northeastern Brazilian coast and oceanic islands were visited less frequently. Macroalgae were vigorously shaken to dislodge epiphytic microalgae and the epiphyte solution was preserved with neutral lugol iodine solution. Dinoflagellate species were identified using optical, fluorescence and scanning electron microcopies. Cell abundance of each species or genus was determined from each site using sedimentation chambers or Sedgewick-Rafter counting chambers and observation in light microscopy. Dinoflagellate cells were isolated and laboratory cultures were established. Morphological and genetic analysis of isolated strains was performed. The species found include *Ostreopsis* cf. *ovata*, *Prorocentrum lima*, *Prorocentrum caipirignum*, *Prorocentrum hoffmaniannum*, *Prorocentrum mexicanum*, *Prorocentrum emarginatum*, *Prorocentrum borbonicum*, *Gambierdiscus excentricus*, *Gambierdiscus silvae*, *Gambierdiscus belizeanus*, *Gambierdiscus carolinianus*, *Fukuyoa* sp., *Coolia malayensis* and *Coolia tropicalis*. Abundance values of each genus in each sampling site will be presented. *Ostreopsis* cf. *ovata* was found in bloom abundances at Rio de Janeiro, St. Paul Rocks and Forte beach, Bahia. The study revealed a high diversity of epibenthic dinoflagellates in Brazil and highlights the need of studying the potential ecological impacts of these harmful species in coastal and oceanic food webs as well as the potential threats to human health.

Disclosure of Interest: None Declared

Taxonomy

O-108

The use of High-Throughput Sequencing in the study of the diversity of toxigenic cyanobacteria

N. Salmaso [1,*], L. Cerasino [1], S. Martens [1]
[1]Research and Innovation Centre, Fondazione Edmund Mach, S. Michele all'Adige, Italy

Abstract: The study of cyanobacterial diversity in aquatic environments has been traditionally carried out through the microscopic examination of samples and the use of molecular techniques based on culture-dependent approaches. A correct identification of cyanobacteria is of the utmost importance, due to the ability of these organisms to produce a wide variety of toxins. Nonetheless, in environmental studies the traditional approaches have many limitations, because they underestimate the number of cyanobacterial taxa, whereas identifications might be affected by taxonomic ambiguities. This is especially true in the detection of rarest or smaller individuals. This issue in only partially softened by the use of traditional and expensive and time-consuming cultivation-independent approaches, such as gel electrophoresis based methods, CARD-FISH, and clone and sequencing. This situation has radically changed with the advent of new cultivation-independent high-throughput sequencing (HTS) technologies, which opened new perspectives in the evaluation of both biological and functional diversity. In this contribution, the application of marker gene amplification metagenomics in the study of cyanobacterial diversity will be evaluated with practical examples. A recent study carried out in Lake Garda highlighted pros and cons of this approach based on the amplification of 16S rRNA genes. Results obtained from two years investigations allowed discovering a wide cyanobacterial diversity. The most abundant OTUs identified coincided with the most abundant and larger taxa quantified using traditional microscopic and molecular approaches (e.g. the toxic *Tychonema bourrellyi* and *Planktothrix rubescens*, and non-toxic *Dolichospermum lemmermannii*), as well as metabolomic profiling of cyanotoxins. In addition, HTS identified many other abundant but smaller Synechococcales and Chroococcales, along with other rare Nostocales and the new non-photosynthetic cyanobacterial groups ML635J-21 and Melainabacteria, never identified so far in the large lakes south of the Alps. The potential of these techniques to unravel the bacterial and metabolomic diversity in non axenic cultures will be further evaluated considering a few approaches used to study algal inocula for photo-bioreactors (project H2020-MSCA-RISE AlgaeCeuticals). The application of marker gene amplification is not free of complications, e.g. due to the short length of the most used 16S rRNA gene sequences obtained with Illumina MiSeq technologies, the scarsity of information available in reference databases for other molecular markers, and limits inherent to the most common bioinformatic pipelines. The use of less widespread approaches, such as full shotgun sequencing, represents powerful complementary tools to disentangle biodiversity and functions of cyanobacteria and plankton.

Disclosure of Interest: None Declared

Taxonomy

O-109

Towards a stable classification of Cyanobacteria?

M. Gugger [1,*]

[1]Head of Laboratory, Institut Pasteur, Paris, France

Abstract: Among Bacteria, the Cyanobacteria constitute a robust monophyletic group of oxygenic phototrophic prokaryotes. They exhibit a wide range of morphological features and colonize strikingly different ecosystems. Consequently, they also differ greatly with respect to physiological traits. Their traditional classification is mainly based on morphological characteristics, which is insufficient to deduce the true diversity of these organisms. On the other hand, the global picture of cyanobacterial interrelationships inferred from analyses of complete or partial 16S rDNA sequences is still very incomplete, and most of the data has been obtained on isolates for which little or no physiological and genetic information is available, or on environmental samples restricted to very specific ecosystems.

The extensive genomic data now available for oceanic and marine cyanobacteria of the genera *Prochlorococcus* and *Synechococcus* clearly revealed the coherence of several internal subgroups in this clade, in agreement with the physiological and ecological diversity of their representatives. In contrast, the limited sampling of other cyanobacterial groups for genome sequencing projects still severely hinders our understanding of the evolution in this phylum as a whole.

In order better illustrate the genetic diversity of Cyanobacteria, we sequence the genomes of the Pasteur Culture Collection of Cyanobacteria (PCC). Applying phylogeny on genomic and proteomic datasets, we delineate stable clades, not challenged anymore by sequence added. The data obtained provide a new framework for the improvement of an urgently needed modern classification of the Cyanobacteria, and help to choose appropriate reference candidates.

Disclosure of Interest: None Declared

Taxonomy

O-110

Phylogenomic diversity and co-dominance of Arthrospira fusiformis (Cyanobacteria) and Picocystis salinarum (Chlorophyta) in a soda lake

C. Bernard [1,*], M. Cellamare [1 2], C. Djediat [1 3], M. Hugoni [4], A. Escalas [1], H. Agogué [5], C. Carré [6], N. Touibi [1], C. Duval [1], N. Villeriot [1 6], P. Got [6], G. Sarazin [7], D. Jézéquel [7], C. Leboulanger [6], M. Ader [7], M. Troussellier [6]

[1]UMR 7245 Molécules de Communication et Adaptations des Microorganismes, Muséum National d'Histoire Naturelle, [2]Phyto-Quality, [3]Platform of electronic microscopy, Muséum National d'Histoire Naturelle, Paris, [4]UMR 5557 Ecologie Microbienne, Université Lyon 1 - CNRS - INRA, Villeurbanne, [5]UMR 7266 LIENSs , Université de La Rochelle – CNRS, La Rochelle, [6]UMR 9190 MARBEC, CNRS-Université de Montpellier -IRD - IFREMER, Montpellier, [7]UMR 7154 Institut de Physique du Globe, Sorbonne Paris Cité, Univ. Paris Diderot, Paris, France

Abstract: Lake Dziani Dzaha (Mayotte) is a lake, where water column environmental conditions are highly contrasted (e.g. oxic *vs* anoxic, chlorophyll a from 6 to 800 µg L^{-1}, salinity from 35 to 70 psu and H_2S concentration from 10 to 2000 µM*)*. The diversity and dynamics of phytoplanktonic communities from the Lake Dziani Dzaha water column were investigated using environmental metabarcoding approaches and strains isolation. From this extreme environment, we isolated 55 cyanobacterial and microalgal strains. All the strains were taxonomically characterized using polyphasic approaches and we described two new genera and four new cyanobacteria species belonging to the orders Oscillatoriales (*Desertifilum dzianense* sp. nov.) and Synechococcales (*Sodalinema komarekii* gen. nov., sp. nov., *Sodaleptolyngbya stromatolitii* gen. nov., sp. nov. and *Haloleptolyngbya elongata* sp. nov.). These new taxa have been further used as taxonomic references. High-throughput sequencing showed that among the 455 bacterial, cyanobacterial and eukaryotic OTUs retrieved from 16S rRNA genes analysis (18,747,809 sequences), only 15 OTUs were affiliated to cyanobacteria or microalgae. Among the latter, two hyper-dominant phylotypes were recorded: *Arthrospira fusiformis*, straight, large filamentous cyanobacteria (99.99% of cyanobacterial sequences) and *Picocystis salinarum* a small picoeukaryotic Chlorophyta (99.99 % of photosynthetic eukaryotes sequences). The vertical distribution of these two co-dominant taxa from the surface to the bottom of the water column was modelled using cell abundance. The models revealed that below the surface layer, *A. fusiformis* and *P. salinarum* abundances dropped under the simultaneous influence of salinity increase, anoxia and high concentrations of H_2S and NH_4^+. Our results provide a new support to the species coexistence theory and highlight the different adaptations that these two very distinct taxa, both from a phylogenetic and functional point of view, may use to fulfil the extreme environmental niches they encountered in this lake.

Disclosure of Interest: None Declared

Taxonomy

O-111

An undescribed kareniacean dinoflagellate harboring peridinin-type chloroplast with an eyespot

K. Takahashi [1,*], G. Benico [2], W. M. Lum [2], M. Iwataki [1]

[1]Asian Natural Environmental Science Center, [2]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

Abstract: In unarmored dinoflagellates of the family Kareniaceae, all reported species possessed chloroplasts that contain 19'-acyloxyfucoxanthins (19'-AFs) and lack peridinin. A culture strain of an undescribed kareniacean dinoflagellate was established from Japanese coast, and cells were examined by LM, SEM, TEM and HPLC. Phylogeny of the host was inferred from nucleus-encoded rDNAs (SSU rDNA, ITS and LSU rDNA), and the chloroplast from chloroplast-encoded *psbA* and *psbC* sequences. Under LM, cells were 5.9–9.5 µm long, with the smaller epicone. Nucleus was located in the hypocone and a chloroplast was positioned periphery in the hypocone. An eyespot, which has not been reported in any described kareniaceans, was observed in the sulcal region. SEM showed the straight apical groove that resembles to those in *Karenia* and *Karlodinium*. Under TEM, the number of chloroplast enveloping membranes was usually two, unlike the common three membranes found in the peridinin-type. An eyespot was composed of a single layer of osmiophilic globules in chloroplast (type A). HPLC revealed the main pigment was peridinin, with no trace of 19'-AFs found in kareniaceans. Phylogenetic trees based on rDNAs showed core position of this species in the Kareniaceae, but not included within clades of any described genera (*Karenia*, *Karlodinium* and *Takayama*). Chloroplast *psbA* and *psbC* phylogeny demonstrated that chloroplast of this species was derived from a peridinin-type chloroplast of dinoflagellate, whereas 19'-AF-type of other kareniaceans originated from a single endosymbiotic event of a haptophyte. These results suggested the chloroplast of this undescribed kareniacean was replaced from 19'-AF-type to peridinin-type.

Disclosure of Interest: None Declared

Taxonomy

O-112

Characterizing the raphidophyte, Chattonella sp. associated with massive fish mortality on the east coast of Peninsular Malaysia

C. P. Leaw [1,*], W. L. S. Lau [1], I. K. Law [1], H. C. Lim [2], S. T. Teng [3], S. C. Y. Leong [4], K. Takahasi [5], M. Iwataki [5], H. Gu [6], P. T. Lim [1]

[1]Bachok Marine Research Station, Institute of Ocean and Earth Sciences, University of Malaya, Bachok, [2]Tunku Abdul Rahman University College, 85000 Segamat, [3]Department of Aquatic Science, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia, [4]Tropical Marine Science Institute, National University of Singapore, Kent Ridge Road, Singapore, [5]Asian natural Environmental Science Center, The University of Tokyo, Tokyo, Japan, [6]Third Institute of Oceanography, Xiamen, China

Abstract: Blooms of raphydophyte *Chattonella* spp. that often linked to massive fish kills, are not unusual in the Southeast Asian region. In 2016, a fish kill event along the northeastern coast of Peninsular Malaysia was found concomitantly with the high densities of *Chattonella* in the water. Dead fishes collected were observed and Chattonella cells were found accumulated in the fish gills and gut. The cell density of *Chattonella* up to 143,000 cells L^{-1} was observed in the water samples collected. Live samples were used for microscopic observations and culture establishment. Morphological characteristics of the specimen were observed based on cell shape, sizes and chloroplast arrangement. Under LM observation, cells appear ovoid-shaped, with round anterior and pointed posterior ends, sometimes appear globular. It has smaller cell sizes as compared to *C. marina* var. *antiqua* but similar to that of *C. subsalsa*. Sequences of the large subunit ribosomal RNA gene (LSU-rDNA) and the internal transcribed spacer (ITS) were obtained by single-cell PCR and used for phylogenetic analyses. The phylogenetic inferences revealed a distinct lineage with some degree of genetic heterogeneity, the ribotype forms a sister clade with its sister species, *C. marina* and *C. subsalsa*.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

O-113

The REPHY monitoring network in France: thirty years of data on phytoplankton, associated physico-chemistry, and phycotoxins

C. BELIN [1,*], M. LEMOINE [1], D. SOUDANT [1]

[1]Ifremer, 44311 NANTES cedex 03, France

Abstract: Monitoring of phytoplankton and phycotoxins has been carried out on the French coast since 1984 by the REPHY network implemented by Ifremer. The data acquired has been banked since 1987 in a national database, which ensures its durability, homogeneity and consistency with international standards such as WoRMS for taxonomic data. Thirty years of data are available, including counts of all phytoplankton taxa which can be recognized by light microscopy, physicochemical measurements, and quantification of toxins belonging to the three families present in French sea shellfish (DSP-diarrheic, PSP-paralytic, ASP-amnesic). An optimization of monitoring conducted in recent years has resulted in a major restructuring of REPHY in 2016, with emphasis on the observation part and the preservation of long-term data sets. The new monitoring pattern, now separated into REPHY (phytoplankton and physical chemistry) and REPHYTOX (phycotoxins) will be briefly described. The scientific interest of these thirty years of data, and their contribution to the improvement of knowledge will then be approached and illustrated with the most convincing examples. For phytoplankton, the treatment of the data according to different points of view made it possible to identify the emblematic species for each of the three parts of the French coast (Channel, Atlantic, Mediterranean). These points of view were: (i) taxa that have regularly made blooms at high or very high concentrations, (ii) taxa considered as "dominant" in the sense of the dominance indices used in ecology, (iii) the results of the assessment carried out under the European WFD (Water Framework Directive) for biomass and phytoplankton abundance, (iv) toxic or harmful species, with a focus on the three main groups of species producing toxins. For physico-chemistry, the temperature data acquired over the last thirty years clearly show the trace of climate change, highlighted by the modeling according to a dynamic linear model (DLM). Finally, the history of toxicity episodes in sea shellfish has made it possible to identify characteristics that are very specific to each of the three families of toxins: (i) lipophilic toxins (DSP) affect numerous areas and diversified shellfish every year on the whole coast, (ii) PSP toxins affect a few areas some years, (iii) ASP toxins affect mainly scallops in a number of areas quite variable from one year to another.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

O-114

Ichthyotoxic skeleton-forming silicoflagellates in British Columbia, Canada; Data from the Harmful Algae Monitoring Program, 1999–2017

N. Haigh [1,*], T. Brown [1], D. Johnson [1]
[1]Microthalassia Consultants Inc, Nanaimo, Canada

Abstract: The silicoflagellates *Octactis speculum* and *Dictyocha fibula* are regular components of the phytoplankton community in British Columbia (BC) waters (46-76% of monitored weeks), with *O. speculum* being particularly prevalent throughout the year. Data from the BC salmon aquaculture industry's Harmful Algae Monitoring Program indicate blooms of both *O. speculum* and *D. fibula* cause mortalities of farmed salmon, including Atlantic (*Salmo salar*) and Chinook (*Oncorhynchus tshawytscha*) salmon. Fish-kills occurred with cell counts of 300 – 500 cells mL^{-1} of either species of algae. Blooms of *O. speculum* were more limited in duration and extent than those of *D. fibula*. *O. speculum* blooms were most often observed in the spring (April to June), whereas *D. fibula* blooms occurred in summer to early autumn (July to September). *O. speculum* was generally observed in the skeletal form, but *D. fibula* cells observed were predominantly the non-skeletal form, with intermediate and skeletal forms more common later in the blooms. Not all thick blooms were associated with fish kills; mortalities were more often observed when significant concentrations of the algae were seen throughout the water column (surface to 15m depth) than with surface blooms only. In all reported fish kill events dissolved oxygen levels were not limiting, and pathological signs in salmon mortalities were consistent with a toxic mechanism. Significant bloom years for *O. speculum* during this period were 2004, 2005, 2012, and 2013; significant bloom years for *D. fibula* were 2001, 2003, 2016, and 2017.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

O-115

HAB monitoring in Okhotsk Sea in Japan by the metagenetic approach

S. Nagai [1,*], N. Nishi [2], Y. Kawakami [2], S. Sildever [1], A. Shiomoto [3], S. Katakura [4]

[1]National Research Institute of Fisheries Science, Japan Fisheries Research and Education Agency, Yokohama,Kanagawa Pref., [2]AXIOHELIX Co. Ltd., Tokyo, [3]Bioindustry, Tokyo University of Agriculture, Setagaya-ku, Tokyo, [4]City of Mombetsu , Hokkaido, Japan

Abstract: Next generation sequencing technologies have made it possible to obtain millions of sequence reads in a single experiment, and massively parallel sequencing (MPS) is a currently revolutionizing survey of eukaryotic diversity including HAB species. This technology enables us to detect several hundreds of operational taxonomic units (OTUs) of eukaryotes from seawater samples and facilitates the detection of low-abundance populations in complex eukaryote communities. We have employed the MPS-based technology for HAB monitoring such as the genus *Alexandrium*, *Chattonella*, *Karenia*, *Pseudo-nitzschia* mainly in Japanese coastal waters. The detection sensitivity of MPS-based technology was evaluated with two different techniques, i.e. a multiplex-PCR and a DNA chromatography chip, using natural plankton assemblages. There was no significant difference in the detection sensitivity among the three techniques, suggesting MPS-based technology can be readily used to monitor the HAB species.

The eastern part of Hokkaido facing the Okhotsk Sea, Japan is where the coldest water (−2°C) flows in from Sakhalin in winter. Therefore, the perturbations in the marine ecosystem have been considered as an environmental sensor, and Mombetsu city is especially recognized as the most important monitoring base for observing plankton biodiversity in the coldest waters of Japan. To study the relationship between the change in biodiversity and global warming, weekly monitoring has been carried out using MPS-based technology in the Okhotsk Sea at one location off the coast of Mombetsu city from Apr 2012 to Mar 2017 (n = 265). As the result of metagenetic analysis using amplicon-seqs of 18S-rRNA gene (V7-9 regions), we detected 3,577 OTUs. We succeeded in identifying 849 species, i.e. 96, 80, 126, 184, 26, 185, and 152 species in Dinophyceae, Ciliophora, Bacillariophyceae, Metazoa, Rhizaria, other microalgae, and other eukaryotes, respectively. The frequency of OTUs and MPS, which were identified as a single species, were 11.9–41.2% and 30.9–70.4%, respectively. The taxonomic identification power was high for Archaeplastida, Opisthokonta, and Viridiplantae, but relatively low for Alveolata and Rhizaria. Surprisingly, we detected a number of HAB species such as the genera *Chattonella*, *Cochlodinium*, *Karenia*, *Karlodinium*, *Pseudo-chattonella*, and *Takayama* in Northeastern Hokkaido coastal area. All these are the new records of the detection there. The relative abundances of these HAB species showed clear seasonal patterns. For example, *K. mikimotoi* was detected from March and November, reflecting the appearance patterns in Kyushu region and the long transfer of the populations by Tsushima warm current. Currently, we are analyzing the amplicon-seqs of 28S-rRNA gene to demonstrate the more data at the conference.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

O-116

Regional changes in harmful algal events in the North Atlantic area over the last two decades documented using the HAEDAT database

E. Bresnan [1,*], P. Andersen [2], D. Anderson [3], S. Angles [4], L. Arin [4], C. Belin [5], M. Branco [6], A. Cembella [7], K. Davidson [8], M. Delgado [4], M. De Rijke [9], J. Diogene [10], W. Eikrem [11], L. Fernandez [12], R. Fernandez [12], M. Fernandez-Tejedor [10], E. Garces [4], M. Gordillo [12], H. Guðfinnsson [13], D. Jean [12], B. Karlson [14], J. Kobos [15], A. Kremp [16], D. Kulis [3], M. Lemione [5], L. Maman [12], H. Mazur-Marzec [15], C. McKenzie [17], A. McKinney [18], S. Milligan [19], L. J. Naustvoll [20], A. Ocana [4], Y. Pazos [21], M. Poelman [22], P. Provoost [23], B. Reguera [24], N. Sampedro [4], R. Siano [25], J. Silke [26], A. Silva [6], M. Vila [4], L. Viure [4], H. Enevoldsen [27]

[1]Marine Scotland Science, Aberdeen, United Kingdom, [2]NIRAS, Aarhus, Denmark, [3]Wood's Hole Oceanographic Institute (WHOI), Wood's Hole, USA, [4]Institut de Ciències del Mar, Barcelona, Spain, [5]Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), Nantes, France, [6]Instituto Português do Mar e da Atmosfera, (IPMA), Lisbon , Portugal, [7]Alfred Wegner Institute for Polar and Marine Research (AWI), Bremerhaven, Germany, [8]Scottish Association for Marine Science, Oban, United Kingdom, [9]Vlaams Instituut voor de Zee vzw Flanders Marine Institute, Ostend, Belgium, [10]IRTA, Sant Carles, Spain, [11]Norwegian Institute for Water Research, Oslo, Norway, [12]Laboratorio de Control de Calidad de los Recursos Pesqueros, Cartaya, Spain, [13]Marine and Freshwater Research Institute, Reykjavík, Iceland, [14]Swedish Meterological and Hydrological Institute (SMHI), Gothenburg, Sweden, [15]Institute of Oceanography, Gdynia, Poland, [16]Leibnitz Institute for Baltic Sea Research Warnemuende, Roshtock, Germany, [17]Department of Fisheries and Oceans Canada, Newfoundland, Canada, [18]Agri-Food and Biosciences Institute, Belfast, [19]Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Lowestoft, United Kingdom, [20]Institute of Marine Research, Flødevigen, Norway, [21]Instituto Tecnoloxio Para o Control do Medio Marina de Galicia (INTECMAR), Galicia, Spain, [22]Institute for Marine Resources and Ecosystem Studies (IMARES), Yerseke, Netherlands, [23]International Oceanographic Data and Information Exchange (IODE), Ostend, Belgium, [24]Instituto Español de Oceanografia (IEO), Vigo, Spain, [25]Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), Brest, France, [26]Marine Institute, Galway, Ireland, [27]IOC Science and Communication Centre on Harmful Algae, Copenhagen, Denmark

Abstract: The International Council for the Exploration of the Sea (ICES) - Intergovernmental Oceanographic Commission of UNESCO (IOC) Working Group on Harmful Algal Bloom Dynamics (WGHABD) has entered data into the Harmful Algal Event (HAEDAT) database for the last 20 years. These entries report information about harmful algal events that result in management actions such as closures of shellfish harvesting areas, have a negative impact on human health (e.g. illness) or the environment (e.g. fish kills, mortalities of marine mammals). These data, collected mainly from routine monitoring programmes, provide a wealth of information that is not routinely accessible for scientific assessment. WGHABD is producing a status report based on the incidence of HAB events in the ICES area which will contribute to a Global HAB Status Report. These HAEDAT data reveal a regional distribution in harmful algal events in the North Atlantic area. On the east coast of the USA and Canada, the majority of issues have been caused by paralytic and amnesic shellfish toxins (PST, AST). In contrast, diarrhetic shellfish toxins (DST) has been the dominant cause of problems in Europe while cyanobacteria events were largely restricted to the Baltic. HAEDAT also provides information about changes in the incidence of harmful algal events over time, e.g. the recent increased recording of DST events in the USA, a reduction in the incidence of PST closures in Scottish waters at the beginning of this century. HAEDAT is an essential source of information to support investigation of environmental drivers such as climate change on the incidence of harmful algal events on a regional scale.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

O-117

Analysis of Phytoplankton bloom phenology by identification of gaussian mixture in the southern bight of the North Sea (1989-2014).

A. Lefebvre [1,*], E. Poisson Caillault [2]

[1]ODE/UL, IFREMER, Boulogne sur Mer, [2]LISIC, Universté du Littoral Côte d'Opale, Calais, France

Abstract: Phytoplankton sampled weekly within the IGA monitoring programme since 1989, correspond to one of the longest time series in France. This time series is significant to further knowledge on phytoplankton bloom dynamics in the context of the regional massive outburst of the *Prymnesiophyceae Phaeocystis globosa*.

We provide a four steps innovative method of missing data completion and classification applied to *Chlorophyll-a (Chl-a) time* series in order to define various typology of algae blooms and their phenology. These schemes are then analysed according to taxonomic composition.

First step corresponds to data pre-processing: data validation, time shifting and missing data completion using fuzzy logic.

Second step consists of identifying characteristic frequencies for multi-scale modelling. In our *Chl-a* case study, conventional approaches like correlation techniques, trend or mode decomposition allow extracting main cycles when considering phytoplankton blooms. Nevertheless, they are ineffective for analysis at small scales, closer to the real temporal dynamics of phytoplankton blooms. Spectral classification is used to identify sets of yearly shape-profiles.

Third step concerns the identification of recurrent or rare events that look like an asymmetric bell-shaped curve. As first study, we consider these events as Gauss curve. So, an algorithm to estimate Gaussian mixture is proposed to model seasonal series and each yearly *Chl-a* series. A comparison between model from the seasonal decomposition and obtained models per year show an important variability of the number of Gaussians, of their shapes and also of their dates of beginning and end of the bloom, duration.

Fourth step is the correspondence analysis between obtained Gauss curves and the associated phytoplankton composition. Three Gaussians are characterized by one to three taxa (>95% of abundance) and a dominance of *Phaeocystis Globosa*. Other Gaussians are relative to *Bacillariophyceae* dynamics.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-118

Gambierdiscus and Ciguatera Poisoning: A Retrospective to Inform the Future

P. Tester [1,*]

[1]Ocean Tester, Beaufort, USA

Abstract: From the earliest reports by the surgeon's mate on the HMS Resolution in 1774 to the 2017 review on the clinical, epidemiological, environmental and public health management of Ciguatera Fish Poisoning (CFP), this malady has largely been considered the potential cost of eating fish from tropical regions. However, within the last decade the intensity and quality of *Gambierdiscus* and CFP related research has provided excellent tools for the detection of species and the discovery new ones, improved toxin detection and quantification techniques and established standardized sampling protocols. Too, progress has been made on understanding species specific habitat requirements and how this can help inform monitoring programs. Ciguatera poisoning has attracted the attention of the World Health Organization and Food and Agriculture Organization of the United Nations and activities are underway to include ciguatoxins in the Codex Alimentarius.

Monitoring programs have been established in some CFP indigenous regions while implementation plans are being developed for *Gambierdiscus* cell abundance or ciguatoxin testing in other areas. Too, important progress is being made on one of the major impediments to testing programs, that of affordable ciguatoxin standards. Yet, before we congratulate ourselves and pronounce "mission accomplished" we must consider the effectiveness of the transfer of techniques, methods, and protocols to those who are responsible for implementing and managing monitoring programs. No single "model" surveillance program will suffice for the needs of all CFP endemic areas or regions of emerging CFP risk. So the continued collaboration and cooperation of national and international researchers, health officials, resource managers and seafood providers are essential for the effective management of CFP.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-119

Habitat complexity affects benthic harmful dinoflagellate assemblages in the fringing reef of Rawa Island, Malaysia

P. T. Lim [1,*], H. L. Yong [1], L. K. Lee [1], Z. F. Lim [1], N. I. Mustapa [1], T. H. Tan [1], G. Usup [2], H. Gu [3], W. R. Litaker [4], P. Tester [5], C. P. Leaw [1]

[1]Bachok Marine Research Station, Institute of Ocean and Earth Sciences, University of Malaya, Bachok, [2]Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Malaysia, [3]Third Institute of Oceanography, Xiamen, China, [4]National Ocean Service, National Centers for Coastal Ocean Science, Center for Coastal Fisheries and Habitat Research, national Oceanic and Atmospheric Adminstration, Beaufort, [5]Ocean Tester LLC, 381 Glillikin Road, Beaufort, NC28516, USA

Abstract: Benthic harmful Algal blooms (BHABs) have been the focus of research owing to the increased incidence of ciguatera fish poisoning (CFP) worldwide. To determine how microhabitats of coral reefs affect the distribution and abundance of *Gambierdicus* and other major BHAB dinoflagellates, a field survey was undertaken between April 2015 and May 2017 on a shallow fringing reef flat of Rawa Island (Terengganu, Malaysia), an island with no CFP record. A sampling of benthic dinoflagellates was carried out by an artificial substrate sampling method utilizing fiberglass screens. Five taxonomic groups of benthic harmful dinoflagellates, *Ostreopsis*, *Gambierdiscus*, *Prorocentrum*, *Amphidinium*, and *Coolia* were identified, and cells were enumerated under a light microscope. Microhabitats of the studied reef area were characterized simultaneously based on the bottom substrate types. The results showed that *Gambierdiscus* was less abundant than the other genera throughout the study period, with maximum abundance of 1.2×10^3 cells 100 cm^{-2}. The abundance of *Gambierdiscus* reported in this study was at least a magnitude lower than areas with known CFP cases. This implied low CFP risk in the study area. Higher cell abundances were observed in reefs with high turf algal cover and coral rubble. *Ostreopsis* was found in high abundance up to 3.4×10^4 cells 100 cm^{-2} in habitats with high coral cover, but no blooms or “bottom mat” was observed in this study. Microhabitat heterogeneity was identified as a key factor governing the benthic harmful dinoflagellate assemblage. The results will serve as a fundamental basis of consideration in BHAB monitoring strategies in potential CFP prone areas.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-120

What we have learned from the challenges of assessing ciguatoxin risk in South Eastern Australia

H. Farrell [1,*], S. Murray [2], A. Edwards [1], A. Zammit [1]

[1]NSW Food Authority, Newington, Australia, [2]Climate Change Cluster (C3), University of Technology Sydney, Sydney, Australia

Abstract: There is growing global awareness of ciguatera fish poisoning (CFP), particularly in areas not previously affected by the disease. Incidence of CFP in New South Wales, Australia appears to be increasing. Since 2014 we have investigated six incidents of CFP linked to Spanish Mackerel caught in NSW and, since 2015, four incidents of CFP linked to imported fish. Across these ten incidents, forty-one individuals were affected by the illness. Prior to this, reports of CFP in NSW were few. The two potential sources of ciguatoxin contaminated fish, either from imported or local catch, present similar challenges for seafood safety risk management strategies. The central issue, in Australia and worldwide, is the limited capacity for routinely testing ciguatoxins in seafood. There is also concern regarding misdiagnosis of CFP, given the complexity of CFP symptoms. As CFP risk management is currently restricted to fishing bans and awareness campaigns for fishers and consumers, we used the NSW investigations and associated data from those affected by CFP in the region to develop practical guidelines for consumers to reduce their potential exposure to ciguatoxins and to improve surveillance methodologies to increase our understanding of the disease.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-121

Diversity and habitat associations of *Gambierdiscus* and *Fukuyoa* spp. in the Pacific Island nation of Tonga

P. Argyle[1 2 3,*], K. Smith[3], T. Halafihi[4], L. Rhodes[3], T. Harwood[5], I. Marsden[2], T. Halafihi[6]

[1]University of Technology Sydney, Sydney, Australia, [2]University of Canterbury, Christchurch, [3]Cawthron Institute, Nelson, New Zealand, [4]Ministry of Fisheries , Nuku'alofa, Tonga, [5]Cawthon Institute, Nelson, New Zealand, [6]Ministry of Fisheries, Nukuʻalofa, Tonga

Abstract: This study is the first ecological study of *Gambierdiscus* spp. in Tonga, a Pacific Island nation where ciguatera fish poisoning is commonly reported. This study examined the distribution and abundance of the genus across a wide diversity of coastal tropical habitats. Habitat surveys were conducted at 15 sites across Tongatapu, Ha’apai, and Vava’u island groups to characterise seagrass, macro-algae, and coral species assemblages. Micro-algae were collected at the same sites using artificial substrates deployed for 24 hours. The most common habitat were dominated by seagrass and mixed macro-algae. Other habitat types included *Halimeda* dominant, algal turf dominant, and highly turbid lagoons. A DNA meta-barcoding approach was used to identify *Gambierdiscus* and *Fukuyoa* species present. One *Fukuyoa* and six *Gambierdiscus* species in total were identified, with at least one of these present at 14 of the 15 sites. *G. pacificus* was the most frequently observed species, at 11 sites, and *G. carpenteri* was found at 9 sites. Recently described species *G. chelonii* and *G. honu* were found and 6 and 5 sites respectively. The least common were *G. australes* and *G. polynesiensis* (2 sites each). The highest diversity of species was found at sites in Ha’apai, where ciguatera is most commonly reported. Corresponding microscope counts also identified high concentrations of *Gambierdiscus* cells at all Ha’apai sites, but also in 2 Vava’u sites. This study shows that mixed communities of *Gambierdiscus* and *Fukuyoa* spp. are not restricted to specific habitat types and are present in high and low energy coastal sites around Tonga. This study forms a baseline for future work and management of these toxic species.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-122

Water quality, *Gambierdiscus* species, ciguatoxins and maitotoxins in lagoon fish in a ciguatera hotspot, Rarotonga, Cook Islands

S. Murray [1,*], K. Smith [2], L. Rhodes [2], B. Curley [3], A. Verma [1], G. Kohli [1], A. L. Kretzschmar [1], T. Harwood [2], S. Murray [2], T. Rongo [4]

[1]University of Technology Sydney, Sydney, Australia, [2]Cawthron Institute, Nelson, New Zealand, [3]Department of Primary Industries, Sydney, Australia, [4]Climate Change Division, Rarotonga, Cook Islands

Abstract: The Cook Islands is estimated to have the world's highest per capita incidence of Ciguatera Fish Poisoning (CFP). Recent research has shown a marked correlation between CFP incidences and disturbances to coral reefs in the region. In this study, we examined water quality variables, and conducted a study of the distribution of ~8 species of *Gambierdiscus*, as measured using metabarcoding of regions of ribosomal RNA and qPCR, and CTX and MTX levels in a small herbivorous fish, maito (*Ctenochaetus striatus*), with a home range localised to the area around the sampling site. We cultured species of *Gambierdiscus* from the area and examined them for known CTX and MTXs using LCMS. We found that *Gambierdiscus polynesiensis* was the only species that produced known CTX analogs, while other species produced MTXs. The abundance of *G. polynesiensis* differed amongst sites, as did the amount of P-CTX-3B in maito and other fish. This study shows that CTXs in lagoon fish continue to pose a health risk in the Cook Islands, and that new methods of monitoring may allow for more targeted CFP prevention strategies.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-123

Contribution of marine invertebrates to Ciguatera

H. T. Darius[1,*], M. Roué[2], M. Sibat[3], J. Viallon[1], C. M. I. Gatti[1], M. W. Vandersea[4], P. A. Tester[5], R. W. Litaker[4], Z. Amzil[3], P. Hess[3], M. Chinain[1]

[1]Insitut Louis Malardé - UMR 241 EIO, [2]Institut de Recherche pour le Développement - UMR 241 EIO, Papeete, French Polynesia, [3]Phycotoxins Laboratory, Ifremer, Nantes, France, [4]Centers for Coastal Ocean Science, National Oceanic and Atmospheric Administration, [5]Ocean Tester, Beaufort, NC, USA

Abstract: Ciguatera Fish Poisoning (CFP), the most prominent non-bacterial seafood poisoning worldwide, is widespread in tropical and subtropical regions, and results from the consumption of coral reef fish contaminated with ciguatoxins (CTXs) produced by *Gambierdiscus* spp. While residents in endemic areas are familiar with CFP symptoms after ingesting ciguatoxic fish, they are often unaware that marine invertebrates also bioaccumulate these potent marine biotoxins and can pose a similar health hazard. Indeed, over the past decade, marine invertebrates such as *Tridacna maxima* (bivalve mollusk), *Tectus niloticus* (gastropod) and *Tripneustes gratilla* (sea urchin) have been repeatedly involved in “ciguatera-like” episodes in French Polynesia, New Caledonia and the Cook Islands. All patients experienced severe gastrointestinal, neurological and cardiovascular disorders, typical of ciguatera. This type of poisoning was also distinguishable by its unusual severity and the rapid onset of symptoms.

Since marine invertebrates constitute a valuable source of protein and revenue for the communities of Pacific Island Countries and Territories it is important to document these atypical forms of ciguatera. *Tectus niloticus* and *Tripneustes gratilla* specimens were collected from Nuku Hiva Island (Marquesas archipelago, French Polynesia), extracted and analyzed using the neuroblastoma cell based assay (CBA-N2a) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Several known CTXs analogs were detected in toxic samples, i.e. the predominantly P-CTX3B, followed by P-CTX3C and to a lesser extent, P-CTX4A and P-CTX4B, all known to be directly produced by *Gambierdiscus* spp. This suggests that these dinoflagellates are the likely source of CTXs detected in these organisms. Sea urchins also contained a significant amount of 51-OH-P-CTX3C, an analog considered to be favored through metabolization in the trophic food web. These findings are also consistent with observations in *T. maxima* experimentally fed *Gambierdiscus polynesiensis,* showing bioaccumulation of P-CTX3B and P-CTX3C in tissues of these giant clams (viscera, flesh, and mantle).

Given that most CFP monitoring programs usually rely on the survey of *Gambierdiscus* cell densities and species composition and/or the monitoring of CTXs in fish, these findings point to the importance of also taking into account the toxicity of marine invertebrates which can represent a significant risk for human health in CFP prone areas.

Disclosure of Interest: None Declared

Taxonomy

O-124

Genetic diversity, distribution, and DSP toxin production of *Prorocentrum caipirignum* and *P. lima* complex in Japanese coastal areas

T. Nishimura [1], H. Uchida [2], T. Suzuki [2], H. Funaki [3], S. Arimitsu [1], C. Ihara [1], K. Hagino [1], K. Hashimoto [1], K. Tanaka [1], I. Yanagida [4], M. Adachi [1,*]

[1]Kochi University, Nankoku, [2]National Research Institute of Fisheries Science, Yokohama, [3]Ehime University, Matsuyama, [4]NPO INO, Okinawa, Japan

Abstract: Among the benthic species of the genus *Prorocentrum* (class Dinophyceae), *P. caipirignum* and *P. lima* complex are known as the main species which produce diarrhetic shellfish toxins (DSTs). Although molecular phylogenetic analyses based on rDNA have been conducted to classify these species, the phylogeny, distribution, and DST production of these species from the Japanese coastal areas have not been well characterized to date. In this study, the phylogeny, distribution, and DST production of *P. caipirignum* and *P. lima* complex from Japanese coastal areas were investigated. Between 2014 and 2017, macroalgal samples were collected at 27 sites (subboreal, temperate, and subtropical areas, <3–30 m depths), and 244 clonal strains were established from 13 sites (temperate and subtropical areas). Phylogenetic analysis based on the LSU rDNA D1/D2 region revealed that the Japanese strains could be separated into 4 distinct groups: *P. caipirignum* (number of strains [n] = 39); *P. lima* complex (n = 203); a new group *Prorocentrum* sp. type 1 (n = 1), which is a sister taxon to the *P. caipirignum*, *P.* cf. *lima*, and *P. hoffmannianum* complex groups; and *Prorocentrum* sp. type 2 (n = 1), which is a sister taxon to the *P. caipirignum* and *P.* cf. *lima* groups. The Japanese strains of the *P. lima* complex could be separated into 5 clades: 1, 2, 3, 4, and 6. Furthermore, the clades 1 and 6 could be separated into 6 subclades (1a, 1c, 1d, 1e, 1f, and 1g) and 3 subclades (6a, 6b, and 6c), respectively. The Japanese strains of *P. caipirignum* were separated into 2 subclades: b and e. The distributions of these clades were related to the sampling regions and depths, e.g., *P. lima* complex clades 1e and 2 were distributed in temperate areas, whereas *P. lima* complex clade 3 was distributed in subtropical areas. The production of DSTs by all the strains at a stationary phase was assessed by LC/MS/MS analysis with the exception of a single strain of *Prorocentrum* sp. type 1. The results revealed that all strains produced okadaic acid (OA), and among them, the OA contents per cell of *P. caipirignum* subclades b (28.2 ± 9.40 pg/cell) and e (22.4 ± 13.5 pg/cell) and *P. lima* complex clade 3 (18.8 ± 10.8 pg/cell) were higher than those of other clades/subclades. The strains of *P. lima* complex clades 1a, 1e, 2, and 3 produced dinophysistoxin 1 (DTX1) and OA. DTX1 contents per cell of *P. lima* complex clades 1e (15.5 ± 9.28 pg/cell) and 2 (12.8 ± 11.0 pg/cell) were higher than those of other clades/subclades ($p < 0.05$).

Disclosure of Interest: None Declared

Taxonomy

O-125

Semiautonomous, image-based identification of *Gambierdiscus* spp.

C. E. Tibirica [1,*], I. Leite [1], L. Fernandes [2], L. Mafra Jr. [1]
[1]Centro de Estudos do Mar, UFPR/Brazil, Pontal do Paraná, [2]Departamento de Botânica, UFPR/Brazil, Curitiba, Brazil

Abstract: Species identification can be especially challenging for some genera of toxic dinoflagellates, frequently requiring the aid of experienced taxonomists and/or molecular tools. The size, shape and distribution pattern of thecal plates may be a useful feature for species identification within thecate genera, including *Gambierdiscus* spp. However, with new species being constantly described, using characteristics of only one or a few thecal plates for microscopic identification can be ambiguous and lead to frequent taxonomic misidentification. In order to make *Gambierdiscus* taxonomy easier, we developed a database that compares multiple proportions between the lengths of thecal plate sutures among different *Gambierdiscus* species. This first Excel-based version of the database includes suture proportions from 49 apical and 47 antapical pictures available in 13 scientific papers, from all 15 *Gambierdiscus* species described so far. From each analyzed cell, we considered up to 52 proportions that were easier to obtain by image analysis software, ignoring the curved thecal plates near the sulcus. As the database only uses proportions between the size of adjacent sutures rather than absolute length values, no scaling is necessary, thus eliminating problems related to the high cell size variability present in the genus. The application of the database includes three simple steps: (i) obtaining a good-quality (showing as much thecal plate sutures as possible) scanning electron or epifluorescent microscopy picture from apical or antapical view, avoiding sloping cells; (ii) exporting the picture to an image analysis software and measuring the length of thecal plates sutures; and (iii) transferring the measurements to an answering sheet – the automated database interface. The database instantaneously compares the proportions measured in the unidentified *Gambierdiscus* cell with the mean proportions already recorded for all species, calculating the absolute differences among them. As a result, dissimilarity indices are shown for all candidate species, with the lowest indices corresponding to the most likely *Gambierdiscus* species. We tested the method using 94 pictures from either apical or antapical views of previously identified (in scientific papers) *Gambierdiscus* cells, and the identification provided by the answering sheet was correct in 86% of the time. Using composed specimens, with pictures from both sides of the cell, only 2 out of 42 cells were misidentified. Most of the mistakes were due to the shortage of measurable suture in the analyzed pictures, or because the number of measurements composing the database is still insufficient for some species. This method can be greatly improved by adding new pictures from known *Gambierdiscus* species, and can be also adapted for other thecate dinoflagellate genera.

Disclosure of Interest: None Declared

Taxonomy

O-126

Genetic relatedness of Japanese isolates of *Alexandrium ostenfeldii* with global isolates

S. Sildever [1,*], J. Jerney [2], A. Kremp [9], H. Oikawa [1], S. Sakamoto [3], M. Yamaguchi [4], K. Baba [5], A. Mori [6], L. Mackenzie [7], D. Anderson [8], S. Nagai [1]

[1]National Research Institute of Fisheries Science, Yokohama, Japan, [2]Marine Research Centre, Finnish Environment Institute, Helsinki, Finland, [3]National Research Institute of Fisheries and Environment of Inland Sea, Hatsukaichi, [4]Kitazato University, Sagamihara, [5]Hakodate Fisheries Research Institute, Hokkaido Research Organization, Hakodate, [6]Tottori Prefectural Institute of Public Health and Environmental Sciences, Tottori, Japan, [7]Cawthron Institute, Nelson, New Zealand, [8]Woods Hole Oceanographic Institution, Woods Hole, USA, 9Leibnitz Institute for Baltic Sea Research Warnemuende, Roshtock, Germany

Abstract: *Alexandrium ostenfeldii* (Paulsen) Balech and Tangen, 1985, is a globally distributed marine planktonic dinoflagellate that produces three different types of neurotoxins. Dense blooms of *A. ostenfeldii* have been reported from coastal areas around the world, including from Japan. In the northern part of Japan, the species blooms every year, whereas in western Japan, *A. ostenfeldii* blooms were first recorded in 2013 in a small harbor located in an estuary. In a previous phylogenetic analysis, the *A. ostenfeldii* strains from northern Japan grouped separately from strains originating from other geographical locations. Our aim is to further investigate the phylogenetic relationship between *A. ostenfeldii* strains from different geographical origins by adding new strains from Japan and from the Baltic Sea. We sequenced the ITS and LSU (D1D2) and compared the results with other *A. ostenfeldii* isolates around the world by constructing phylogenetic trees. For strains originating from northern Japan (Funka Bay, Hokkaido) no identical sequences were found from Genebank and the sequence similarity with the most similar sequence was only 98% (684/696 bp) in LSU (as *A. peruvianum*, JF921196) and 94 % (576/610 bp) in ITS (as *A. peruvianum*, JX113683). However, the sequences obtained from the Funka Bay strains isolated in different years were identical, suggesting the settlement of locally adapted and genetically unique population. Two different ITS sequences were obtained from the brackish population of western Japan. One sequence was similar to a strain from China (521/522 bp as *A. ostenfeldii*, JN173268), and the other matched completely with the strain from the Baltic Sea (541/541 bp as *A. ostenfeldii*, KT732904). This suggests that the *A. ostenfeldii* from the western part of Japan is transported there from China either by ships' ballast water or by currents. It has been suggested that the *A. ostenfeldii* strain isolated from a Chinese seaport located in an estuary was transported there via ships' ballast water from the Baltic Sea. The origin of the strains from northern Japan is still unknown. Further analyses based on the full length LSU and microsatellites will be carried out by using more strains.

Disclosure of Interest: None Declared

Taxonomy

O-127

Sympatric occurrence of two *Azadinium poporum* ribotypes in the Eastern Mediterranean Sea

H. Gu [1,*], Z. Luo [1], B. Krock [2], L. Wang [1], A. Giannakourou [3], A. Venetsanopoulou [3], K. Pagou [3], U. Tillmann [2]
[1]Third Institute of Oceanography, Xiamen, China, [2]Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, [3]Hellenic Center for Marine Research, Institute of Oceanography, Attica, Greece

Abstract: The marine dinoflagellate Azadinium poporum produce azaspiracids (AZA) and has been recorded widely in the world. However, information on its biogeography is still limited, especially in view of the fact that A. poporum comprises several genetically differentiated groups. A total of 18 strains of A. poporum were obtained from the Eastern Mediterranean area by incubating surface sediment collected from Ionian Sea of Greece. The morphology of these strains was examined with light microscopy and scanning electron microscopy. Small subunit ribosomal DNA (SSU rDNA), large subunit ribosomal DNA (LSU rDNA) and internal transcribed spacer (ITS) sequences were obtained from all cultured strains. Molecular phylogeny based on concatenated SSU, LSU and ITS sequences confirmed three ribotypes within A. poporum and revealed two subclades within ribotypes A and C. Greek strains of A. poporum ribotype A were nested within ribotype A2 together with strains from Western Mediterranean Sea and French Atlantic, and Greek strains of A. poporum ribotype C were nested within ribotype C2 together with a strain from the Gulf of Mexico. Growth experiments on four selected strains revealed that ribotypes A and C from Greece differed in their growth rates in response to temperature, indicating that they are physiologically differentiated. Azaspiracid profiles were analyzed for 15 cultured A. poporum strains using LC-MS/MS and demonstrate that the A. poporum ribotype A from Greece produce low level or no AZA and A. poporum ribotype C from Greece produces predominantly AZA-40 (9.6-30.2 fg cell-1) followed by AZA-2 (2.1-2.6 fg cell-1). The first record of AZA-40 producing A. poporum from the Mediterranean suggests that this species is a potential source for azaspiracid contaminations in shellfish from the Eastern Mediterranean Sea.

Disclosure of Interest: None Declared

Taxonomy

O-128

Morphological, molecular and toxicological data on *Ostreospsis* cf. *siamensis* (Dinophyceae): proposal of *Ostreopsis rinoi* sp. nov.

H. David [1 2,*], M. Santos [1 3], A. Laza-Martínez [4], M. F. Caeiro [2 5], C. Dell'Aversano [6], A. Penna [7], A. Amorim [1 2]

[1]MARE, [2]Department of Plant Biology, University of Lisbon, [3]IPMA, Lisbon, Portugal, [4]Department of Plant Biology and Ecology, University of the Basque Country (UPV/EHU), Leioa, Spain, [5]CESAM, University of Lisbon, Lisbon, Portugal, [6]Department of Pharmacy, University of Naples Federico II, Naples, [7]Department of Biomolecular Sciences, University of Urbino, Pesaro, Italy

Abstract: *Ostreopsis*, a toxic genus of epibenthic dinoflagellates, includes 11 currently accepted nominal species. Nevertheless, species delimitation in this genus has always been controversial. The original description of *O. siamensis*, the type species of the genus, was based only on morphological characters. However, the (semi)cryptic nature of the *Ostreopsis* species, makes the assignation of a specimen to a certain species based only on morphological features complicate. Lately, phylogenetic, morphological and chemical analyses combined, have allowed to solve certain taxonomical aspects of this genus leading to the description of new *Ostreopsis* species. Molecular studies recently performed in the type locality of *O. siamensis*, the Gulf of Thailand, have confirmed that the clade, so far known as *O*. cf. *siamensis,* is not present in the tropical area. In fact, the *O*. cf. *siamensis* clade includes ribosomal sequences from the Atlantic Iberian Peninsula, Mediterranean Sea, New Zealand and Australia. The Atlantic Iberian coast is known to have at least two species of the genus *Ostreopsis,* as *O.* cf. *siamensis* and *O.* cf. *ovata*, but, the most common is *O*. cf. *siamensis* for its distribution and frequency of occurrence. In recent years, several samples have been collected in the Iberian area and a total of 19 strains of *O*. cf. *siamensis* were established. Given the absence of this clade in the type locality of *O. siamensis,* a new species should be assigned to this clade. In this study, we propose to redescribe the ribotype known as *O*. cf. *siamensis* as *Ostreopsis rinoi* based on previous studies and new morphological, molecular and toxicology data.

Disclosure of Interest: None Declared

Taxonomy

O-129

Ostreopsis **cf.** ***ovata*** **and** ***Ostreopsis lenticularis*** **in the Galápagos Islands**

O. Carnicer[1,*], M. García-Altares[2], I. Keith[3], Y. Okolodkov[4], K. B. Andree[5], J. Diogène[5], M. Fernández-Tejedor[5]

[1]Escuela de Gestión Ambiental, Pontificia Universidad Católica del Ecuador, sede Esmeraldas, Esmeraldas, Ecuador, [2]Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany, [3]Charles Darwin Foundation, Isla Santa Cruz, Gálapagos, Ecuador, [4]Laboratorio de Botánica Marina y Planctología, Instituto de Ciencias Marinas y Pesquerías, Universidad Veracruzana, Veracruz, Mexico, [5]Institut de Recerca i Tecnologia Agroalimentària, IRTA, Sant Carles de la Ràpita, Tarragona, Spain

Abstract: Toxic benthic dinoflagellates and in particular *Ostreopsis* species are known for their implication in seafood poisoning and for their negative impacts in coastal marine ecosystems. In the last decades, the use of molecular techniques has revealed new cryptic species impossible to discriminate with light and scanning electron microscopy. Our expanding knowledge on the specific diversity of *Ostreopsis* requires a fast adaptation of their taxonomy. In that sense, *Ostreopsis* populations from remote areas, such as the Galapagos Islands, not studied before could provide critical data as they are renowned for their unique biological diversity and high level of endemism. The present study was undertaken to characterise the morphology, molecular biology and toxinology of *Ostreopsis* strains found in the area. Samples were collected from macroalgae and bottom invertebrates at three sites of two southern islands in the Galapagos Marine Reserve (Santa Cruz and Santa Fé) on April 6 and May 29, 2017. *Ostreopsis* was dominant in 10 samples of macrophytes, the maximum abundances were found on *Dictyopteris* sp. (33,405 cells/g wet weight). Three different morphotypes based on dorsoventral diameter (DV) and width (W) were distinguished in field samples and a total of 16 strains were isolated. The PCR amplifications of 5.8S and ITS regions clustered 13 strains in *O*. cf. *ovata* Atlantic/Indian/Pacific clade (DV = 44.73 ± 5.62 µm; W = 32.32 ± 5.35 µm, n = 477), and three strains in *O. sp 5* (DV = 88.49 ±7.22 µm; W = 67.29 ± 6.1 µm, n = 61). The presence of two types of thecal pores (large pores 0.20-0.35 µm, and small thecal pores 0.04-0.12 µm) as a discriminative morphological feature of O. *lenticularis* allowed us to identify the latter, although in the published literature it has been reported also for *O. ovata* and *O. siamensis*, which raises a question about its value for species identification. During the analysis of SEM micrographs, the emphasis was put on the morphology of the 1', 3' and 1p plates. The strains proved to be non-toxic according to the haemolytic assay, which was supported by the absence of palytoxin-like compounds, known or unknown, in the analysis by LC-HRMS. These results represent the first evidence of *Ostreopsis* in the Galapagos Islands and highlight the importance of taxonomic revision of *O. lenticularis* since it belongs to two genetic clades.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

O-130

Cyanobacterial community and a description of the cases of the Huff disease on Lake Kotokel located near Lake Baikal

Z. Namsaraev [1,*], A. Nedoluzhko [1], F. Sharko [2], A. Melnikova [1], A. Komova [1], D. Tsyrenova [3], D. Barkhutova [3]
[1]NRC "Kurchatov Institute", [2]Research Center of Biotechnology RAS, Moscow, [3]Institute of General and Experimental Biology SB RAS, Ulan-Ude, Russian Federation

Abstract: In the period from 2008 to 2011 in the Republic of Buryatia (Russia), there were 18 cases of poisoning after eating thermally processed fish caught in the lake Kotokel. The patients were diagnosed with alimentary-toxic paroxysmal myoglobinuria (Haff disease). One person died of acute kidney failure - myoglobinuric nephrosis.

Haff disease is a disorder caused by injury to skeletal muscle that results in the release of muscle cells. Liver, kidney and nervous system also affected. The first outbreak was recognized in 1924 in Germany. Also, outbreaks were registered in Sweden, the US and the USSR.

Lake Kotokel (52.8112 N, 108.2047 E) is located one kilometer from the eastern shore of Lake Baikal, which contains 20% of the world's fresh water. Lake Kotokel has a size of 15 by 6 km and a depth of 4 meters. This lake is a popular holiday destination for the local population due to the higher water temperature compared to Lake Baikal. The lake is eutrophic with a chlorophyll concentration of up to 172 mg/m3 and a total phosphorus content of up to 0.037 mg/l. The plankton in various years was dominated by cyanobacteria of the genera *Aphanocapsa, Woronichinia, Anabaena, Microcystis*, as well as colonial green algae. In addition, the hepatotoxin Microcystin-LR was detected in phytoplankton biomass in the lake and its outlet by HPLC. However, the presence of microcystin does not explain the observed clinical status of the people.

We collected samples of water from the lake Kotokel in summer 2017 and performed preliminary 16S and 18S rRNA gene sequencing analysis for three of them using Illumina MiSeq. RNA which was extracted from one of these samples was sequenced using the same technology. The bioinformatics analysis showed that the highest number of sequences belonged to OTUs which included cultured representatives of the *Cyanobium sp.*, *Anabaena circinalis, Aphanizomenon flos-aquae* and *Microcystis aeruginosa*. The analysis of RNA sequences showed a presence of genes encoding several types of cyanotoxins: microcystin, nodularin, and saxitoxin.

Since Lake Kotokel is connected to Lake Baikal by a river, there is a risk of spreading potentially toxic algae species from Lake Kotokel to warm lagoons and bays on the eastern shore of Lake Baikal.

The work was supported by the Russian Foundation for Basic Research (grant #17-29-05103).

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

O-131

Increasing occurrence of the proliferations of toxic benthic cyanobacteria in French rivers, a state of knowledge

I. Echenique-Subiabre [1 2], K. Tambosco [3 4], C. Durimel [2], J.-F. Humbert [4], C. Quiblier [2 3,*]

[1]GEMA Center for Genomics, Universidad Mayor. Camino La Pirámide, Santiago, Chile, [2]National Museum of Natural History, [3]University Paris Diderot, [4]Institute of Ecology and Environmental Sciences, UPMC, Paris, France

Abstract: If a large amount of work has been done on planktonic cyanobacteria and on their potential toxicity in the past three decades, only few studies have investigated the proliferations of benthic cyanobacteria, which are also potentially toxic. Indeed, the accidental ingestion of benthic mats has killed many terrestrial animals, in particular dogs, during the last decade. Most of these works dealing with benthic cyanobacteria have been performed in New Zealand, where many rivers host proliferations of these microorganisms. In France, almost all the recorded cases of dog intoxication caused by benthic cyanobacteria concerned the Tarn River since the beginning of the 2000's. Recently this phenomenon has been also observed in several new rivers in France and in the rest of the world, raising the issue of the increasing expansion of benthic cyanobacterial blooms and of the sanitary risks associated with these blooms.

Our presentation aims to report the state of knowledge concerning the environmental conditions allowing the development of *Phormidium* proliferations in French rivers and the variations in their potential toxicity. The presentation will be based on data acquired from (i) the monitoring during two summer seasons in the Tarn River, located in the South of France and (ii) preliminary studies performed on two rivers (Loire and Cher), located in the center of France where exceptional proliferations occurred in the summer of 2017. These last proliferations were associated with the mortality of at least 11 dogs.

The nature of the substrates covered by *Phormidium* biofilms, the morphologic and hydrodynamic characteristics (depth, flow velocity, temperature...) favorable for the development of these biofilms were very similar in the Tarn River compared to New Zealand rivers. On the other hand, in the Loire and the Cher rivers, *Phormidium* biofilms were found on very various substrates, including coarse sandy to gravely sediments but also macrophytes and were not limited to riffle areas, as found in the Tarn River and in New Zealand.

Concerning the potential toxicity of *Phormidium* biofilms, only anatoxin-a was detected in France with concentrations up to more than 200 $\mu g.g^{-1}$ of biofilm DW, while homoanatoxin-a was dominant in New Zealand. Finally, a marked temporal and spatial variability in the potential toxicity of biofilms from the Tarn River was observed, anatoxin-a being produced by mature biofilms at the end of summer, with the highest concentrations detected in the upstream part of the sampling area.

All these findings suggest that proliferations of toxic *Phormidium* biofilms leading to animal mortalities might potentially occur in a large range of rivers in France and elsewhere, and consequently a monitoring of these biofilms is needed in order to limit the risks for animal and human populations.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

O-132

Dog fatalities associated with tychoplanktic, anatoxin-a producing *Tychonema* sp. in a Berlin lake recovering from eutrophication

J. Fastner [1,*], C. Beulker [2], B. Geiser [3], A. Hoffmann [2], R. Kröger [4] on behalf of Water Hygiene & Environmental Health, K. Teske [5], L. Mundhenk [5], H. Neurath [6], D. Sagebiel [4] on behalf of Water Hygiene & Environmental Health, I. Chorus [1]

[1]German Environment Agency, [2]Berlin Brandenburg State Laboratory Abt. IV, [3]Nusshäherstr. 17A, [4]State Office for Health & Social Affairs (LAGeSo), [5]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Freie Universität Berlin, Berlin, [6]Toxicological Laboratory, Medical University Center, Göttingen, Germany

Abstract: In May 2017, at least 12 dogs showed signs of acute neurotoxicosis (and several dogs died) after swimming in or drinking from Lake Tegel, a mesotrophic lake in Berlin, Germany. Cyanobacterial surface blooms were absent, but detached and floating water moss (*Fontinalis antipyretica*) harbouring high amounts of *Tychonema* sp., a potential anatoxin-a (ATX) producing cyanobacterium, was found near the beaches where the dogs had been swimming and playing. Necropsies of two of the dogs revealed no specific lesions beside the anamnestic neurotoxicosis. ATX was detected in concentrations up to 8700 µg L^{-1} in the stomach contents, while other (neuro)toxic substances were not found. In the aqueous fraction of *Fontinalis*/*Tychonema* clumps sampled after the casualties, ATX was found in concentrations up to 1870 µg L^{-1}. This is the first report of a dense population of ATX producing *Tychonema* sp. in stands of *Fontinalis*. The findings emphasizes the need for further investigation of potentially toxic, non-bloom forming cyanobacteria in less eutrophic water bodies, but also present a challenge for appropriate surveillance of bathing sites.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

O-133

Novel DSP-Like Toxins Associated with a Bloom of *Dinophysis norvegica* in Maine, USA with Implications for Shellfish Management.

J. Deeds [1,*], J. MacLeod [2], A. Hamilton Vailea [2], B. Lewis [2], K. Kanwit [2], W. Stutts [1], P. McCarron [3], J. Borchert [4], S. Lankford [5], B.-T. Eberhart [6], C. Rauschenberg [7], C. Burnell [7], S. Archer [7]

[1]Center for Food Safety and Applied Nutrition, US Food and Drug Administration, College Park, [2]Maine Department of Natural Resources, West Boothbay Harbor, USA, [3]Institute for Marine Biosciences, National Research Council of Canada, Halifax, Canada, [4]Office of Environmental Health and Safety, Washington State Department of Health, Olympia, [5]Public Health Laboratories, Washington State Department of Health , Shoreline, [6]Northwest Fisheries Science Center, National Oceanic and Atmospheric Administration, Seattle, [7]Bigelow Analytical Services, Bigelow Laboratory for Ocean Sciences, East Boothbay Harbor, USA

Abstract: Shellfish harvesting closures due to Diarrhetic Shellfish Toxins (DSTs) in excess of the 0.16 ppm regulatory level are a relatively recent occurrence in the United States. The first such closure occurred in the state of Texas (Gulf of Mexico region) in 2008 due to a bloom of *Dinophysis ovum*. The first closure in the state of Washington (west coast Puget Sound region) occurred in 2011 due to a mixture of species, primarily *D. acuminata*. On the east coast of the US, *D. acuminata* has been documented in the Mid-Atlantic region with DSTs >0.16 ppm in shellfish (non-commercial) occurring only sporadically since 2011. To date, no shellfish harvesting closures have occurred in this region. In the east coast New England region, single shellfish harvesting closures have occurred in the states of Massachusetts in 2015, due to a bloom of *D. acuminata*, and in Maine in 2016, due to a bloom of *D. norvegica*. The 2016 harvesting closure in Maine was the result of a large monospecific bloom of *D. norvegica* that lasted from July 5 – August 29 and covered much of the central portion of the coast. During this time, multiple samples with cell concentrations >2,000 cells/L were observed with a maximum concentration of 54,300 cells/L recorded on July 17. Shellfish collected from several sites within the bloom area showed total DSTs >0.16 ppm OA eq. using a commercial protein phosphatase inhibition assay (PPIA) resulting in a shellfish harvesting closure on July 20, but confirmatory testing by liquid chromatography tandem mass spectrometry (LC-MS/MS) only confirmed trace concentrations of DTX-1, with no OA or DTX-2 detected. Testing in three additional laboratories using both methods provided the same results. Further LC-MS/MS testing for additional lipophilic shellfish toxins as well as high resolution LC-MS testing for known DST diol esters were negative. Testing for freshwater microcystins, also known to inhibit protein phosphatases, was also negative. Therefore, the harvesting closure was lifted on August 5 and the PPIA results were considered as false positives. We show here that subsequent testing of shellfish collected during this event, as well as a filtered water sample collected during the bloom, continued to show strong PPIA activity after both base hydrolysis and hexane washing, and results correlated to two different antibody-based commercial kits (a qualitative lateral flow kit and a quantitative ELISA) suggesting that the PPIA activity was due to the *D. norvegica* bloom and that DSP-like toxins, likely previously undescribed, were present. Bioassay guided purification is currently in progress to confirm this hypothesis and determine the identity of the responsible toxins. Findings from this study will have important implications for the management of DSP in the US, and possibly other regions globally, where *D. norvegica* occurs.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

O-134

An increase in the numbers of the genus Pseudo-nitzschia in the North Sea will have implications for the aquaculture industry

C. Whyte [1,*], K. Davidson [1], P. Tett [1], S. Swan [1], M. Edwards [2]
[1]SAMS, Oban, [2]Sir Alistair Hardy Foundation for Ocean Science, Plymouth, United Kingdom

Abstract: The plankton community index (PI(mp) is a quantitative method for evaluating changes in the community structure of phytoplankton communities using a state-space perspective. Here we report on a PI(mp) based evaluation of changes in the harmful genera Pseudo-nitzschia and Dinophysis within the North Sea using a long term dataset spanning the period 1975 to 2016 collected by the Sir Alistair Hardy Foundation for Ocean Science using the Continuous Plankton Recorder. Analysing the data with the PI (mp) we detected no evidence for a change in the numbers of *Dinophysis*. We did however find a significant increase in the numbers of *Pseudo-nitzschia*. This increase has ramifications for aquaculture around the North sea, both in terms of caged fish and shellfish as *Pseudo-nitzschia* can cause mechanical insult to fish gills and will intoxicate shellfish through the production of domoic acid, a cause of Amnesic shellfish Poisoning (ASP).

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

O-135

Comprehensive study of the occurrence and distribution of lipophilic marine toxins in shellfish from production areas in Chile

B. A. Suarez-Isla [1,*], F. Barrera [2], D. Carrasco [2], L. Cigarra [2], A. M. Lopez-Rivera [2], I. Rubilar [2], C. Alcayaga [2], V. Contreras [2], M. Seguel [3]

[1]Laboratory of Marine Toxins, Faculty of Medicine University of Chile, [2]Laboratory of Marine Toxins, Faculty of Medicine University of Chile, Santiago, [3]CERAM, Universidad Austral de Chile, Puerto Montt, Chile

Abstract: Lipophilic marine biotoxins accumulate in shellfish and can produce negative impacts in human health and local economies. In the southernmost regions of Aysén and Magallanes (44°-54°LS) persistent presence of *Dinophysis acuta* and accumulation of okadaic acid and dinophysistoxins in benthic shellfish have been reported since 1994. Concurrent blooms of a PSP producing species (*Alexandrium catenella*) have severely impacted local industries. In contrast, the contiguous Los Lagos Region (41°-43°30´LS) has been affected by separate PSP events during the summers of 2002, 2016 and 2018 and has developed as an important mussel aquaculture area with over 300,000 landed tons of mussels (*Mytilus chilensis*) in 2017.

Application of an UHPLC-MS/MS method in Los Lagos Region for the determination of lipophilic toxins (EURLMB SOP, 2015) as part of a national shellfish sanitation program, generated a detailed survey of their occurrence and distribution in over 112 sampling stations. In samples obtained from October 2015 to March 2018, toxins were detected in 10.1% of all samples (N = 922 of 9132). Yessotoxin accounted for 83.4% of all positive samples (769/922) and this correlated well with the abundance of *Protoceratium reticulatum* in some locations. Pectenotoxin-2 was detected in 13.6% of positive samples (125/922) and correlated with the presence of *Dinophysis acuminata*. Much fewer samples containing okadaic acid, dinophysistoxin-1 and -2 (1.5%; 14/922) and azaspiracid-1 and 2 (1.5%; 14/922) could be observed. Interestingly, all detected samples had toxin levels below the maximum permitted levels and no precautionary closures needed to be enforced.

Distribution analyses of phytoplankton abundance vs. No of detections of lipophilic toxins per sampling station and toxicity levels, showed a clear North-South latitudinal distribution gradient for *P. reticulatum* abundance and YTX levels. Maximum abundances of *P. reticulatum*, number of YTX detections and toxicity levels were consistently found in sampling stations to the north of the Los Lagos Region, in areas of significant shellfish production located near the town of Calbuco (41°30´). A similar latitudinal trend was observed for PTX2 and *D. acuminata*. In contrast, in aquaculture areas located in the central part of the Island of Chiloé (42°) that provide over 65% of landed tons of mussels per year, all parameters were much lower as compared to northern areas.

These results indicate that the Los Lagos Region is characterized by: a) a latitudinal asymmetric distribution of *P. reticulatum* and *D. acuminata*, b) a very low prevalence of diarrheagenic lipophilic toxins and their causative species and, c) low accumulation levels of these toxins in mussels and clams.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-136

Beyond the Transcriptomes: Biochemical and Proteomic Validation of the PKS Machinery Involved in Sterolysin Production.

A. R. Place [1,*], E. Williams [2], S. Haq [2], T. Bachvaroff [1]
[1]Professor, [2]UMCES@IMET, Baltimore, USA

Abstract: We have uncovered a single large transcript (>14Kb) with three consecutive KS modules, that are conserved across three species of dinoflagellates (*Karlodinium veneficum* and *Amphidinium carterae*, have known sterolysin toxins , and *Akashiwo sanguinea*, which does not produce a sterolysin), as well as many single module KS subunits using Illumina sequencing analysis. When comparing the three species, the acyl transferase (AT) subunit in the triple module KS was present only in the non-toxic species and missing in the toxin producing species. To test the functionality of PKS in these species, we added cerulenin, an inhibitor that covalently binds to the KS subunits to an exponential phase culture of *A. carterae* and *A. sanguinea*. Using ^{14}C labeled acetate and liquid chromatography mass spectrometry (LC/MS), we found that cerulenin inhibition resulted in the cessation of both fatty acid and sterolysin production. This shows KS plays a role in both fat and sterolysin synthesis leading us to our hypothesis: The triple module KS acts as a scaffold for both sterolysin and fatty acid production where the final product is mediated by accessory *trans*-AT subunits, which exist in parallel with the KS. To substantiate that the triple KS transcript produces a protein in *A. carterae,* we generated antibodies to three epitopes: KS1, KR2 and TE domain. Western analysis clearly finds evidence that a partial multi-module protein (~285,000 daltons, KR2 and TE domains) is expressed and is predicted to catalyze two carbon additions to a growing fatty acid/polyketide chain. However, an antibody to KS1 domain found a discrete protein containing the KS1 and KS2 domains but not the KS3 domain indicating post-translational processing. To further substantiate this triple module KS is involved in both sterolysin and fatty acid production we mapped the cerulenin modified residues using mass spectrometry. In *A. carterae*, there are 11 single *trans*-AT domain proteins which could provide the missing AT activity. We are currently investigating the interacting partners with this triple KS scaffold to determine how fatty acid and sterolysin are selected as final products.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-137

The biosynthesis of potent neurotoxin domoic acid in *Pseudo-nitzschia multiseries* diatoms

S. McKinnie [1,*], J. Brunson [1 2], J. Chekan [1], V. Bielinski [3], Z. Miles [1], A. Allen [2 4], B. Moore [1 5]
[1]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography at University of California, San Diego, [2]Microbial and Environmental Genomics Group, [3]Synthetic Biology and Bioenergy Group, J. Craig Venter Institute, [4]Integrative Oceanography Division, Scripps Institution of Oceanography at University of California, San Diego, [5]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, USA

Abstract: Oceanic harmful algal blooms of *Pseudo-nitzschia* diatoms produce the potent mammalian neurotoxin domoic acid (DA). Despite decades of research, the molecular basis for its biosynthesis is not known. By employing growth conditions known to induce production in *Pseudo-nitzschia multiseries*, we implemented transcriptome sequencing to identify DA biosynthesis genes that co-localize in a genomic four-gene cluster similar to a canonical prokaryotic biosynthetic gene cluster. We have heterologously expressed and biochemically interrogated the recombinant DA biosynthetic enzymes, identifying novel enzymology and chemistry. Moreover, their unique activities have successfully constructed the diagnostic pyrrolidine skeleton of DA from simple metabolic precursors, thereby establishing a model for DA biosynthesis. Knowledge of the genetic basis for toxin production provides an orthogonal approach to bloom monitoring and enables a greater understanding of the cellular mechanisms that drives oceanic DA production.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-138

Exploring domoic acid biosynthesis in a heterologous diatom host, *Phaeodactylum tricornutum*

J. K. Brunson [1 2,*], S. McKinnie [1], J. Chekan [1], V. Bielinski [3], A. Allen [1 2], B. Moore [1]

[1]Scripps Institution of Oceanography, UC San Diego, [2]Environmental Genomics, [3]Synthetic Biology, J. Craig Venter Institute, San Diego, USA

Abstract: Domoic acid (DA) is a potent neurotoxin produced by harmful algal blooms (HABs) of the diatom genus *Pseudo-nitzschia*. Until recently, the genetic components of DA biosynthesis remained undescribed, limiting our ability to understand the cellular mechanisms and compartmentalization of toxin production. Classic stable isotope labeling studies have suggested a compartmentalized biosynthesis, but the absence of a genetic model for biosynthesis has precluded further validation of this hypothesis. Our discovery of the DA biosynthetic (*dab*) genes enables us to apply them in a heterologous *in vivo* system to study toxin production under physiological conditions with a particular focus on subcellular compartmentalization. Here, we use an episomal expression system in the model pennate diatom *Phaeodactylum tricornutum* in order to further interrogate and characterize the *dab* genes. Our initial investigations have focused on DabA, which catalyzes the condensation of *N*-geranyl-L-glutamate from geranyl pyrophosphate and L-glutamate and represents the first committed step of DA biosynthesis. Preliminary results based on confocal fluorescence microscopy indicate that DabA enters periplastidic vesicular trafficking similar to many characterized chloroplast localized proteins. Further development of this system will allow us to more fully characterize the recently discovered DA biosynthetic steps, as well as those biological processes such as toxin export that remain to be described.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-139

TRANSCRIPTOMC AND PHYSIOLOGICAL RESPONSES OF *DOLICHOSPERMUM* SP. 54, AN ANATOXIN-PRODUCING CYANOBACTERIUM, TO DIFFERENT NUTRIENT REGIMES

B. J. Kramer [1,*], M. Harke [2], J. Jankowiak [3], M. Ladds [3], C. Gobler [3]

[1]Graduate Student, Stony Brook University, Southampton, [2]Lamont Doherty Earth Observatory, Columbia University, Palisades, [3]School of Marine and Atmospheric Sciences, Stony Brook University, Southampton, USA

Abstract: Anthropogenic activities that enrich freshwater ecosystems with nitrogen (N) and phosphorus (P) have been linked to the emergence of cyanobacterial blooms that deleteriously impact human and animal health. These trends have been largely observed in genera that produce the hepatotoxin microcystin, such as *Microcystis* and *Planktothrix*, yet comparatively less is known regarding how other groups of toxin-producing cyanobacteria, particularly diazotrophs such as *Dolichosperumum* (*Anabaena*) spp., respond to eutrophication. It has also been assumed that the ability of these cyanobacteria to fix dinitrogen (N_2) gas makes them strictly P-limited, although there is evidence to suggest that N_2-fixation is more metabolically demanding than use of exogenous N sources which may support more rapid growth and toxin production. Here we report on how the anatoxin-producing strain *Dolichospermum* sp. (strain 54) responds when grown under different nutrient regimes, particularly different species of nitrogen (NO_3^-, NH_4^+, or Urea) as well as the absence of nitrogen (NO_3^-) and/or phosphorus (PO_4^{3-}). Results indicated that *Dolichospermum* sp. (54) grew significantly faster with NH_4^+than without exogenous N. N_2-fixation rates were highest when grown on NO_3^- or without exogenous N. Interestingly, despite similar N_2-fixation rates between cultures treated with NO_3^- and PO_4^{3-} or neither, only in the latter treatment did *Dolichospermum* sp. (54) exhibit upregulation of certain genes (*nifB* and *nifK*) associated with N_2-fixation, whereas in the former treatment there was no differential expression of genes belonging to the *nif* cassette. Cellular anatoxin content (fg cell^{-1}) of *Dolichospermum* was highest when cultures were deprived of nitrogen and phosphorus, suggesting that the production of this neurotoxin is representative of a stress response. There were, however, no significant differences in the expression of all genes in the anatoxin synthetase cluster in any of the treatments, suggesting that toxin production was being post-transcriptionally regulated. Collectively, these data provide insight regarding how cyanobacterial diazotrophs form toxic blooms under different nutrient conditions. Future work will compare responses in microcystin producing diazotrophs to similar conditions.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-140

Gene expression in *Pseudo-nitzschia* reveals the methylerythriol phosphate metabolic pathway in the synthesis of domoic acid

S. Hardardottir [1,*], S. Wohlrab [2], D. Marie Hjort [3], B. Krock [4], T. Nielsen [5], U. John [4], N. Lundholm [1]

[1]Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark, [2]Helmholtz Centre for Polar and Marine Research, Alfred Wegener Institute, Bremenhafen, Germany, [3]The Geological Survey of Denmark and Greenland, Copenhagen, Denmark, [4]Alfred Wegener Institute, Bremenhafen, Germany, [5]National Institute of Aquatic Resources, Copenhagen, Denmark

Abstract: Even though it has been decades since the discovery of *Pseudo-nitzschia* as a source of the marine biotoxin domoic acid (DA), the biosynthetic pathway has only partly been revealed. Using functional genomic approaches we acquired information on genes involved in biosynthesis and regulatory functions for DA production in *P. seriata*. In our study, we used predator cues to induce DA production in P.seriata and compared the response with a control not exposed to predator cues, and a parallel study with a non-toxic diatom, *Fragilariopsis* sp. Over 1000 genes were differently expressed in P.seriata cells exposed to predator cues for two days compared to the control. The majority (>70%) of these genes were up regulated including several genes involved in major cellular processes and metabolism such as amino acid and carbohydrate metabolism as well as genetic information processing and environmental signal transduction mechanisms. Previous studies suggested that the cellular route for DA production is a fusion of two unknown precursors, one deriving from the isoprenoid pathway and the other from the citric acid cycle. We found a clear signal for the methylerythriol phosphate metabolic pathway to be the isoprenoid pathway for precursor synthesis of geranyl pyrophosphate to form DA. Six out of eight genes involved in this pathway were upregulated but none in the conventional mevalonate pathway. Fragilariopsis surprisingly did not change gene expression compared to control conditions. These findings indicate that the two species have evolutionary distinct strategies for coping with grazer threats and that only *Pseudo-nitzschia* cells receive and respond to the predator cues.

Disclosure of Interest: None Declared

HABs causing aerosol and/or direct contact toxicity

O-141

HABs causing aerosol and/or direct contact toxicity

E. Berdalet [1,*], L. Fleming [2], R. Lemée [3], M. Burford [4], L. Backer [5]

[1]Marine Biology and Oceanography, Institute of Marine Sciences (ICM-CSIC), Barcelona, Spain, [2]European Centre for Environment and Human Health, University of Exeter Medical School, Truro, Cornwall Tr1 3HD, United Kingdom, [3]Laboratoire d'Océanographie de Villefranche (LOV), UMR 7093, Sorbonne Université - CNRS, Villefranche-sur-mer, France, [4]Australian Rivers Institute, Griffith University, Nathan, Queensland, Australia, [5]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA , USA

Abstract: Human respiratory irritation associated with HABs was first documented by Taylor (1917) during his observations of *Karenia brevis* (formerly *Gymnodinium breve*) blooms in the west coast of Florida, also causing massive fish mortalities. Later, Woodcock (1948) provided the first evidences of the link between respiratory symptoms and aerosolized compounds produced by the dinoflagellate. In the last 30 years, concerted well-funded interdisciplinary and multi-agency research has been intensively conducted to understand *K. brevis* blooms (also know as Florida red tides) dynamics, and the exposure and health effects of aerosolized toxins (brevetoxins). Advances include early warning systems monitoring the presence of *Karenia brevis* and its bloom dynamics (combining *in situ* sampling, autonomous vehicles, remote sensing), a detailed knowledge on the involved toxins (mainly brevetoxins PbTx-2 and PbTx-3 in both water and aerosols) and their mode of action, the relevance of meteorology factors in the transport of aerosolized toxins, and a better understanding of the human exposures and health effects. At the same time, substantial work has gone into prevention of this exposure and health effects including community and expert education, beach warnings and involving Poison Control Centers.

In the last 20 years, a new environmental and human health threat seems to be emerging in temperate latitudes, and specially in Mediterranean coasts. Blooms of the marine benthic dinoflagellate genus *Ostreopsis* are linked to cutaneous and/or respiratory problems on beach goers and people that work or inhabit in areas where this species thrives, and were in contact with benthic or planktonic toxic cells and/or exposed for certain hours to aerosols. Although the impacts are mild by now, alarm has been raised because this species produces palytoxins (PLTX) and analogues, being PLTX potent biotoxins associated to fatal seafood borne intoxications in the tropics. Whether those toxins are at the origin of cutaneous and respiratory health problems is still unknown. Before *Ostreopsis* could constitute a bigger problem, multidisciplinary investigation has been and it is conducted nowadays by different research teams and a communication network has been implemented with environmental and public health authorities in the affected countries.

In this presentation we will compare the available information about the harmful benthic (*Ostreopsis* spp.) and planktonic (*Karenia brevis*) blooms, with the aim of identifying gaps in knowledge and further steps to progress and design appropriate human health prevention plans. Analogous comparison will also be done concerning freshwater toxic cyanobacteria blooms causing cutaneous and respiratory irritative symptoms.

Disclosure of Interest: None Declared

HABs causing aerosol and/or direct contact toxicity

O-142

Toxicity and Pathophysiology of Palytoxin Congeners after Intraperitoneal and Aerosol Administration in Rats

M. Poli [1,*], P. Ruiz-Olvera [1], A. Nalca [2], S. Ruiz [2], V. Livingston [2], O. Frick [2], D. Dyer [2], C. Schellhase [3], J. Raymond [3], D. Kulis [4], D. Anderson [4], S. McGrath [5], J. Deeds [5]

[1]Diagnostic Systems Division, [2]Aerobiology Division, [3]Pathology Division, USAMRIID, Frederick, [4]Biology Department, Woods Hole Oceanographic Institution, Woods Hole, [5]Center for Food Safety and Applied Nutrition, US FDA, College Park, USA

Abstract: Preparations of palytoxin (PLTX, derived from Japanese *Palythoa tuberculosa*) and the congeners 42-OH-PLTX (from Hawaiian *P. toxica*) and ovatoxin-a (isolated from a Japanese strain of *Ostreopsis ovata*), as well as a 50:50 mixture of PLTX and 42-OH-PLTX derived from Hawaiian *P. tuberculosa* were characterized as to their concentration, composition, *in-vitro* potency and interaction with an anti-PLTX monoclonal antibody (mAb), after which they were evaluated for lethality and pathophysiological effects by intraperitoneal (IP) and aerosol administration to rats. Once each preparation was characterized as to its toxin composition by LC-HRMS and normalized to a total PLTX/OVTX concentration using HPLC-UV, all four preparations showed similar potency towards mouse erythrocytes in the erythrocyte hemolysis assay and interactions with the anti-PLTX mAb. The IP LD_{50} values derived from these experiments (1-3 µg/kg for all) were consistent with published values, although some differences from the published literature were seen. The aerosol LD_{50} values (0.03-0.06 µg/kg) confirmed the exquisite potency of PLTX suggested by the literature. The pathophysiological effects of the different toxin preparations by IP and aerosol administration were similar, albeit with some differences. Most commonly affected tissues were the lungs, liver, heart, salivary glands, and adrenal glands. Despite some differences, these results suggest commonalities in potency and mechanism of action among these PLTX congeners.

Disclosure of Interest: None Declared

HABs causing aerosol and/or direct contact toxicity

O-143

An update on the *in vitro* toxicity and detection of ovatoxin-a

M. Pelin [1], S. Sosa [1], L. Tartaglione [2], G. Honsell [3], L. Fusco [1], M. Poli [4], M. Prato [5], C. Dell'Aversano [2], A. Tubaro [6,*]

[1]Department of Life Sciences, University of Trieste, Trieste, [2]Department of Pharmacy, University of Napoli "Federico II", Napoli, [3]Department of Agricultural and Environmental Sciences, University of Udine, Udine, Italy, [4]U.S. Army Medical Research Institute of Infectious Diseases, Ft Detrick, USA, [5]Department of Chemical and Pharmaceutical Sciences, University of Trieste, [6]Full Professor, Department of Life Sciences, University of Trieste, Trieste, Italy

Abstract: In the last two decades, *Ostreopsis* cf. *ovata* blooms have been recorded with increasing frequency and intensity along the Mediterranean coasts where they have been shown to produce palytoxin (PLTX)-like compounds, principally ovatoxin-a (OVTX-a). Simultaneously, episodes of adverse effects in humans (e.g. cough, fever, dyspnea, rhinorrhea and dermatitis) after inhalation and/or skin contact with marine aerosols or direct contact to seawater were also reported. However, to date no cases of human poisonings linked to OVTXs-contaminated seafood have been documented in this area. Considering the increasing sanitary problems ascribable to OVTXs during *Ostreopsis* blooms, development of rapid detection methods for risk assessment is of particular importance.

Due to the lack of sufficient toxin for *in vivo* studies, OVTX-a was evaluated for its potential toxicity *in vitro* using skin HaCaT keratinocytes, one of the most sensitive cell models to the reference toxin PLTX. OVTX-a was about 100-fold less potent than PLTX in reducing HaCaT cell viability (EC_{50}=1.1x10^{-9} M vs 1.8x10^{-11} M, MTT test), consistent with their binding affinities to these cells (K_d=1.2x10^{-9} vs 2.7x10^{-11} M, saturation experiments on intact cells). Accordingly, OVTX-a hemolytic activity was lower than that of PLTX (EC_{50}=3.4x10^{-8} M vs 5.9x10^{-9} M). Although OVTX-a cytotoxicity appears to be lower than that of the reference compound PLTX, its significant effect at nanomolar concentrations raises significant concerns for human health.

A sandwich immunoassay (ELISA) previously developed and characterized for PLTX, was assessed for its suitability to quantify OVTX-a. The assay was able to detect OVTX-a in a sensitive and accurate manner (Bias: 0.3%), in both *O.* cf. *ovata* extracts (LOQ = 9.6 ng/ml) and contaminated mussels (LOQ = 11 mg/kg). As such, it is a suitable screening method for OVTX-a detection within monitoring programs. The anti-PLTX antibodies used in the ELISA also allowed OVTX-a immunolocalization in *O.* cf. *ovata* samples. As expected, toxins were not detectable in the dinoflagellte *Coolia monotis,* frequently found associated with *Ostreopsis* blooms. To increase the sensitivity of the ELISA, an ultrasensitive electrochemiluminescence-based sensor for PLTXs detection was developed, taking advantage of the specificity provided by anti-PLTX antibodies, the good conductive properties of carbon nanotubes, and the excellent sensitivity achieved by a luminescence based transducer.

Disclosure of Interest: None Declared

HABs causing aerosol and/or direct contact toxicity

O-144

Mixotrophy of the toxic benthic dinoflagellate Ostreopsis

B. Lee [1,*], M. G. Park [1]

[1]Department of Oceanography, Chonnam National University, Gwangju, Republic of Korea

Abstract: Phagotrophy of the harmful benthic dinoflagellates of the genus *Ostreopsis* has long been inferred based on observations of food particles present inside the cell, but the prey was not identified yet. This study aimed to investigate seasonal dynamics of the benthic dinoflagellates *Ostreopsis* spp.in temperate Korean coastal sites, with special emphasis on their phagotrophy. To further identify the prey, the ingested food particles were extracted from single *Ostreopsis* cells and their *rbc*L and *psa*A genes sequences were determined. High concentration of *Ostreopsis* cells was observed between June and October at all sites, when water temperatures were higher than 19°C, exhibiting a marked temporal seasonality. The percentage of *Ostreopsis* cells containing the ingested food particles exhibited the large spatial and temporal variations among sampling sites, ranging from undetectable level to 29.5%, and was not always associated with *Ostreopsis* cell abundance. Phylogenetic analyses using both plastid-encoded *rbc*L and *psa*A genes revealed that all sequences obtained from the ingested food particles of *Ostreopsis* cells grouped within the class Florideophyceae, rhodophyta. Our result clearly demonstrates that *Ostreopsis* species consume various macroalgae from the rhodophyta, not protists which have long been thought to be potential prey. The result from this study provides a basis for better understanding mixotrophic behavior and nutritional ecology of the benthic harmful dinoflagellate *Ostreopsis* species.

Disclosure of Interest: None Declared

HABs causing aerosol and/or direct contact toxicity

O-145

Assessing human exposure to background levels of algal toxins via sea spray aerosols

M. De Rijcke [1,*], E. Van Acker [2], S. Huysman [3], M. Vandegehuchte [1], L. Vanhaecke [3], K. A. De Schamphelaere [2], C. R. Janssen [2]
[1]Flanders Marine Institute, Oostende, [2]Laboratory of Environmental Toxicology and Aquatic Ecology, Faculty of Bioscience Engineering, [3]Laboratory of Chemical Analysis, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium

Abstract: Airborne exposure of man to marine natural substances via sea spray aerosols (SSAs) is gaining attention. The inhalation of low concentrations of biogenic compounds was hypothesized to be a human health promoting mechanism[1] that contributes to the epidemiologically observed beneficial coastal health effect[2]. Due to their specific modes of action, phycotoxins like okadaic acid (OA) and yessotoxin (YTX) are potentially relevant effect chemicals within this so called “biogenics hypothesis”. However, these specific phycotoxins have never been observed in SSAs, nor was aerosolisation ever suggested. Brevetoxin (PbTx) and ovatoxin (OVTX) are known to aerosolize during severe blooms of *Karenia brevis* and *Ostreopsis ovata*. Under favorable aerosolisation conditions, high air concentrations of PbTx ($\leq$ 180 ng m^{-3})[3] and OVTX ($\leq$ 2 ng m^{-3})[4] are generated during blooms, often leading to respiratory distress in exposed coastal populations. These elevated aerial concentrations are exceptional. Background concentrations of toxins are presumably much lower, though the quantification of these low air concentrations is hampered by the analytical limits of detection. As a result, little is known about the potential for frequent exposure to low doses of aerosolized phycotoxins, as is required for the biogenics hypothesis

This study explores the potential for human exposure to low-level aerosolized phycotoxins. To this end, new analytical methods to simultaneously quantify multiple phycotoxins in SSAs, in both direct and indirect ways, were developed. SSA phycotoxin concentrations were analysed by combining two analytical techniques: ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS), and inductively coupled plasma optical emission spectrometry (ICP-OES). With these analytical techniques and the artificial production of SSAs using a marine aerosol reference tank[5], specific phycotoxins were not only detected and quantified for the first time in an (natural) aerosol phase, but the aerosolisation process itself was also studied. Using these newly developed methods in the field, the first data are now being collected to assess the environmental air concentrations of phycotoxins which are suggested as potential health-promoting chemicals within the biogenics hypothesis.

[1] M. N. Moore 2015. *Environ. Res.*, vol. 140, pp. 65–75.
[2] B. W. Wheeler *et al.* 2012. *Heal. Place*, vol. 18, no. 5, pp. 1198–1201.
[3] R. H. Pierce *et al.* 2003. *Bull. Environ. Contam. Toxicol.*, vol. 70, no. 1, pp. 161–165.
[4] P. Ciminiello *et al.* 2004. *Environ. Sci. Technol.*, vol. 48, no. 6, pp. 3532–3540.
[5] M. D. Stokes *et al.* 2013. *Atmos. Meas. Tech.*, vol. 6, no. 4, pp. 1085–1094.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-146

Spatial and temporal abundance of ichthyotoxic microalgae in Todos Santos Bay, Baja California, Mexico

M. Fimbres-Martínez [1,*], E. García-Mendoza [1], A. Olivos-Ortiz [2], D. A. Rivas-Camargo [1], J. Helenes-Escamilla [3]
[1]Biological Oceanography, Ensenada, [2]Universidad de Colima, Colima, [3]CICESE, Ensenada, Mexico

Abstract: Mortalities of cultivated fish associated to ichthyotoxic microalgae is one the main economically negative effects of harmful algae blooms (HABs). Ichthyotoxic microalgae produce hemolysins, hemagglutinins, polyunsaturated fatty acids (PFA) and reactive oxygen species (ROS) that damage the gills and provoke the death of the fish by suffocation. Species of Chattonella, a raphidophyte and the dinoflagellate Margalefidinium are ichthyotoxic microalgae associated with mortalities of wild and cultivated fish worldwide. In 2016 in Todos Santos Bay (TSB), Baja California, an algae bloom of Chattonella marina was present from June to September and another bloom of Margalefidinium c.f. fulvescens was detected in November. These blooms caused die-offs of cultivated blue fin tuna (Thunnus thynnus) affecting importantly the industry. HABs of ichthyotoxic have not been reported in the region and the factors that promote the increase in the abundance and distribution of C. marina and M. c.f fulvescens are unknown. Therefore, in this work we characterized the environmental and circulation conditions from June 2016 to Agust 2017 in the TSB region and was related to the abundance of ichthyotoxic species. Also, we present data of an oceanographic cruise realized in the region in March 2017. A bloom of M. c.f. fulvescens was detected on this month. We propose that the increase in C. marina abundance is related to an increase in surface temperature and stratification of the water column in the region associated with “El Nino/Southern Oscillation”. Conversely, M. c.f. fulvescens will predominate with “La Nina” conditions. This work is the first report of C. marina and M. c.f. fulvescens HABs in the region. We discuss the importance of the presence of abnormally environmental condition with the appearance of C. marina and M. c.f. fulvescens.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-147

Dinoflagellate adaptation to coastal ecosystem variations revealed through metabolomic analyses of revived strains

R. Siano[1,*], F. Mondeguer[2], M. Latimier[1], J. Quéré[1], M. Sibat[2], M. Le Gac[1], P. Hess[2]
[1]Ifremer DYNECO Pelagos, Brest, [2]Ifremer DYNECO Phyc, Nantes, France

Abstract: Dinoflagellate cyst may persist in marine sediments for decades and ancient phenotypes can be revived from living archives. It can be presumed that ancient cysts have preserved the vegetative form physiological traits fitting the time and ecosystem in which the species lived. Consequently, the adaptations of dinoflagellate populations to environmental changes could be inferred by comparing *in vitro* the physiology of modern and ancient strains revived from cysts. Over the last century, Brittany (NW Atlantic, France) coastal ecosystems experienced variations in nutrient ratios due to changes in agricultural politics and management. An increase in nutrient inputs was observed until the ’90s, when a mitigation strategy has been applied to limit the increasing eutrophication of coastal areas. Over this time, phytoplankton communities varied and harmful algal blooms events increased. Phosphorous was identified as a limiting factor for species and community development, while nitrogen is still at non-limiting concentrations.

Seeking for signs of dinoflagellates population adaptations to the environmental changes occurred in Brittany across the last century, we revived strains of the toxic dinoflagellate *Alexandrium minutum* and non-toxic species *Scrippsiella donghaienis* from up to ca. 20 and ca. 30 years-old sediments of the bays of Morlaix and Brest, respectively. The revived strains were exposed to culture conditions mimicking dystrophic conditions that populations could have experienced over the century. Non-axenic, batch cultures (3 strains of two ages for each of the 2 species) were established during 16 days. Physiological responses to phosphate (P) limitation were analyzed with a non-targeted metabolomics approach. Lipophilic and hydrophilic secondary metabolites were analyzed at 4 different growth phases.

Contrarily to ancient strains (1986), modern strains (2006-2010) of *S. donghaienis* were coherently able to restart growing after a P spike injected at the moment of limitation. Except for one strain, neither modern (2006) nor ancient (1998) *A. minutum* strains were able to restart growing. The differences in growth dynamics between ancient and modern strains of *S. donghaienis* were mirrored by differences in their metabolomics profiles. These variations were more important between strains of different ages than between growth phases. Metabolites specifically produced by ancient strains were identified.

This study suggests an intra-specific variability of the phosphate utilization between phenotypes of dinoflagellate of different ages in Brittany. The associated genetic diversity and the existence of other differential physiological traits between strains of different age are being sought. The final picture could illustrate an adaptation of dinoflagellate populations to changes in abiotic conditions that occurred in a coastal ecosystem.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-148

HOW DOES CLIMATE CHANGE AFFECT THE PHYSIOLOGICAL ECOLOGY OF KARLODINIUM VENEFICUM AND ITS CONSEQUENCES ON TROPHIC TRANSFER?

N. Vidyarathna [1,*], L. Smith [1], J. Cohen [1], K. Coyne [1], M. Warner [2]

[1]College of Earth, Ocean, and Environment, [2]University of Delaware, Lewes, USA

Abstract: Shifts in global temperature as well as carbon dioxide are expected to have potentially profound effects on phytoplankton communities, including harmful algal species, with possible cascading consequences for marine food webs. We studied the combined effects of elevated temperature and CO_2 on the physiological response of the dinoflagellate *Karlodinium veneficum*, originally collected from the Delaware Inland Bays. Algae were first acclimated to the ambient (25 °C / 400 ppm CO_2) and climate change conditions (29 °C / 1000 ppm CO_2) predicted for year 2100, using automated pH stated continuous cultures. When grown under high temperature and CO_2, cell growth improved and Primary production increased significantly (x 1.6 times), as compared to ambient conditions. Similarly, high temperature and CO_2 resulted in the cellular quota for carbohydrate, protein, total lipids and fatty acids also increasing by 3.1, 1.8, 1.1 and 2-fold, respectively. Cells grown under high temperature and CO_2 produced a higher percentage of saturated and a lower percentage of mono-and polyunsaturated fatty acids compared to those grown at ambient conditions. Cell toxicities measured as %fish gill cell mortality were significantly higher in the cells grown at high temperature and CO_2, as compared to the ambient conditions while the hemolytic activities did not differ significantly. This work is in tandem to grazing and respiration analyses (in progress) with the common estuarine copepod *Acartia tonsa*, while grown under the same temperature and CO_2 conditions. Ingestion, respiration, and egg production values will be converted to carbon equivalents to assess changes in carbon allocation in *A. tonsa* and thereby to understand the effect of altered physiology of *K. veneficum* on trophic transfer in marine food webs in a climate change scenario.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-149

Physiological responses and toxins production of *Microcystis aeruginosa* acclimated to different salinities.

M. Georges Des Aulnois [1,*], P. Roux [1], F. Malo [1], A. Caruana [1], E. Briand [1], M. Bormans [2], Z. Amzil [1]
[1]IFREMER, Nantes, [2]UMR CNRS 6553 ECOBIO, Université de Rennes 1, Rennes, France

Abstract: Proliferation of *Microcystis* is a concern worldwide as the detection of hepatotoxic microcystins is often associated with *Microcystis* blooms. As a consequence of eutrophication and climate changes, the intensity and occurrence of *Microcystis* blooms is expected to increase. The combined impacts of climate change and anthropogenic activities are increasing the salinity levels of freshwater bodies at a rate that is expected to threaten the functioning of natural ecosystems. Freshwater salinization is mainly related to summer droughts, rising sea levels and agricultural use of freshwater. Harmful effect of *Microcystis* proliferation concerns mainly freshwater ecosystems, however, in recent years, an increasing number of studies pointed out the occurrence of *Microcystis* proliferation in brackish water. Surprisingly, few studies have investigated the response of acclimated *Microcystis* to elevated salinity. Therefore, in this study, two strains of *Microcystis aeruginosa* (PCC 7806 and PCC 7820) were progressively acclimated to elevated salinities according to their limits of tolerance (14.8 and 7.5 respectively). At each dilution step, the salinity of *M. aeruginosa* batch cultures was gradually increased. After acclimation period, monitoring of growth, toxin production and photosynthetic activity were recorded between the different growth phases. Maximum growth rate (PCC 7806: 0.37; PCC 7820: 0.33) was reduced at high salinity for both strains (PCC 7806: 0.23; PCC 7820: 0.18) in comparison with the control condition of zero salinity. However, the photosynthetic activity remained stable between the different salinity conditions for both strains suggesting a progressive acclimation. Early results conveyed that increasing salinity reduces microcystins production for both strains while keeping the same toxin profile. Such results suggest that long term salinity increase in freshwater ecosystems will potentially decrease growth rate and toxins production of *M. aeruginosa*. Upcoming approaches using a continuous culture system combined with metabolomics and transcriptomic analyses allow to compare more deeply the physiology of toxic *M. aeruginosa* strains at high salinity.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-150

EFFECTS OF ELEVATED WATER TEMPERATURES ON THE ABUNDANCE OF ALEXANDRIUM CATENELLA IN KACHEMAK BAY AND LOWER COOK INLET, ALASKA

M. Vandersea[1], S. Kibler[1], P. Tester[2], D. Hondolero[3], K. Holderied[4], K. Powell Schuster[5], S. Baird[5], A. Doroff[6], D. Dugan[7], W. Litaker[1,*]

[1]National Oceanic and Atmospheric Administration, [2]Ocean Tester, LLC, Beaufort NC 28516, [3]National Oceanic and Atmospheric Administration, Seldovia AK 99663, [4]National Oceanic and Atmospheric Administration, Homer AK 99603, USA, [5]Kachemak Bay National Estuarine Research Reserve, National Oceanic and Atmospheric Administration, Homer AK 99603, [6]South Slough National Estuarine Research Reserve, National Oceanic and Atmospheric Administration, Charleston OR 97420, [7]Alaska Ocean Observing System, Anchorage AK 99501, USA

Abstract: Despite the long history of paralytic shellfish poisoning (PSP) events in Alaska, little is known about the seasonal distribution and abundance of the causative genus, *Alexandrium*, or the environmental factors that govern the development of its toxic blooms. To address this issue, we undertook a 5-year study (2012 – 2017) in Kachemak Bay and lower Cook Inlet, Alaska to determine how the occurrence of *Alexandrium catenella*, the dominant PSP-causing *Alexandrium* species in Alaska, was influenced by temperature, salinity, nutrient concentrations, and other environmental factors. *A. catenella* cell concentrations from 572 surface water samples were estimated using quantitative PCR. Monthly sampling across sampling years revealed a seasonal pattern of *A. catenella* bloom development that was positively correlated with the water temperatures. Prevailing salinity conditions did not significantly affect abundance, nor was nutrient limitation a direct factor. Bloom concentrations ranging from 100 to 3,050 cell eq. L^{-1} were detected in 35 samples (8%) from Kachemak Bay. In contrast, maximum abundances reached only 67 cell eq. L^{-1} in samples from lower Cook Inlet sites. The cell distribution patterns indicated *A. catenella* blooms originate within Kachemak Bay rather than from Cook Inlet. Approximately 7-8 °C appears to represent the temperature threshold for significant bloom development in Kachemak Bay, with the greatest risk of shellfish toxicity occurring when temperatures exceed 10-12 °C. The monitoring data also showed average water temperatures in Kachemak Bay were ~2°C warmer in 2014-2016 compared to 2012-2013. These increased water temperatures coincided with the general warming trend observed in the eastern Pacific at that same time and were accompanied by increases in both *A. catenella* abundance and shellfish toxicity relative to 2012-2013. The association between increased temperatures and elevated PSP saxitoxin levels in shellfish was supported by time series data from the Alaska Coastal Current (station GAK1) and Kachemak Bay relative to shellfish toxicity. The data showed summertime shellfish toxicity events in Kachemak Bay generally followed periods of anomalously high water temperatures. Consequently, monitoring changes in water temperatures may serve as an early warning signal for subsequent increases in shellfish toxicity within Kachemak Bay.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-151

First report of *Gambierdiscus* and *Ostreopsis* genera from São Vicente Island, Cape Verde.

E. Soler Onís [1,*], J. Fernández Zabala [2]

[1]Researcher and Taxonomist, [2]researcher, Banco Español de Algas, Telde, Spain

Abstract: In general, there is a good knowledge about benthic dinoflagellates worldwide, in particular from temperate and tropical areas. This fact contrasts with the lack of information in the Macaronesian Region, where the data regarding the benthic dinoflagellates and its distribution is very recent, sparse or even absent, as it occurs in Cape Verde.

To date, the genus *Ostreopsis* (Ostreopsidaceae) is represented in the Macaronesian Region by 4 species, namely *O. heptagona, O. ovata, O siamensis* in Azores (Silva *et al.,* 2008), *O. ovata* in Madeira (Fraga *et al.,* 2002) and *O. ovata* (Ojeda, 2013) and *O. fattorussoi* (Soler Onís *et al.,* 2017) in Canary Islands. However, this genus had not been previously reported in Cape Verde.

On the other hand, the genus *Gambierdiscus* (Ostreopsidaceae) is presented by 6 species in this biogeographical region located in the middle North-East Altltantic area: *G. excentricus* (Fraga *et al*., 2005) and *Gambierdiscus* sp. (Kaufmann, 2013) in Madeira and *G. excentricus* (Fraga *et al.,* 2011), *G. australes* (Fraga *et al.,* 2014), *G. silvae* (Fraga and Rodríguez, 2014), *G. caribaeus* (Soler Onís *et al.*, 2016) and *G. carolinianus* (Rodríguez *et al.,* 2017) in the Canarian Archipelago. In Cape Verde, the species reported by Silva in 1956 as *Goniodoma* sp. observed at Boavista island in 1948 is, according to Fraga and Rodríguez (2014), *G. silvae*, the first report of this genus in the Cape Verde archipelago.

The composition, abundance and morphology of some of the most abundant benthic dinoflagellates was investigated in November 2017 at 8 localities of São Vicente island (Cape Verde). In this study the presence of *Gambierdiscus* genus is confirmed in Cape Verde with two species. Also new information on *Ostreopsis* is given with the first record *O. lenticularis* in the East Atlantic Ocean. In one location, *O. lenticularis* along with *O. ovata* showed bloom levels with maximum abundances of 220.079 cel gr^{-1} fresh weight macroalgae and 323.529 cel 100 cm^{-2} on fiberglass screens. Several species of *Prorocentrum, Coolia, Amphidinium, Sinophysis, Vulcanodinium* and *Heterocapsa* were also found in the same samples in all the localities examined. The detection of *Gambierdiscus* and *Ostreopsis* species in the coastal areas of São Vicente island constitutes the first record of the potentially causative agent of ciguatera (CTXs) and PLTX-analogs in Cape Verde archipelago.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-152

Gene expression variation provides key insights into trait evolution in *Alexandrium ostenfeldii* populations

S. Wohlrab [1 2,*], K. Brandenburg [3], D. van de Waal [3], J. Jerney [4], A. Kremp [5], U. John [2]

[1]HIFMB, Oldenburg, [2]AWI, Bremerhaven, Germany, [3]NIOO-KNAW, Wageningen, Netherlands, [4]SYKE, Helsinki, Finland, 5Leibnitz Institute for Baltic Sea Research Warnemuende, Roshtock, Germany

Abstract: Populations of the toxigenic dinoflagellate *Alexandrium ostenfeldii* occur worldwide in temperate waters were they have adapted to the respective environments. On the genetic level, such local adaptations can express themselves by different variant forms of proteins, by differences in gene duplication events retained in the genome and by differences in regulatory DNA regions. The latter two can cause specific gene expression variation patterns in the respective populations. Several studies have indicated that evolution might depend more strongly on such variations in gene expression than on variant forms of proteins. Estimating gene expression variation has therefore been applied to assess the type of selection pressure acting on traits represented by genes or groups of genes between species or populations, i.e. stabilizing, directional and balancing selection or neutral evolution. We used the potential of gene expression variation analysis to assess the selective pressures on genetic traits in two *A. ostenfeldii* populations. The investigated genotypes are from a Baltic Sea population, which is relatively large and already established between 8000-3000 BP, and from a much smaller and only recently established population in a small creek in the Netherlands. The Baltic Sea population exhibits considerable higher phenotypic trait variation compared to the younger population. Both populations differ significantly in trait expression related to growth, size and physiology (e.g. nitrogen uptake kinetics, secondary metabolite synthesis). Our results highlight genetic traits which are under various selective pressures and further, how gene expression variation changes in the younger population by comparing variation patterns between genotypes from two successive years. Additionally, we give an estimate of traits that became fixed in the respective population and traits that remain neutral. We further analyzed if gene expression variation is potentially caused by gene duplication events and if duplicated genes are under purifying, conserved or neutral evolution. We conclude that gene expression variation can be used to assess 'the evolutionary direction and potential' of harmful algal bloom species and to assess if genes with unknown functions are important for the autecology of the respective population.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-153

Unraveling the molecular mechanism in response to changing phosphorus in a dinoflagellate *Alexandrium catenella* with proteomics

S.-F. Zhang [1,*], Y. Chen [1], Z.-X. Xie [1], H. Zhang [1], L. Lin [1], D.-Z. Wang [1]
[1]Xiamen University, Xiamen, China

Abstract: Phosphorus (P) is a key macronutrient limiting cell growth and bloom formation of marine dinoflagellates. Physiological responses to ambient P changing have been intensively investigated in dinoflagellates, however, little is known about the molecular mechanisms behind these responses. Here, we compared protein expression profiles of *Aexandrium catenella* grown in inorganic P-replete, P-deficient, and inorganic- and organic-P resupplied conditions using an iTRAQ-based quantitative proteomic approach. P-deficiency inhibited cell growth and enhanced alkaline phosphatase activity (APA), but had no effect on photosynthetic efficiency. After resupplement of P, the P-deficient cells recovered growth rapidly and APA decreased. Proteins involved in sphingolipid metabolism, organic-P utilization, starch and sucrose metabolism, and photosynthesis were up-regulated while proteins associated with protein synthesis, nutrient assimilation and energy metabolism were down-regulated in the P-deficient cells. Responses of P-deficient *A. catenella* to organic- and inorganic-P presented significant differences: more biological processes were enhanced in the organic P-resupplement cells compared with the inorganic P-resupplement cells, and *A. catenella* might directly utilize G-6-P for nucleic acids synthesis through the pentose phosphate pathway. Our results indicated that *A. catenella* had evolved diverse adaptive mechanisms to ambient P-deficiency, i.e. enhancing acid phosphatase expression, utilizing non-phospholipids and reallocating intracellular energy. This study provides new insights into the response mechanisms of marine dinoflagellate to ambient P changing.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-154

De novo assembly and characterisation of transcriptomes from *Amphidinium* species

G. S. Kohli [1 2,*], J. Jose Dorantes-Aranda [3], T. Bachvaroff [4], A. Place [4], A. Verma [1], S. Murray [1]

[1]Climate Change Cluster, University of Technology Sydney, Sydney, Australia, [2]Alfred Wegener Institute, Bremerhaven, Germany, [3]Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, Australia, [4]Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Sciences, Baltimore, USA

Abstract: *Amphidinium* Claparede et Lachmann is one of the most abundant and diverse dinoflagellate genera found in marine benthic, sand-dwelling and pelagic habitats. Species of *Amphidinium* have been widely studied for their potential to produce natural products, in particular, long chain and linear polyketide and macrolide compounds. More than 40 compounds produced by different species of *Amphidinium* have been isolated. These compounds with varying bioactivities are being investigated for their efficacy as antimicrobial and/or therapeutic agents. Some compounds produced by *Amphidinium* species have bioactivities against fish gill cells and may lead to fish kills. Structural elucidation of many toxic compounds produced by dinoflagellates suggests that they may be based on a polyether ladder backbone, and that polyketide synthase (PKS) enzymes may have a role in their biosynthesis. The aim of our study was to screen *Amphidinium* species for the production of polyketide compounds and test their toxicity. We conducted a transcriptomic sequencing study to determine the presence of PKS genes (both mono-functionally expressed catalytic domains and genes encoding multimodular PKS enzyme complexes) in *Amphidinium*. The ketosynthase domain encoding transcripts were found to form distinct clades in the phylogenetic analysis, in comparison to similar genes from other organisms. We also show the presence of genes encoding six key enzymes essential for fatty acid production in *Amphidinium*. The results presented here are a step forward towards recognising the genes encoding critical steps in toxin biosynthesis and designing tools to monitor for and mitigate human illnesses due to harmful algal blooms.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-155

Polyketide synthase gene clusters in cryptic sub-tropical *Ostreopsis* species

A. Verma [1,*], G. Kohli [2], K. Smith [3], L. Rhodes [3], S. Murray [1]

[1]Climate Change Cluster, University of Technology Sydney, Sydney, Australia, [2]Alfred Wegener Institute for Polar and Marine Research, Bremerhaen, Germany, [3]Cawthron Institute, Nelson, New Zealand

Abstract: Dinoflagellates play vital ecological roles in marine and freshwater ecosystems and are known for their ability to produce complex secondary metabolites that have toxic impacts on co-occurring phytoplankton, fisheries, and humans. Species of genus *Ostreopsis* Schmidt are common in tropical and temperate benthic environments and produce complex toxic palytoxin molecules (PLTX) and/or its analogs, which are associated with benthic blooms, human skin and eye irritations, and poisonings through the consumption of contaminated fish and shellfish. Structural elucidation of such toxic compounds suggests a polyether ladder backbone with polyketide synthase (PKS) enzymes driving their biosynthesis. In this study, we isolated two cryptic *Ostreopsis* clades, *Ostreopsis* sp. 3 and sp.5 from macroalgae collected in Rarotonga, Cook Islands. We report their molecular phylogeny using LSU-ITS-5.8S rDNA regions as well as describe the key domains involved in the production of polyketide molecules. We conducted a *de novo* transcriptomic sequencing study to determine the presence of both mono-and multi-modular PKS enzyme complexes in these species and compared them to other closely related dinoflagellate species. The ketosynthase (KS) domains were putatively identified and found to form discrete phylogenetic clades when compared to similar genes from other toxic and non-toxic organisms. The results presented in this study add to our currently scarce knowledge of toxin biosynthesis in dinoflagellates and will aid in developing better diagnostic screening tools for algal blooms in the future.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-156

Molecular mechanisms regulating sexual reproduction and mating type determination in the diatom *Pseudo-nitzschia multistriata*

M. I. Ferrante [1,*], M. T. Russo [1], L. Vitale [1], C. Borgonuovo [1], R. Annunziata [1], K. Anestis [1], F. Romano [1], R. Sanges [2], M. Montresor [1]

[1]Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, [2]Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy

Abstract: *Pseudo-nitzschia* is an important genus of marine diatoms responsible for blooms in both coastal and oceanic waters. We selected *Pseudo-nitzschia multistriata* as a model system to explore the process of sexual reproduction, which is especially important in diatoms since it is linked to the formation of large-sized cells within the cell size reduction/restitution cycle. *P. multistriata*, as most pennate diatoms, is a heterothallic species in which sex is induced when cells of opposite mating type get in contact. We recently sequenced the *P. multistriata* genome, defined its meiotic toolkit and elucidated the global gene expression changes occurring in cells of opposite mating type during the early stages of sexual reproduction.

To further refine our knowledge of this process, we are now focusing on a set of genes that are preferentially expressed in one or the other of the two mating types. These mating type-related genes, identified using a transcriptomic-based approach, play a role in defining the mating type and in governing cell behaviour during sexual reproduction. By using genetic transformation we are reconstructing the genetic network that connects them. Most importantly, we identified structural differences in the genomic locus for one of these gene, and demonstrated that it acts as the sex determinant in *P. multistriata*.

A refined knowledge of the genes and of the mechanisms regulating the life cycle for a toxic *Pseudo-nitzschia* species will increase our understanding of the dynamics regulating harmful algal blooms.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-157

From the lab to the real word: issues and challenges in detecting parasite-mediated HAB dynamics in natural populations

C. Alves-de-Souza [1,*]

[1]Algal Resources Collection, MARBIONC at Crest Research Park, University of North Carolina Wilmington, Wilmington, USA

Abstract: Biotic control by eukaryote parasites has been suggested as a major factor affecting the formation and demise of Harmful Algal Blooms (HABs). However, information on the quantitative relevance of eukaryote parasites to HAB dynamics in the field remains scarce. This is due mainly to the inconspicuous nature of parasites, which untimely preclude their detection during traditional environmental surveys. In addition, the understanding of parasite-mediated HAB dynamics requires the detection of parasites within a community context since parasite and host populations do not exist as isolated pairs. Rather, they exist as parts of multispecies systems involving biotic interactions others than parasitism such as predation and allelopathy. While valuable insights on plankton host-parasite interactions have been gained in the last years thanks to network analysis of high-throughput sequencing data, caution should be taken when interpreting the obtained results as correlation does not necessarily imply causality and different patterns can be detected depending on the sampling frequency. Here, we review the main aspects to take into account when investigating the relevance of host-parasite interactions for HAB dynamics in natural populations. Based on this overview, an integrative approach combining field and experimental data is explored.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-158

Viral infection process in dinoflagellate cells

Y. Takano [1], Y. Tomaru [2], K. Nagasaki [1,*]

[1]Team Auqa-Virus, Kochi University, Kochi, [2]National Research Institute of Fisheries and Environment of Inland Sea, Japan Fisheries Research and Education Agency, Hiroshima, Japan

Abstract: Dinoflagellates are one of the most abundant and diverse groups of phytoplankton; still, viral impacts on dinoflagellates have been only partially clarified so far. HcDNAV is the first-isolated large dsDNA virus infecting *Heterocapsa circularisquama*, which is a noxious bloom-forming marine dinoflagellate. Previous studies showed (1) HcDNAV is an icosahedral virus ca. 180-210 nm in diameter; (2) it propagates within the host cytoplasm where virions are associated with the viroplasm (i.e. virus factory); (3) phylogenetic analysis of DNA polymerase gene indicates the close relationship between HcDNAV and African swine fever virus (*Asfaviridae*); (4) according to the classification by ICTV, HcDNAV belongs to the genus Dinodnavirus. In the present study, epifluorescent microscopy (EFM), field emission scanning electron microscopy (FE-SEM), and quantitative PCR technique (Q-PCR) were used to elucidate its infection process.

By FE-SEM, viral attachment was mainly observed at the transverse groove; probably due to the water current caused by the ribbon-like transverse flagellum. Then, the virions appeared to be captured into the host cell. It is likely that the host mucilage blocks the infectivity of HcDNAV. Each vertex of HcDNAV was decorated with a lump， which made the virion easily-distinguishable by FE-SEM. The viral genome signal was detected from manually-isolated host nuclei at 0.5-2 h post-infection (hpi) by means of a Q-PCR designed specific to HcDNAV. By EFM, virus assembly was observed in the host cytoplasm from 11 hpi, and it appeared to be horseshoe- or doughnut-shaped at 15-30 hpi. The three-dimensional inner structure of the viroplasm was precisely observed by FE-SEM; perhaps for the first time in the world.

Considering that the genome draft sequence of HcDNAV has been obtained just recently, one of our next goals is to compare the gene expression pattern and the intracellular morphological change in parallel through the infection process.

This work was partially supported by grants for Scientific Research on Innovative Areas from the Ministry of Education, Culture, Science, Sports and Technology (MEXT) of Japan (Nos. 16H06429, 16K21723, 16H06437), and commissioned project study on "Technological developments for characterization of harmful plankton in the seawater", Ministry of Agriculture, Forestry and Fisheries, Japan.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-159

Prey cells and extraction trigger DSP toxin production by *Dinophysis acuminata*

H. Gao [1,*], M. Tong [1]

[1]Ocean College, Zhejiang University, Zhoushan, China

Abstract: As a causative species of diarrhetic shellfish poisoning (DSP) and/or pectenotoxins (PTXs), Dinophysis acuminata physiological and toxicological characteristics are widely studied by alternating factors, including temperature, illumination, prey quantity and dissolved inorganic nutrient. Prey and light are the essential key factors for growth and toxicity of Dinophysis. However, the role of prey extraction has not been well studied. Therefore, we conducted a series of experiments to investigate how the growth and toxin production of Dinophysis is influenced by the organic material originating from prey extraction, using the prey Mesodinium rubrum and Teleaulax amphioxeia. Our results showed significant growth of D.acuminata (0.12 /d) when fed the M. rubrum extract. The highest growth rates and cell densities of 0.25/d and 3902 cell/mL for Dinophysis were observed in the mixed cultures containing live M. rubrum cells and debris, resulting to the highest total toxin concentration (intracellular + extracellular, amount toxin per mL of culture) of okadaic acid (OA, 14.2 ng/mL) and dinophysistoxin-1 (DTX1, 10.3 ng/mL). OA was actively released during the growth of D. acuminata. The proportion of dissolved OA increased from 67% in the middle exponential phase, to 73% in the early plateau phase and to 82% in the late plateau phase on average of three treatments with ciliates cell addition. DTX1 and pectenotoxin-2 (PTX2) were not actively released but distributed in the medium over 73% and 24%, respectively, at the end of incubation due to the cell lysis. Total OA and DTX1 were significantly greater in the cultures receiving live or cryptophytes debris than those in the monoculture of Dinophysis. Together these investigations suggest that organic material from the prey could be utilized by Dinophysis cells as a nutrient source and the addition of prey cells or extraction, from both ciliates and cryptophytes, could potentially trigger the DSP toxin production of D. acuminata.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-160

Ubiquity and critical role of phagotrophy in phytoplankton community

Z. Yu [1 2 3], L. He [3], J. Chu [4], Y. Tang [2 3], X. Song [1 2 3], X. Cao [2 3], F. Cheng [3], Y. Yuan [3], X. Xu [3], C. Gobler [5], N. Chen [1 2 3 6,*]
[1]University of Chinese Academy of Sciences, Beijing, [2]Laboratory of Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, [3]CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Qingdao, [4]Wuhan Frasergen Bioinformatics Inc., Wuhan, China, [5]School of Marine and Atmospheric Sciences, Stony Brook University, New York, USA, [6]Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, Canada

Abstract: Phagotrophy is an important nutrition strategy for phytoplankton, however, its exact role in phytoplankton succession *in situ* remains poorly understood. Through applying metatranscriptomic analysis of 12 time-series samples collected in Bohai Bay, China, we evaluated the critical role of phagotrophy in phytoplankton community. Our analysis demonstrated that phagosome genes were ubiquitously expressed in essentially all phytoplankton species including diatom species and green algae that were previously thought to be exclusive phototrophs. We further found that the expression intensity of genes related to phagosome can be used to quantitatively evaluate phagotrophy activities in phytoplankton. Additionally, we uncovered that the intensity of phagosome expression was dynamic and highly correlated with the changes of phytoplankton cell densities with a delay, suggesting that phagotrophy plays an important role in phytoplankton succession and bloom formation. Moreover, our analysis illustrated that phagosome expression intensity in individual phytoplankton species was associated with coordinated changes in population dynamics, suggesting that phagotrophy is regulated and plastic during algal succession, likely responding to the interplay between nutrient availability and ecological factors. The findings offer a novel understanding to the role of phagotrophy nutritional strategy in succession of phytoplankton community in marine ecosystems.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-161

The cost of toxicity in microalgae: evidence from light-limited growth of *Alexandrium* species (Dinophyceae)

H. E. Blossom [1,*], N. Daugbjerg [2], B. Markussen [3], B. Krock [4], A. Norlin [1], P. J. Hansen [1]

[1]Marine Biological Section, Department of Biology, University of Copenhagen, Helsingør, [2]Marine Biological Section, Department of Biology, [3]Department of Mathematical Sciences, University of Copenhagen, Copenhagen, Denmark, [4]Alfred-Wegener Institut für Polar- und Meeresforschung, Bremerhaven, Germany

Abstract: Empirical evidence of the cost of producing toxic compounds in harmful microalgae is completely lacking. Yet costs are often assumed to be high, forming the foundation of a widely accepted cost-benefit interpretation of the evolution of toxin production. To study potential fitness costs of toxin production, sixteen strains including 3 species of the former *Alexandrium tamarense* species complex were grown under both unlimited and light limiting conditions (carbon limited). We measured growth rates, levels of intracellular paralytic shellfish poisoning toxins (PSTs) and effects of lytic compounds. We provide trade-off curves of toxicity for both PST and lytic toxicity under unlimited, and carbon limitation where we found direct fitness costs in terms of reduced growth rates of PST production under high light (300 µmol photons m-2sec-1), but no obvious costs of PST production were observed under light limitation. On the other hand, the cost of production of lytic compounds was highest under light limitation, but only in strains producing PST. The results show that in order to further understand the evolutionary role and ecological function of toxins, costs on intrinsic growth rates may be significant and should be accounted for in relation to quantifying benefits such as grazer avoidance and toxin mediated prey capture in natural food-web settings.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-162

Large-scale climatic patterns and relationship with Harmful Algal Blooms (HABs) in fjords and canals of Southern Chile

R. Martínez [1,*], L. Guzmán [1], M. J. Calderón [1], J. Cáceres [1], G. Labra [1], L. Iriarte [1], V. Muñoz [1], C. Alarcón [1], H. Pacheco [1]
[1]Centro de Estudios de Algas Nocivas (CREAN) - IFOP, Puerto Montt, Chile

Abstract: The ocean plays a key role in the global climate system, as well as in turn, is directly affected by different disturbances in climate regimes causing drastic changes in the structure and function of marine ecosystems. The Southern Chilean canals and fjords ecosystem is characterized by a complex oceanographic and biological dynamic, with a wide variety of HABs, which have some characteristic patterns of abundance and distribution in the area. However, abnormal changes in these parameters can result in an increase in the occurrence, density and geographic coverage of HABs, with serious consequences for coastal resources, local economy and public health, so large-scale events such as El Niño–Southern Oscillation (ENSO), Decadal Pacific Oscillation (PDO) and the Antarctic Oscillation (AAO) could have a direct relationship with these changes. Therefore, the objective of this work is to show the dynamics of HABs and their relationship with large-scale events through standardized anomalies of physical parameters (temperature-salinity, Light-PAR, wind speed, barometric pressure) and biological data (dinoflagellates total abundance, relative abundance of *Alexandrium catenella* and *Dinophysis acuminata* species) obtained from a time series between 2007-2017 in 30 sampling sites distributed over a wide geographical area ranging from -41º20ˈS to -55º58ˈS, and its correlation with different climatological index (PDO, AAO, SOI). It is important to mention that is the first approximation we have of the entire Chilean fjord system. Our results indicate that the responses to these large-scale climate patterns are not always consistent and are subject to different factors depending on the latitude and area where the sampling stations are located. Most parameters had a stronger statistical relationship with the PDO than with the SOI, however the complement of both index PDO and SOI is a better predictor of biological and physical conditions than either index alone. As for the biological variables, we can conclude that the different harmful blooms do not present a clear pattern that could relate them to the presence of ENSO, but we can conclude that the PDO is the most important phenomenon in the first two regions, while the AAO acquires more relevance as the latitude increases.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-163

Toxic Cyanobacteria Blooms in the Finger Lakes of Central New York – New Climate, New Rules.

G. Boyer [1,*], K. Slodysko [1], D. Derminio [1], Z. Smith [1], B. Wei [1], N. Mueller [2], M. Perkins [3], R. Gorney [4], S. Kishbaugh [4]

[1]State University of New York, College of Environmental Science and Forestsry, Syracuse, [2]New York State Federation of Lake Associations, LaFayette, [3]Upstate Freshwater Institute, Syracuse, [4]New York State Department of Environmental Conservation, Albany, USA

Abstract: The 11 Finger Lakes located in Central New York (USA) are the crown jewel of the region. They serve as important resources for drinking water, recreation, and tourism. While the lakes share a similar geological origin they vary in hydrology, land use, and trophic status. Several lakes (i.e. Canandaigua Lake and Skaneateles Lake) are historically oligotrophic (total phosphorus (TP) < 10 µg/L) whereas other lakes (i.e. Honeoye Lake and Otisco Lake) are eutrophic (TP > 20 µg/L). In 2016, Owasco Lake, a meso-oligotrophic lake suffered a severe cyanobacterial HAB dominated by *Microcystis aeruginosa* with microcystin concentrations in the lake reaching 2500 µg/L. Detectable levels of the toxin were found in the City of Auburn's finished drinking water, which draws its water from Owasco Lake. In 2017, all 11 Finger Lakes developed cyanobacteria HABs for the first time on record. Peak microcystin concentrations reached 200 and 400 µg/L in "oligotrophic" Skaneateles Lake and mesotrophic Seneca Lake, and >1,800 µg/L in Owasco Lake. Historically, management of these lakes has focused on reducing the overall total phosphorus concentrations, which has suppressed algal growth in these systems. However in 2017, intense episodic rain events led to a sudden influx of terrestrially derived nutrients. Coupled with an extremely mild autumn that extended the growing season, intense sunshine and warmer temperatures, these conditions released the previously constrained algal populations to bloom. Driven by prevailing winds, these blooms accumulated along the shorelines of the lake, resulting in widespread public health concerns. These regional conditions that led to the first near-simultaneous occurrence of cyanobacterial HABs in all 11 Finger Lakes are unlikely to be a unique event. Current climate change models predict that the region will be characterized by more frequent episodic spring rains, earlier spring turnover and later fall turnover leading to an extended growing season, and more late-summer days characterized by calm wind conditions and bright sunshine. These factors should all combine to lead to more HAB events in lakes previously considered immune to these blooms. Prior monitoring efforts have focused on higher phosphorus lakes. Agricultural practices were designed to reduce the overall nutrient concentrations in these lakes while still rapidly removing storm water from fields to prevent damage to crops. These efforts will need to be revisited to in view of the effects of these episodic rain events on what were previously considered to be oligo- or meso-trophic systems.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-164

The implementation of different tools for understanding and managing harmful algal blooms: case studies in the Mediterranean Sea

A. Penna [1,*], E. Valbi [1], R. Fabio [1], S. Casabianca [1], S. Capellacci [1], C. Dell'Aversano [2], L. Tartaglione [2], M. G. Giacobbe [3], M. Scardi [4]

[1]DISB, University of Urbino, Pesaro, [2]Department of Pharmacy, University of Napoli Federico II, Naples, [3]ISMAR, CNR, Messina, [4]Department of Biology, University of Tor Vergata, Rome, Italy

Abstract: Climatic change conditions and anthropogenic pressures have strong impact on the harmful bloom development along the coasts worldwide. The PSP toxin producing *Alexandrium minutum* causes harmful blooms in the Mediterranean coastal areas with its frequency increasing and capacity of dispersal due of resting stages production and settling in new areas. Therefore, there is an urgent need for new methods for managing and forecasting these harmful algal blooms. Early warning and forecasting systems for HABs are shown to be useful tools together with rapid and species-specific accurate detection of cells and toxins in the seawater, sediments and seafood chain. Mathematical models based on Machine Learning techniques and principally those based on Random Forests, ensemble techniques that combine many classification trees, are very promising both at regional and at wider scale. We trained a Random Forest based on environmental predictive data and *A. minutum* molecular abundance from the sampling area close to aquaculture farms. This technique proved to be effective and allowed developing a qualitative predictive model for the toxic *A. minutum* forecasting, although most relationships among variables are still unknown. The improved molecular methods of qPCR applied to species-specific quantification of harmful dinoflagellates based on *sxt* genes allowed to rapidly determine the STX–producing cell concentration of *Alexandrium* species, especially at early warning stage of a bloom, in coastal area highly exploited by maritime activities. The concomitant molecular quantification of *A. minutum* resting cysts in harbour sediments offered further information on the seed bed size and the germination potential. Toxin contamination of both seawater and shellfish samples was assessed by hydrophilic interaction liquid chromatography combined with high resolution mass spectrometry and correlation between toxicity data with molecular abundance of *A. minutum* were used to determine the likelihood of risk from PSP toxins in bivalve molluscs harvested in southern Mediterranean Bay. Monitoring and predicting strategies for HABs by combined different methodologies could play a fundamental role in preventing and control health and economic risks related to toxin-producing species blooms in coastal areas.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-165

A continental scale multilake survey of cyanobacteria, toxin synthetase genes and toxins during a heatwave summer

K. Panksep[1 2,*], E. Mantzouki[3], M. Lurling[4], J. Fastner[5], P. Visser[6], H. Tammert[2 7], B. Ibelings[3]

[1]Institute of Ecology and Earth Sciences, University of Tartu, [2]Chair of Hydrobiology and Fishery, Estonian University of Life Sciences, Tartu, Estonia, [3]Institute F.A. Forel, University of Geneva, Geneva, Switzerland, [4]Department of Environmental Sciences, Wageningen University, Wageningen , Netherlands, [5]Unit Drinking Water Resources and Water Treatment, German Environment Agency, Berlin, Germany, [6]Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, Netherlands, [7]Institute of Technology, University of Tartu, Tartu, Estonia

Abstract: In eutrophic systems, the phytoplankton community is often dominated by bloom-forming cyanobacteria. Due to ongoing eutrophication and climate change, the frequency and extent of the blooms are predicted to increase, making prevention, detection, and mitigation of blooms extremely important. During summer 2015, in the frame of a CyanoCOST and NETLAKE joint project, the …European Multi Lake Survey (EMLS), a large-scale snapshot study of toxic cyanobacteria across wide geographical and climatic regions in Europe was executed. A total of 369 lakes were sampled in the survey during the locally, historically two warmest weeks in summer. Sampling and in-situ measurements of environmental variables followed standardized protocols. All samples were analysed centrally for nutrients, pigments, toxins and DNA. 200 lakes out of 369 were sampled for DNA analysis. The aim of this study was to determine the distribution of potentially toxic cyanobacteria in European water bodies. Quantitative PCR (qPCR) was used to detect and quantify potential microcystin, nodularin, anatoxin and cylindrospermopsin producers, and liquid chromatography with tandem mass spectrometry (LC-MS/MS) to detect and quantify cyanotoxins. This is the first study of cyanobacteria that applies the molecular detection methods over such a large latitudinal, longitudinal gradient encompassing lakes with different trophic status and depth. The results of the molecular approach of EMLS and the effects of environmental factors affecting the distribution of toxin-producing cyanobacteria in Europe will be presented.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-166

Three Decades of Canadian Marine Harmful Algal Events- Reviewed and Evaluated Using Information from the HAEDAT Database

C. H. Mckenzie [1,*], J. L. Martin [2], S. S. Bates [3], N. Haigh [4]

[1]Northwest Atlantic Fisheries Centre, Fisheries and Oceans Canada, St. John's , [2]St. Andrews Biological Station, Fisheries and Oceans Canada, St. Andrews, [3]Gulf Fisheries Centre, Fisheries and Oceans Canada, Moncton, [4]Microthalassia Consultants Inc., Nanaimo, Canada

Abstract: Three decades of marine harmful algal events (1987 to 2017) in Canada are reviewed and evaluated, spatially and temporally, using data from the Intergovernmental Oceanographic Commission of UNESCO (IOC) harmful algal event database (HAEDAT). Canada has been contributing to HAEDAT through the International Council for the Exploration of the Seas/IOC Working Group on Harmful Algal Bloom Dynamics. A harmful algal event is defined as having a documented impact, such as a management action (closures of harvest sites, beaches) or environmental impacts, such as mortalities of fish or other marine species or human illness. Harmful events from the Canadian Pacific coast have also been included. This database is an important spatial and temporal record of the blooms, toxins and closures in Canada. For Canada, highlights of the database include: the first detection of ASP toxins (domoic acid) anywhere in the world and identification of the causative alga species (*Pseudo-nitzschia multiseries*) in Prince Edward Island (1987); closures of shellfish harvesting in the Bay of Fundy, New Brunswick (1988, *P. pseudodelicatissima*); the first proven case of DSP in North America (1990); the first closures of shellfish harvesting due to DSP toxins (*Dinophysis norvegica)* in Nova Scotia (1992) and Newfoundland (1993); the recurrent PSP toxin closures associated with *Alexandrium* species along portions of the Atlantic (Bay of Fundy, southern Nova Scotia, northeastern Newfoundland and the Gulf of St. Lawrence) and Pacific coasts; and the mass mortalities of fish, birds and mammals in the St. Lawrence Estuary (2008) due to an *A. tamarense* bloom and high levels of PSP toxins. Mortalities of both wild and cultured finfish associated with *Alexandrium* spp. have been reported on the Atlantic coast, whereas fish mortality events on the Pacific coast have only been reported from salmon aquacultures sites, due to this data being supplied by the industry-sponsored Harmful Algae Monitoring Program. Multiple algae species have been associated with fish-killing blooms in British Columbia, including the diatoms *Chaetoceros convolutus* and *C. concavicornis*, the Raphidophyte *Heterosigma akashiwo*, the dinoflagellates *Cochlodinium fulvescens* and *A. catenella*, and the silicoflagellates *Dictyocha fibula*, *Octactis speculum* and *Pseudochattonella verruculosa*. During 2015, a large algal bloom in the Pacific that extended from California to Alaska resulted in the closure of several shellfish harvesting areas in British Columbia due to *Pseudo-nitzschia* spp. and ASP toxins. This caused serious concern among the First Nation groups and fish harvesters regarding impacts on human health and the Pacific coast ecosystem. Information from this database is being used in an assessment of the potential impact of harmful algae as a stressor on Canadian marine ecosystems.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-167

Early detection and monitoring of HABs trough a regional network in Latin American and Caribbean countries

T. D. C. Cuellar Martinez [1,*], M.-Y. Dechraoui-Bottein [2], A. C. Ruiz Fernández [3], H. Enevoldsen [4], O. Amaya [5], L. Asencio [6], C. Alonso [6], R. Quintanilla [5]

[1]Student, Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Mazatlán, Mexico, [2]International Atomic Energy Agency, Environment Laboratories, 4a Quai Antoine 1er, 98000 , Monaco, Monaco, [3]Unidad Académica Mazatlán, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, 82040, Mazatlan, Mexico, [4]IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Copenhagen, Denmark, [5]Marine Toxins Laboratory of the University of El Salvador LABTOX-UES. Faculty of Natural Sciences and Mathematics. End 25 north Avenue, University City, San Salvador, El Salvador, [6]Centro de Estudios Ambientales de Cienfuegos (CEAC), Cienfuegos, Cuba

Abstract: Harmful algal blooms (HABs) constitute a worldwide problem, affecting aquatic ecosystems, public health and local economies. From 1970 to 2007, in Latin America and the Caribbean (LAC) ~5000 people have been poisoned and 67 died owing to the toxins associated with the occurrence of HABs (mainly Paralytic Shellfish Poisoning by *Pyrodinium bahamense* in the Pacific, and Ciguatera Fish Poisoning by *Gambierdiscus* spp. in the Atlantic). Algal blooms have affected more than one nation at a time, as is the case of Mexico and Central America, which highlights the regional scope of this problem. The scarcity of qualified personnel and properly equipped laboratories have acted as factors against to the establishment of prevention and mitigation measures. Argentina, Brazil, Chile, Colombia, Costa Rica, Cuba, Dominican Republic, El Salvador, Haiti, Honduras, Jamaica, Mexico, Nicaragua, Panama, Uruguay and Venezuela supported by the International Atomic Energy Agency established a regional network to develop capacities for early detection of HABs, to evaluate its toxicity and its historical trends, by using nuclear techniques (receptor binding assay and isotopic dating). With the support IOC-UNESCO, IAEA projects developed the "Guide for designing and implementing a plan to monitor toxin-producing microalgae" which introduced standardized sampling protocols and analytical techniques for microalgae and biotoxins vectors and facilitate interlaboratory comparison. The network recognized the lack of HAB records in LAC and found synergy with the IOC Harmful Algae database (HAEDAT), which reports 180 HABs from 1970 to 2018, within the countries from the network (between 1 and 49 records per country). It is evident that much more HABs have occurred but are not registered yet, which implies that efforts are needed to improve either the monitoring or the report of HABs, to maximize the benefits of the database in coastal management. Increasing HAB occurrence around the world during the past decades has been frequently attributed to contamination of the coastal zone; but the study of dinocysts in ^{210}Pb dated sediment cores have shown that some harmful species have been present in the region since more than 100 years ago, and that besides seawater contamination, climate variability might be an important driver for HAB occurrence. The IAEA projects have been effective to include 68% of the coastal Latin American countries and efficient to strength the HAB and associated biotoxins monitoring capabilities in LAC, to develop reference centers in the region (El Salvador and Cuba), which facilitate south-south collaboration and allow the creation or improvement of routine monitoring programs. Currently there are about seventy microalgae species that have produced HAB in the region, from which *Gymnodinium catenatum* and *Alexandrium catenella* are the most frequently reported.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-168

Identification of seven possible biosynthetic intermediates of tetrodotoxin in marine environments isolated from pufferfish

M. Yotsu-Yamashita [1,*], N. Ueyama [1], K. Sugimoto [1], Y. Yaegashi [1], Y. Kudo [1], K.-I. Onodera [2], Y. Cho [1], K. Konoki [1], T. Nishikawa [3]

[1]Graduate School of Agricultural Science, Tohoku University, Sendai, [2]Faculty of Agriculture and Marine Sciences, Kochi University, Kochi, [3]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

Abstract: Tetrodotoxin (TTX) is a potent and specific inhibitor to voltage gated sodium channel. This toxin is widely found in both of terrestrial (newts, frogs, and toads) and marine animals (crabs, octopuses, flatworms, etc). Recently, TTX was detected in European shellfish in low concentration. However, the biosynthetic pathway and genes for TTX have not yet been elucidated. Previously, we proposed that TTX originates from a monoterpene, based on the structures of cyclic guanidino compounds commonly found in toxic newts.[1] However, these compounds have not been detected in marine organisms. Instead, a series of deoxy analogues of TTX were found in toxic marine animals.[2] Thus, we further screened for TTX-related compounds in marine animals. As the result, we isolated seven novel spiro bicyclic guanidino compounds from the pufferfish *Tetraodon biocellatus*.[3] In six out of seven compounds, a six membered cyclic guanidino amide is spiro-fused with 2,4-dimethyl cyclohexane, whereas in one compound, the same cyclic guanidino amide is spiro-fused with 2,3,5-trimethyl cyclopentane. The six compounds have the same carbon skeleton structure and relative configuration as those of TTX. Thus, we propose that these compounds are biosynthetic intermediates of TTX in marine environments. TTX could be biosynthetically derived from these compounds, through several oxidations, amide hydrolysis, and formation of the hemiaminal and lactone found in 5,6,11-trideoxyTTX, the major TTX analogue, whereas two compounds in them might be shunt products. LC-MS analysis confirmed wide distribution of two of these compounds in TTX-containing marine animals, namely five species in three genera of pufferfish, a crab, an octopus, and a flatworm. Furthermore, another similar guanidino compound has been also detected in *T. biocellatus*.

References

1. Y. Kudo, T. Yasumoto, D. Mebs, Y. Cho, K. Konoki, M. Yotsu-Yamashita, *Angew. Chem. Int. Ed.*, *55*, 8728–8731, **2016**.
2. M. Yotsu-Yamashita, Y. Abe, Y. Kudo, R. Ritson-Williams, V. J. Paul, K. Konoki, Y. Cho, M. Adachi, T. Imazu, T. Nishikawa, M. Isobe, *Mar. Drugs*, *11*, 2799–2813, **2013**.
3. N. Ueyama, K. Sugimoto, Y. Kudo, K. Onodera, Y. Cho, K. Konoki, T. Nishikawa, M. Yotsu-Yamashita, *Chem. Eur. J.* in press, **2018**.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-169

Studying the regulation of secondary metabolites in cyanobacteria using targeted and untargeted metabolomic approaches

S. Kim Tiam [1,*], A. Ardilio [1], C. Dalle [2], C. Duval [1], M. Gugger [2], S. Le Manach [1], B. Marie [1], C. Yéprémian [1], C. Bernard [1]
[1]UMR 7245 Molécules de Communication et Adaptations des Microorganismes, Muséum National d'Histoire Naturelle, [2]Collection des Cyanobactéries, Institut Pasteur, Paris, France

Abstract: Cyanobacteria have a key role in ecosystem functioning but are also a source of concern since they can produce secondary metabolites with different bioactivities, including a variety of toxic compounds. Among them, the microcystins are the most common as they are produced by the most common bloom-forming species *Microcystis* and *Planktothrix*. In recent years, we evidenced that the production of microcystins is not always linearly correlated to growth (Bortoli *et al*. 2014) and that their synthesis is regulated by environmental factors (e.g. temperature, light, nutrient availability) (Pearson et *al*. 2016). Nevertheless, these studies on microcystins did not consider the overall metabolome. A broad survey of secondary metabolites can be helpful to better understand the biosynthesis and regulation of microcystins. With the coming of high-resolution mass spectrum technologies, such a survey is now possible. In this context, we investigated the regulation of metabolite production in *Planktothrix* during growth and under stress conditions.

We analysed the variation in metabolites production by cyanobacteria during the different growth phases. Univariate analysis indicated that time had a significant effect on the production of more than 50% of the metabolites. Moreover, metabolic profiling revealed three distinct temporal patterns of production characterizing the exponential, stationary and declining phases.

The cultures exposed to stress conditions (high light intensity of 150 µmol/m^2/s or high temperature of 33°C) showed different metabolomic responses of *Planktothrix*. The identification of metabolites presenting an increased production after high temperature or high light exposure could permit to unravel the biological role of secondary metabolites.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-170

Untargeted metabolomics-based analysis of HAB-forming dinoflagellate cultures

C. Alves-de-Souza [1], R. York [1], C. wingert [1], C. Tomas [1], J. L. Wright [2], W. Strangman [2,*]
[1]Algal Resources Collection, MARBIONC , [2]Biomolecular Discovery Group, MARBIONC , University of North Carolina Wilmington, Wilmington, USA

Abstract: Marine dinoflagellates are extremely complex organisms. They have large, highly divergent genomes that greatly hinder their ability to be analyzed through traditional sequencing methods. However, insight into their metabolite production is of critical importance as they play a large ecosystem role and produce a wide array of extremely potent and chemically diverse toxins. Mass spectrometry-based untargeted metabolomic analysis is emerging as one of the best ways to broadly examine the diversity of toxins and other compounds. To this end, we have examined a large sampling of diverse dinoflagellate genera and species currently in culture in the Algal Resources Collection at UNCW's MARBIONC. Primary metabolomic analysis gives us an idea of chemical diversity within the collection. By diving deeper into strain specific statistical data, we can then highlight compounds playing a significant role in the organism's chemical production. Further screening of these compounds against electronic libraries enables us to rapidly identify known products and highlight those that may be novel. This process is further enhanced through the application of molecular networking, which automates fragmentation matching to more accurately assign identities and chemical relationships between compounds. Preliminary results have demonstrated the diversity of metabolite production across taxa and in-depth investigations have uncovered novel marine toxins including new spirolide cyclic imine toxins and the second reported member of the portimine family of cytotoxins. Results of this ground-breaking study, including the application of molecular networking to compound identification and discovery will be presented.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-171

Combining genomic and metabolomic approaches to study the diversity of cyanobacterial secondary metabolites

B. Marie [1,*], S. Kim Tiam [1], M. Gugger [2], J. Demay [1], S. Le Manach [1], C. Duval [1], C. Bernard [1]
[1]UMR 7245 MCAM, CNRS / MNHN, [2]Collection de cyanobactéries, Institut Pasteur, Paris, France

Abstract: Cyanobacteria are an ancient lineage of slow-growing photosynthetic bacteria and a prolific source of natural products with diverse chemical structures and potent biological activities, such as acute toxicity. However, the chemical identification of these compounds remains a major bottleneck. Strategies to prioritize the most prolific strains and novel compounds are of great interest. Here we combine chemical analysis and genomics to investigate the chemodiversity of produced secondary metabolites based on their pattern of distribution within selected cyanobacteria within the same genus.

The chemical diversity of the metabolites produced by four strains was evaluated using an untargeted metabolomics strategy with high-resolution LC-MS analyses. Metabolite profiles were correlated with whole-genome sequences of the respective strains. Although, the strains present a global similarity in term biosynthetic cluster gene, comprising microcystins, microginin, anabaenopeptins, aeruginosin, prenylagaramide, microviridin and others, we found remarkable specific strain chemodiversity. Features were additionally dereplicated by tandem mass spectrometry (MS/MS) networking to identify molecular families of the same biosynthetic origin, as well as to group analysed molecules with known standards in similarity MS/MS-fragment pattern clouds, and to propose annotations for a large set of corresponding unknown compounds. Only few of the chemical features were common to several strains.

In conclusion, we demonstrated the usefulness of this combined approach in a group of four closely related strains of *Planktothrix*, a genus known to produce a broad spectrum of toxins and bioactive compounds. This efficient, integrative strategy allows to elucidate the chemical richness of cyanobacteria and link the data obtained from analytical chemistry to bio-synthetic genes of cyanobacteria.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-172

Metabolic profiles of cyanobacteria isolated from Italian perialpine lakes

L. Cerasino [1,*], N. Salmaso [1]
[1]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy

Abstract: A comparative profiling of the secondary metabolism of cyanobacteria isolated from Italian perialpine lakes has been attempted. Cultures of *Aphanizomenon flos-aquae*, *Dolichospermum lemmermannii*, *Microcystis aeruginosa*, *Planktothrix rubescens*, and *Tychonema bourrellyi* were obtained from lakes Garda, Idro, and Caldonazzo. LC-MS/MS analysis were used for characterizing the strains’ extracts. A target analyis aimed at assessing the the toxic profile (microcystins, nodularins, anatoxins, cylindrospermopsins, PSP) was performed; it revealed the production of toxic peptides (microcystins) in *M. aeruginosa* and *P. rubescens*, of toxic alkaloids (anatoxin-a and possibly some paralytic shellfish toxins) in *T. Bourrellyi*, and finally no production of toxins in *Aph. flos-aquae* and *D. Lemmermannii*. An untargeted analysis aimed at comparing the capability of the cyanobacterial species to produce peptidic metabolites, allowed the detection of over 300 different peptides, in a mass range between 400 and 2000 Da. The majority of compounds with masses between 500 and 1200 Da (corresponding to many non-ribosomal peptides) was produced by the two species *M. aeruginosa* and *P. rubescens,* which show a much higher capability of producing these compounds compared to the others. Microcystins, aeruginosins and anabaenopeptins were the most represented classes of compounds.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

O-173

Associated bacteria of the bloom-forming cyanobacterium Microcystis : challenging the concept of axenic culture

Y. Terrat [1,*], O. Perez [1], A. Giani [2], N. Fortin [3], N. Tromas [1], J. Shapiro [1]

[1]Biologie, Université de Montréal, Montréal, Canada, [2]Departemento de Botanica, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [3]NRC, Montréal, Canada

Abstract:

So far, *Microcystis* have been considered as single genomic entities that were able to capture occasionally some bacteria in their colonies. However, recent studies revealed that even the putative “axenic” or so-called “pure” cultures host various types of non-cyanobacteria strains. These heterotrophic bacteria may complement cyanobacteria for important functions as nitrogen and sulfur cycles for instance. In our study, we conducted a comprehensive metagenomic analysis of *Microcystis* culture isolates to investigate the diversity of these associated bacteria and to predict their putative functional role. We performed high throughput sequencing of 11 isolates from Brazil and 22 from Canada. None of our isolates were free of bacteria and we identified from 3 up to 12 associated bacteria per sample, representing ~5% up to ~30% of the sequencing data. While a few taxa are almost systematicaly identified in the associated community, thus constituing the core pool, a significant portion of taxa originates from a local pool. This geographical pattern in the bacterial community composition could reflect the local adaptation of the *Microcystis* colonies. In total, we were able to reconstruct ~50 high-quality MAGs (Metagenomic associated genomes) among 80 bins, belonging to more than 20 distinct taxa. The presence of these associated bacteria considerably expand the genomic vocabulary of a single *Microcystis* genome, as demonstrated by ortholog gene clustering analysis. In this study, we also addressed the question of the stability of these associations using sequencing replicates isolated years apart. We demonstrated that most of these associations are highly stable, supporting the hypothesis of mutualistic interactions between *Microcystis* and their associated bacteria. Preliminary results also reveal the existence of horizontal gene transfer events between associated bacteria and *Microcystis* genomes, supporting the existence of a close and long-term biological interaction.

Our current data suggest that the presence of these associated bacteria is not occasional but systematic. These associations clearly challenge the concept of “pure” culture and demonstrate the necessity to study this set of interdependent organisms to be able to fully understand the dynamics of algal blooms.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-174

Marine toxic dinoflagellate release extracellular vesicles

E. Garcés [1,*], E. Alacid [1], R. Gallisai [1], L. López-Puerto [2], A. Jiménez-Alesanco [2], D. Closa [2]
[1]Marine Biology and Oceanography, Institut de Ciències del Mar, [2]Institut d'Investigacions Biomèdiques de Barcelona, CSIC, Barcelona, Spain

Abstract: Effective communication between microorganisms in the marine environment is needed to mediate many ecological processes. Recent research has shown that extracellular vesicles (EVs) are produced by cyanobacteria and haptophytes and these vesicles are reported as vectors to deliver biologically active molecules involved in trophic interactions. But, little is known about the involvement of EVs in microorganism physiology and population dynamics. In this study, we explored the production of EVs by a microalgal culture of *Alexandrium minutum,* dinoflagellate responsible for large-scale coastal blooms and paralytic shellfish poisoning, a human disease that has adversely impacted many coastal ecosystems worldwide. The presence of EVs during the exponential phase of culture growth was assessed in isolated particles that were lipid-stained and then examined by transmission electron microscopy. Fluorescence images revealed stained vesicles, and the micrographs showed spherical vesicles (exosome-like structures) <200 nm in diameter. Our discussion addresses the potential role of EVs in population-relevant processes such as intercellular communication.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-175

Chemically mediated interactions between *Microcystis* and *Planktothrix*: impact on their physiology and metabolic profiles

E. Briand [1,*], S. Reubrecht [2], F. Mondeguer [1], M. Sibat [1], P. Hess [1], Z. Amzil [1], M. Bormans [2]
[1]IFREMER, Nantes, [2]Université de Rennes 1, Rennes, France

Abstract: Freshwater cyanobacteria are well known for their ability to produce a wide variety of bioactive compounds, some of which have been described as allelochemicals. There is growing evidence that these secondary metabolites play an important role in shaping community composition through biotic interactions; however, for the most part, their biological role and mode of regulation of the production are poorly understood. In temperate eutrophic freshwaters, *Microcystis* and *Planktothrix* may co-occur, with *Planktothrix* being an early colonizer and *Microcystis* appearing subsequently. We tested if the production of a range of peptides by co-existing species could be regulated through interspecific interactions. Using a combined approach of co-cultures and analyses of metabolic profiles, we investigated chemically mediated interactions between two cyanobacterial strains, *M. aeruginosa* PCC7806 and *P. agardhii* PCC7805. More precisely, we evaluated changes in growth, morphology and metabolite production and release by both interacting species. Co-culture of *Microcystis* with *Planktothrix* resulted in a reduction of the growth of *Planktothrix* together with a decrease of its trichome size and alterations in the morphology of its cells. However, the production of intracellular compounds by *Planktothrix* was not different between mono and co-culture conditions. Concerning *Microcystis*, the number of intracellular compounds was higher under co-culture condition than under monoculture. Overall, *Microcystis* produced a lower number of intracellular compounds under monoculture than *Planktothrix*, and a higher number of compounds than *Planktothrix* under co-culture condition. Our investigation did not allow us to identify specifically the compounds causing the observed physiological and morphological changes of *Planktothrix* cells. However, altogether, these results suggest that co-culture induces specific compounds as a response by *Microcystis* to the presence of *Planktothrix.* Further studies should be undertaken for identification of such potential allelochemicals.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-176

Release of allelochemicals facilitates nutrient uptake from co-occurring phytoplankton in dinoflagellate *Alexandrium ostenfeldii*.

E. Lindehoff [1,*], E. Svahn [1], J. Jerney [2], C. Legrand [1], S. Suikkanen [2], A. Kremp [3]

[1]Centre for Ecology and Evolution in Microbial Model Systems- EEMiS, Dept of Biology and Environmental Science, Linnæus University, Kalmar, Sweden, [2]Marine Research Centre, Finnish Environment Institute, Helsinki, Finland, [3]Leibnitz Institute for Baltic Sea Research Warnemuende, Roshtock, Germany

Abstract: The toxic dinoflagellate *Alexandrium ostenfeldii* forms dense blooms in shallow waters of the coastal Baltic Sea proper during late summer, when concentrations of inorganic nitrogen (N) are low and dissolved organic N (DON) high. If mixotrophic, i.e have the ability to utilize DON, vegetation or phytoplankton derived DON could drive these *A. ostenfeldii* blooms. Mixotrophy in Baltic *A. ostenfeldii*was confirmed by ^{15}N-tracer experiments that showed uptake and preference for several N species. Phytoplankton derived DON was prepared from co-occurring cryptophyte *Rhinomonas nottbecki*, pre-cultivated on $^{15}NO_3$ and lysed by the released allelochemicals of *A. ostenfeldii*. We visualized the direct uptake of phytoplankton derived ^{15}N-DON using second iron mass spectrometry (SIMS) and screened *A. ostenfeldii* strains isolated from different bloom phases for a correlation between allelopathic activity (EC_{50}) and uptake of phytoplankton derived ^{15}N-DON. Our findings support the concept that dense blooms of *A. ostenfeldii* utilize DON as N source and that it's allelopathic activity facilitates N uptake.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-177

ALLELOPATHIC INTERACTIONS WITHIN A BENTHIC COMMUNITY: EFFECTS OF POLYUNSATURATED ALDEHYDES ON THE DINOFLAGELLATE OSTREOPSIS CF. OVATA

L. Pezzolesi [1,*], S. Accoroni [2], C. Samorì [3], F. Rindi [2], R. Pistocchi [1], C. Totti [2]

[1]Department of Biological, Geological and Environmental Sciences, University of Bologna, Ravenna, [2]Department of Life and Environmental Sciences, Università Politecnica Delle Marche, Ancona, [3]Department of Chemistry «Giacomo Ciamician», University of Bologna, Bologna, Italy

Abstract: A variety of algal species are known to produce chemical compounds involved in the regulation of population dynamics, interactions among microalgal species, regulation of invertebrate epiphytism, lethal and sublethal effects on other organisms, and antimicrobial activities. Diatoms have been shown to produce and release a wide range of secondary metabolites that mediate interactions between organisms of different groups. Among these compounds, fatty acid derived polyunsaturated aldehydes (PUAs) have been related to multiple functions such as intra- or interspecific signals and adverse effects on the reproduction of marine invertebrates. Studies reported that a number of planktonic diatoms produce PUAs, but little is known about the production of these compounds by benthic microalgae and macroalgae.

In the present study, some benthic diatoms and macroalgae of the Conero Riviera (northern Adriatic Sea, Italy) were studied, attesting their capacity to produce several PUAs, including some with four unsaturations, such as decatetraenal, undecatetraenal and tridecatetraenal, or with long carbon chains (i.e. 16 carbon atoms). The intracellular unsaturated aldehydes (UAs) concentration ranged from 1.8 to 154.4 fmol cell^{-1}, varying with the culture stage and among the diatom species. In *Navicula* sp. octenal was the most abundant compound (94%), while hexadienal contributed up to 51 and 39% of the total UAs in *Tabularia affinis* and *Proschkinia complanatoides*, respectively. Among macroalgae, *Dictyopteris polypodioides* and *Ulva rigida* resulted of particular interest for the PUAs diversity (e.g. long-chained compounds) and quantity. Moreover, the effects of some PUAs (heptadienal, octadienal and decadienal) commonly produced by phytoplankton were tested on the toxic benthic dinoflagellate *Ostreopsis* cf. *ovata,* which causes harmful blooms along the Mediterranean coasts and typically along the Conero Riviera in late summer. A decrease of *O*. cf. *ovata* growth was observed, particularly in the presence of longer-chain PUAs than with shorter-chain ones. In addition, microscopic analysis highlighted an increase of abnormal forms (up to 79%) in *Ostreopsis* cells exposed to high concentrations of decadienal (9, 18, 36 µmol L^{-1}), a gradual DNA degradation, and an increase of lipids with all tested PUAs.

This study highlights that various PUAs can be produced and released by diatoms at late growth stage and by macroalgae, potentially affecting benthic invertebrates and/or the succession of other algae, including toxic bloom-forming species, such as *O*. cf. *ovata*. The observed effects of PUAs on the growth, cytological features and cell morphology of this dinoflagellate suggest their potential role in the inhibition of *Ostreopsis* cell proliferation, raising interest on the potential effects of these compounds in the benthic marine environment.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-178

Compositional and functional dynamics of *Microcystis* bloom associated microbiomes in response to nutrient and temperature elevation

J. Jankowiak [1,*], C. Gobler [1]

[1]School of Marine and Atmospheric Science, Stony Brook University, Southampton, USA

Abstract: The toxic cyanobacterium *Microcystis*, one of the most pervasive cyanoHAB genera, naturally occurs in colonies which harbor diverse microbiomes of heterotrophic bacteria and eukaryotic epibionts. Members of this assemblage have been proposed to influence bloom formation, maintenance, and demise as well as toxicity and colony morphology. While extensive research has been conducted examining the effects of nutrient loading and climatic warming on cyanobacteria, there remains a limited understanding of how these environmental drivers alter the structural and functional potential of the microbial assemblages associated with blooms and, in turn, impact cyanoHAB proliferation and toxicity. To gain further insight into these interactions, here next-generation amplicon sequencing of the 16S and 18S ribosomal rRNA genes was used to characterize the bacterial (16S) and eukaryotic (18S) assemblages during *Microcystis* blooms in three temperate US lakes: Lake Erie, Lake Agawam (Long Island, NY) and the Lake in Central Park (Manhattan, NY)), comparing the free living and *Microcystis*-attached fractions. The microbial assemblages were examined through spatial/temporal monitoring to capture nutrient/temperature gradients, paralleled with amendment experiments to assess the direct effects of N, P and T elevation on the naturally occurring communities. Proteobacteria, Planctomycete, Bacteroidetes, and the Armatimonadetes were among the most abundant phyla present in the prokaryotic assemblages, for which non-metric multidimensional scaling analysis identified significant differences between communities within the *Microcystis*-colonies and free-living in the water column. Of the 738 unique prokaryotic genera identified, 45 were significantly enriched in the free-living fraction, including members from the Actinobacteria, Bacteroidetes, Verrucomicrobia, Chlorobi, and Proteobacteria, several of which have been purported to be involved in cell lysis and microcystin degredation. Within the colonies 25 bacterial genera were significantly enriched, largely from the Proteobacteria but including Armatimonadetes, Acidobacteria, Gemmatimonadetes and Plactomycetes, with functions related to macromolecule degradation (i.e. cellulose, amino acids) and N/P cycling, including nitrogen fixation. This attached-assemblage may play a role in the ability of non-diazotrophic *Microcystis* bloom persistence under N deplete conditions. Lower variation was observed among the eukaryotic communities, with only 21 of 608 genera significantly differentially abundant between the free-living and attached communities, 13 of which were enriched within the colonies. Among these genera were predatory amoebas (Flamella) and ciliates (colpodea), parasitic protists (Eugregarinorida), and choanoflagellates which use the colony for buoyancy to feed filter free livening bacteria.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-179

Effects of allelochemical in *Phaeodactylum tricornutum* filtrate on typical marine microalgae growth, morphology and physiology

Q. Xue [1,*], J. Wang [1], R. Wang [2]

[1]Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao, [2]Shenzhen Key Laboratory for the Sustainable Use of Marine Biodiversity, Research Centre for the Oceans and Human Health, City University of Hong Kong Shenzhen Research Institute, Shenzhen, China

Abstract: The effects of an allelochemical extracted from the culture filtrate of diatom *Phaeodactylum tricornutum* on the *Heterosigma akashiwo*, *Prorocentrum donghaiense* and *Dunaliella salina* were investigated by checking a series of morphological, physiological and biochemical characters. Growth experiments showed that *H. akashiwo* and *P. donghaiense* were significantly inhibited after exposure to the allelochemical. The effects of the allelochemical on the surviving cells were explored using Scanning Electron Microscopy (SEM) and Flow cytometry (FCM) for examination of a suite of physiological parameters (membrane integrity, esterase activity, membrane potential).

The results demonstrated that the membrane of *H. akashiwo* and *P. donghaiense* were attacked by the allelochemical directly, causing cell membrane breakage and loss of integrity. Esterase activity was the most sensitive indicator of the impacts of the allelochemical. Membrane potential showed significant decreases following exposure of the *Heterosigma* cells to high concentrations of the allelochemical for 5 and 6 days. The cell size of *H. akashiwo* did not change compared to the *P. donghaiense* which increased. However, the growth of *D. salina* was not inhibited by extracts from the filtrate of *P. triconutum* significantly. Both the cell size and membrane integrity did not change compared with the control cultures, and the extracts only induced negative effects on the esterase activity of *D. salina* after 6 days exposure.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-180

The role of decadal surface water warming in the intensification of marine and freshwater harmful algal blooms

C. J. Gobler [1,*], O. Doherty [2], T. Hattehrath-Lehmann [3], T. Davis [4], A. Griffith [1], Y. Kang [1]

[1]School of Marine and Atmospheric Sciences, Stony Brook University, Southampton, [2]Eagle Rock Analytics, Sacramento, [3]New York State Department of Health, Albany, [4]Biological Sciences, Bowling Green State University, Bowling Green, USA

Abstract: Global temperatures are rising, yet the impacts of such changes on harmful algal blooms (HABs) are not fully understood. We have used high-resolution ocean and lake surface temperature records during the past three decades and temperature-dependent growth rates of multiple HAB species to evaluate recent changes in the phenololgy and growth rates of HABs from marine waters in the North Atlantic and North Pacific Oceans and from the Great Lakes of North America. For toxigenic HABs including *Alexandrium catenella* and *Dinophysis acuminata*, increasing temperatures since 1982 have facilitated increased potential growth rates and a significant increase in the duration of bloom seasons within many coastal Atlantic regions between 40°N and 60°N where incidents of these HABs have emerged and expanded in recent decades. For *Cochlodinium* (*Margalefidinium*) *polykrikoides*, temperature-dependent, bloom-favorable conditions have become established earlier and are persisting longer within the Northeast US. Similar trends were observed for regions in and around the Sea of Japan where potential growth rates and bloom season duration displayed significant positive correlations with rising sea surface temperatures since 1982 and bloom events documented by the Korean government have become more intense. In Lake Erie, blooms of the toxigenic cyanobacteria, *Microcystis*, have intensified this century as temperature increases have facilitated an earlier start to the bloom season and more rapid summer growth rates. Collectively the findings suggest that in ecosystems where requisite physical, chemical, and biological conditions for bloom formation persist, rising temperatures are an important factor facilitating the intensification and expansion of multiple marine and freshwater HABs.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-181

Cyanobacterial dominance challenges the integrity and functioning of phytoplanktonic communities in periurban lakes

A. Escalas [1,*], A. Catherine [1], S. Saloufi [1], M. Cellamare [1 2], S. Hamlaoui [1], C. Yepremian [1], A. Couté [1], C. Louvard [1], M. Troussellier [3], C. Bernard [1]

[1]UMR 7245 Molécules de Communication et Adaptations des Microorganismes, Muséum National d'Histoire Naturelle, [2]Phyto-Quality, PARIS, [3]UMR 9190 MARBEC IRD-CNRS-UM-IFREMER, University of Montpellier, Montpellier, France

Abstract: Occurrences of bloom-forming and potentially toxin-producing Cyanobacteria are expected to increase in the future under the combined influence of climate change and the ever increasing human pressures on natural ecosystems. This is of particular concern in periurban lakes that are close to human populations and support numerous ecosystem services (*e.g.* water supply, fishing and recreational activities). Here, we sampled phytoplanktonic communities across four summer campaigns in a set of 50 lakes from the Île-de-France region in order to determine (1) the level of Cyanobacterial presence and dominance in the region, (2) its impact on the other phytoplanktonic groups and their co-occurrences, and ultimately (3) the consequences for the functioning of these communities. We tested the impact of environmental variables (e.g. temperature, nutrient loads, water transparency, depth, pH) and community characteristics (richness, dominance, cohesion) on communties functioning (biomass production and resource use efficiency) using used multiple regression approach. Then we estimated community cohesion, *i.e.* the amount of inter-species interactions, and constructed co-occurrence networks to determine the effect of cyanobacterial dominance on the integrity of communities. Our results reveal that bloom-forming and potentially toxin-producing Cyanobacteria are widespread in the region (> 33% of the lakes) and represent an important proportion of the biomass in many communities. We show that Cyanobacteria have a weaker interaction potential compared with other phytoplanktonic groups with consequences on the amount of inter-species interactions and the cohesion of primary producers communities. The presence and dominance of Cyanobacteria significantly influences the functioning of phytoplanktonic communities, and modifies their structure by reducing the number of species, increasing drastically the proportion of negative inter-species associations and reorienting the biomass toward non-central nodes in the network. Taken together, these results suggest that cyanobacterial dominance in this region is shifting communities toward trophic dead ends through modification of the structure of association between primary producers.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-182

Dynamics of large-scale dinoflagellate blooms in association with the branch of Kuroshio in the East China Sea

R.-C. Yu [1 2 3,*], Y. Zhao [1 3], Z.-F. Chen [1 3], Z.-X. Zhou [3], F.-Z. Kong [2 3], Q.-C. Zhang [2 3], M.-J. Zhou [3]

[1]University of Chinese Academy of Sciences, Beijing, [2]Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, [3]CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China

Abstract: In the coastal waters adjacent to the Changjiang River estuary, large-scale blooms of dinoflagellates, such as *Prorocentrum donghaiense*, *Karenia mikimotoi* and *Alexandrium tamarense*, started to appear from the beginning of the 21st century. Previous studies found that coastal eutrophication associated with excess nitrate input from the Changjiang River was the most important factor leading to the formation of large-scale dinoflagellate blooms. The sea area adjacent to the Changjiang River estuary, however, is also subjected to the impacts of currents from the open ocean, such as the Nearshore Kuroshio Branch Current (NKBC) in the East China Sea (ECS). Kuroshio is the strongest west boundary current of the Pacific Ocean flowing northeastwards along the steep continental slope of the ECS, and NKBC is a branch of Kuroshio formed in the sea area northeast to the Taiwan Island. Based on the investigations organized in the last 5 years, dynamics of dinoflagellate blooms in association with NKBC was studied. The intrusion of NKBC from the main stream of Kuroshio to the sea area adjacent to the Changjiang River estuary could be observed and clearly indicated by the distribution of cyanobacterium *Prochlorococcus* in the ECS. Examination of phytoplankton samples collected from Kuroshio waters using metagenomic approaches revealed the presence of potential bloom-forming microalgae. Meanwhile, year-round investigations in the sea area adjacent to the Changjiang River estuary from February, 2015 to January, 2016 indicated strong seasonal variation of NKBC, and the occurrence of *Prorocentrum donghaiense* bloom in 2015 was closely associated with the seasonal variation of NKBC. The results suggest that dynamics of dinoflagellate blooms in the coastal waters adjacent to the Changjiang River estuary is under the regulation NKBC, and could be subjected to the interannual variation of NKBC and long-term changes of Kuroshio under the forcing of global climate change. The findings clearly point to the need for a long-term monitoring network to watch both harmful algal blooms and the variation pattern of Kuroshio and its branches in the ECS.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-183

Use of three-dimensional modelling to assess the spatial and temporal dynamics of cyanobacteria in a Mediterranean reservoir

N. Sharaf [1,*], A. Fadel [2], B. Lemaire [3], K. Slim [4], B. Vinçon-Leite [3]

[1]PhD student, LEESU, MA 102, Ecole des Ponts, AgroParisTech, UPEC, Champs-sur-Marne, France, [2]National Center for Remote Sensing, National Council for Scientific Research (CNRS), Beirut, Lebanon, [3]LEESU, MA 102, Ecole des Ponts, AgroParisTech, UPEC, Champs-sur-Marne, France, [4]Laboratory of Microorganisms and Food Irradiation, LAEC, Lebanese Atomic Energy Commission, National Council for Scientific Research , Beirut, Lebanon

Abstract:

The 18th International Conference on Harmful Algae, 21-26 October, Nantes, France

---

Use of three-dimensional modelling to assess the spatial and temporal dynamics of cyanobacteria in a Mediterranean reservoir

Najwa Sharaf[1, 2, 3], Ali Fadel [2], Bruno J. Lemaire [1], Kamal Slim [3], Brigitte Vinçon-Leite [1]

[1] LEESU, MA 102, Ecole des Ponts, AgroParisTech, UPEC, Champs-sur-Marne, France

[2] National Center for Remote Sensing, National Council for Scientific Research (CNRS), P.O. Box 11-8281, Riad El Solh, 1107 2260 Beirut, Lebanon

[3] Laboratory of Microorganisms and Food Irradiation, LAEC, Lebanese Atomic Energy Commission, National Council for Scientific Research (CNRS), P.O. Box 11-8281, Riad El Solh, 1107 2260 Beirut, Lebanon

Abstract

Cyanobacterial blooms deteriorate the water quality and impair the use of lakes and reservoirs. They also threaten human health through toxin production. Therefore, regular monitoring of cyanobacterial biomass is critical for bloom detection. In Karaoun Reservoir, the largest freshwater body in Lebanon, cyanobacteria have been strongly dominating phytoplankton since 2009. In their seasonal trend, *Microcystis aeruginosa* dominates in summer while *Aphanizomenon (Chrysosporum) ovalisporum* occurs in spring and autumn.

The presence of these blooms hampers Karaoun reservoir services including fisheries, recreational uses and irrigation. Understanding how cyanobacteria respond to water level fluctuations and to the thermal structure of the reservoir is important for adequate management. In this study, we aim to use modelling in order to complement field monitoring and determine factors governing cyanobacteria distribution.

In order to calibrate and validate the adopted model, field campaigns were conducted in 2017 and consisted of measuring cyanobacteria distribution with phycocyanin fluorescence profiles, water temperature profiles and water transparency at three locations in the reservoir. These campaigns allowed us to analyze the spatial and temporal variability of cyanobacteria distribution. We used a coupled three dimensional hydrodynamic-ecological model, the Delft 3D suite, to simulate water level fluctuations, water temperature and cyanobacterial biomass. Eventually we compared the spatial distribution of cyanobacteria from field measurements and simulation results. Model simulations helped explain the influence of hydrodynamic processes that led to observed vertical and horizontal heterogeneities of cyanobacteria. We provide a thorough discussion about the contribution of modelling techniques to this study.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-184

Modelled distribution of the toxin-producing dinoflagellate *Alexandrium ostenfeldii* in the Baltic Sea under current and future climates

A. H. Le Tortorec [1 2], A. Kremp [6,*], E. Lindehoff [1 3], E. Virtanen [1], E. Le Tortorec [4], S. G. H. Simis [5]

[1]Finnish Environment Institute, Marine Research Centre, [2]Department of Environmental Sciences, University of Helsinki, Helsinki, Finland, [3]Centre for Ecology and Evolution in Microbial model Systems - EEMiS, Department of Biology and Environmental Science, Linnæus University, Kalmar, Sweden, [4]Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland, [5]Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth, United Kingdom, 6Leibnitz Institute for Baltic Sea Research Warnemuende, Roshtock, Germany

Abstract: The toxin-producing dinoflagellate Alexandrium ostenfeldii has expanded its distribution in coastal waters worldwide during the last decade. In this study , we use species distribution modelling (SDM) to model A. ostenfeldii habitats in Finnish coastal waters based on a diverse set of presence observations (micoscopic, resting stages, bioluminescence, genetic markers) and a set of environmental predictors. Using maximun entropy presence-only modelling it is shown that the primary environmental variables explaining A. ostenfeldii occurence are the ratios of total vs inorganic N and P, depth, depth exposure, distribution of bottom vegetation and temperature. The known habitat for A. ostenfeldii around the Åland island group and Hanko peninsula were well reproduced by the model. In addition, the model indicated previously unknown but potentially suitable habitats in the shallow bays east of Helsinki in the Gulf of Finland and in the Bothnian Sea. Climate change scenarios for salinity and temperature showed a decrease in potential habitats of the species, but these results should be interpreted with caution given the narrow salinity range in present observations in contrast to laboratory studies.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

O-185

Modeling current and future habitat of *Karlodinium veneficum* and *Prorocentrum minimum* in Chesapeake Bay, USA

P. Glibert [1,*], M. Li [1], W. Ni [1], C.-H. M. Lin [1], F. Zhang [1]

[1]University of Maryland Center for Environmental Science, Cambridge, USA

Abstract: *Karlodinium veneficum* and *Prorocentrum minimum* commonly bloom in the mid to upper reaches of Chesapeake Bay, USA, and have been associated with eutrophication. Here, a habitat model was developed for these species based on physical (temperature, salinity) and nutrient criteria, applied in a coupled ROMs (Regional Ocean Modeling System) and biogeochemical model. The criteria for each species were developed based on laboratory relationships; these species differ in their temperature optima (*Karlodinium*- warmer; *Prorocentrum* – cooler) and nutrient preferences (*Karlodinium*- more organic nutrients and mixotrophy; *Prorocentrum*- more inorganic nutrients). The biogeochemical model includes multiple phytoplankton functional groups, separate cycling of N (by form), P, Si and C, and is coupled to a sediment diagenesis model; 20 layers in the water column and 23 biogeochemical variables are simulated. Current modeled habitat based on physical conditions alone compared favorably with the spatial and temporal distribution of these HAB species, based on long term data, but tended to overestimate habitat suitability during some seasons, especially *Prorocentrum* in fall. Using 3 different climate models downscaled for the region, projections were made of future habitat, in 2050 (10 yr average). Future projections suggest wetter, more nutrient rich springs but drier, warmer summers. Nutrient proportions and ratios also change seasonally in these future projections. Based on the modeled future change in habitat, a summer reduction in *Karlodinium* was projected, moving blooms into the upper reaches of the Bay. In contrast, an increase in habitat suitability for *Prorocentrum* blooms was projected, with an earlier spring occurrence. A mechanistic model for these species, including different nutritional modes (autotrophy and mixotrophy) is also being developed to increase predictability. Once fully coupled, these models will provide a useful tool that can be applied to test specific scenarios with regard to how habitat will change temporally and spatially with different nutrient reduction efforts and climate change.

Disclosure of Interest: None Declared

Biotechnology or adding value to toxic and harmful algae

O-186

Fluorescence activated cell-sorting as valuable tool for microalgal biotechnology.

S. D'adamo [1,*], I. T. Dominguez Cabanelas [1], C. Südfeld [1], M. Barbosa [1], R. Wijffels [1]

[1]Bioprocess Engineering & AlgaePARC, Wageningen University and Research, Wageningen, Netherlands

Abstract: Microalgae have caught industrial interest as promising production platforms for several high-value compounds such as fatty acids, hydrocarbons, pigments, toxins and polysaccharides. Although, in order to become an economically feasible chassis, these microorganisms must be optimized in solar energy conversion, carbon capture and utilization, and the partitioning of metabolic fluxes [1]. Genetic engineering could systematically address these obstacles and consolidate bioprocessing organisms that are able to efficiently transform sunlight to refined value-added chemicals. With the advancement of biotechnology, tools and techniques for the genetic manipulation and isolation of microalgae are expanding quickly. These methodologies are still time-consuming, and often it is necessary to wait for several months before selecting cells with improved traits. In this context, technologies exploiting fluorescence-activated cell sorting (FACS) are becoming a popular method in microalgal biotechnology for fast screening of cells with desired characteristics [2]. FACS can sort cells based on multiple fluorescence and light scattering parameters, which makes it a versatile technology. Here we discuss some of the potentials of this emerging technique and report our method development and applications. In particular, we established an efficient staining methods for FACS to detect high-lipid containing cells and to guarantee cellular viability of microalgal strains (i.e. Chlorococcum litorale, Nannochloropsis oceanica), by using BODIPY505/515 fluorescent dye [3]. By taking advantage of the natural genetic variability or, by inducing mutations in a parental population, we were able to screen and sort for cells with abnormal and improved features [4]. We were also able to sort transformant lines in high-throughput mode, by using fluorescent protein expression.

[1] V. H. Work et al., “Biocommodities from photosynthetic microorganisms,” Environ. Prog. Sustain. Energy, vol. 32, no. 4, pp. 989–1001, 2013.
[2] H. Pereira, P. S. C. Schulze, L. M. Schaler, T. Santos, L. Barreira, and J. Varela, “Fluorescence activated cell-sorting principles and applications in microalgal biotechnology,” Algal Res., vol. 30, no. December 2017, pp. 113–120, 2018.
[3] I. T. D. Cabanelas, M. van der Zwart, D. M. M. Kleinegris, M. J. Barbosa, and R. H. Wijffels, “Rapid method to screen and sort lipid accumulating microalgae,” Bioresour. Technol., vol. 184, pp. 47–52, 2015.
[4] I. T. D. Cabanelas, M. Van Der Zwart, D. M. M. Kleinegris, R. H. Wijffels, and M. J. Barbosa, “Sorting cells of the microalga Chlorococcum littorale with increased triacylglycerol productivity,” Biotechnol. Biofuels, vol. 9, no. 1, pp. 1–12, 2016.

Disclosure of Interest: None Declared

Biotechnology or adding value to toxic and harmful algae

O-187

Anaesthetic bioactives production in bioreactors with *Heterosigma akashiwo*

J. J. Gallardo [1,*], V. Avello [2 3], P. Aguilar [1], A. Rivera [2], A. Astuya [2], A. Llanos [2]

[1]Chemical Engineering, [2]Oceanography, University of Concepción, [3]Chemical Engineering, University of Concepcion, Concepción, Chile

Abstract: Toxic marine microalgae are an extraordinary source of high-value bioactives. So far toxicological, environmental and chemical research programs have been carried out on many biomolecules. Additionally, interesting bioactivities have been found although in most of the cases these findings did not lead to licensed drugs. The alleged main problems are the low biomass and bioactive yield, lack of knowledge about bioactive synthesis triggers and the cellular fragility. Indeed, given the cellular fragility of these microorganisms, their massive culture requires the use of bioreactors with specific configurations and modes of operation that eliminate or mitigate cellular damage due to excessive turbulence. In this work, we present a study of the production of anaesthetic and toxic bioactives from *Heterosigma akashiwo* during different growth phases in low-shear bioreactors. Cultures were carried out in control static flasks and in the bioreactor up to a volume of 12L. Bioactivity was assessed *in vitro* (Neuro-2a cell bioassay) and *in vivo* (Zebra fish model). Bioactivity depended on the growth phase for the static cultures. In these cultures, cell toxicity was increasing with culture time whereas a steady STX-like effect was only observed during the exponential phase. On the other hand, agitated cultures produced biomass of similar characteristics in the different growth phases. Bioreactor extract (total methanolic extract; 50 mg/L) showed low cytotoxicity and STX-like effect. In Zebra fish, in 24 h tests no mortality was observed. Although embryotoxicity was found in the order of commercial veterinary anaesthetics. The most probable cause is that the bioreactor hydrodynamic stress terminates aged cells. It is then believed that *H. akashiwo* cells mainly accumulate toxins at the end of the culture when nutrients are scarce and that the anaesthetic bioactives are constantly produced by the studied strain.

Acknowledgement

This research was supported by Conicyt, Chile (Fondecyt 2017, ref. 1170515). We also thank funding from the University of Concepción through the Research Project VRID 216.096.067-1.0IN, the INNBIO Platform and the COPAS Sur Austral (PFB-31/2007) Program.

Disclosure of Interest: None Declared

Biotechnology or adding value to toxic and harmful algae

O-188

Effects of Light, Temperature and Nutrients on Growth and Toxin Production of *A. spinosum*, *P. ima*, and *P. australis*

J. Kilcoyne [1,*], A. McCoy [1], S. Burrell [1], G. Gaiani [1], R. Salas [1], U. Tillmann [2]

[1]Marine Institute, Co. Galway, Ireland, [2]Helmholtz Centre for Polar and Mari, Am Handelshafen 12 (office: C-316), Alfred Wegener Institute, BREMERHAVEN, Germany

Abstract: Most shellfish production sites in Europe are prone to closures due to the accumulation of biotoxins produced by certain phytoplankton that are harmful to human health. Availability of purified toxins for monitoring and research is essential to ensure the long-term sustainability of this highly regulated industry. MARBioFEED is a three year project supported by the First Call for Transnational Research Projects within the Marine Biotechnology ERA-NET. The project involves algal biotechnology for the production of value-added products – marine biotoxin reference materials and fish feed.

One element of the project is focusing on the azaspiracids (AZAs), okadaic acid (OA) group and domoic acid (DA) toxins and their producing organisms. Cultures of a Scottish strain of *Azadinium spinosum* (AZAs) and Irish strains of *Prorocentrum lima* (OA group) and *Pseudonitzchia australis* (DA) were grown under different environmental conditions; light (light intensity and photoperiod), temperature (18 °C and 10 °C) and media (L1, F/2 and f10k) to assess the impact on cell growth and toxin production.

Preliminary results show that the lower temperature was found to significantly impact toxin production in the *A. spinosum* (confirming previous reports). In addition to the reported AZAs found in cultures, some other known and novel AZAs were found in the harvested biomass.

No significant differences were observed on either cell growth or toxin production in the *P. lima* strain when grown under the different temperatures. Cell growth was enhanced using L1 media and a longer photoperiod.

The lower temperature enhanced *P. australis* cell growth while toxin production was highest in the L1 media.

Disclosure of Interest: None Declared

Biotechnology or adding value to toxic and harmful algae

O-189

Metabolic modelling of microalgae during nitrogen starvation

F. Mairet [1,*], C. Baroukh [2], J.-B. Berard [1], G. Carrier [1], A. Charrier [1], M. Garnier [1], R. Kaas [1], E. Lukomska [1], E. Nicolau [1], C. Rouxel [1], N. Schreiber [1], B. Saint-Jean [1], O. Bernard [3], G. Bougaran [1]

[1]Physiology and Biotechnology of Algae Laboratory, Ifremer, Nantes, [2]LIPM, INRA, Castanet-Tolosan, [3]Biocore, Inria, Sophia-Antipolis, France

Abstract: Microalgae are promising microorganisms for cosmetics, pharmaceuticals, agrofood, energy… However, in many fields, production yields are yet to be improved to obtain a sustainable and cost-effective process. This goal can be achieved thanks to the powerful tools of mathematical modeling and optimization, which can help to understand the intracellular mechanisms at place.

Recently, a dynamic metabolic modeling framework – called DRUM – has been proposed in order to handle non-balanced growth condition and hence accumulation of intracellular metabolites (Baroukh et al. 2014). In this framework, we have added metabolic regulations (including organic carbon excretion and dissipation of energy) in order to consider the effect of nitrogen on growth and carbon storage. Our approach is validated with a high-throughput time series concerning the haptophyte *Tisochrysis lutea* submitted to nitrogen starvation and day/night cycles. This study highlights the need for metabolic modeling in dynamic conditions to better understand microalgal metabolism and potentially tune algae production.

Baroukh, C., Muñoz-Tamayo, R., Steyer, J.-P. & Bernard, O. 2014. DRUM : A Newn Framework for Metabolic Modeling under Non- Balanced Growth. Application to the Carbon Metabolism of Unicellular Microalgae. *PLoS One*. 9:e104499.

Disclosure of Interest: None Declared

Biotechnology or adding value to toxic and harmful algae

O-190

An innovative photobioreactor technology for the mass culturing of high value, slow growing toxic microalgae

B. York [1,*], A. Marti [2], W. Strangman [3], A. Cooke [1], C. Tomas [1], C. A. de Souza [1]

[1]Algal Resources Collection, Marbionc, University of North Carolina Wilmington, [2]Marine Science, University of North Carolina Wilmington, [3]Biomolecular Discovery Group, Marbionc, University of North Carolina Wilmington, Wilmington, USA

Abstract: The mass culture of high value, slow growing toxic microalgae in photobioreactors represents a challenging endeavor. As more marine products are being discovered from various microalgae, industry must overcome the problem of producing mass densities for the harvesting of biologically active material while using the fewest resources and space possible. We tested a fully autonomous 10L photobioreactor (IKA Algaemaster 10 control) developed through a collaboration between IKA Works, Inc. and the Algal Resources Collection at the University of North Carolina Wilmington. Cultures (10L) of the raphidophyte *Heterosigma akashiwo* and the dinoflagellates *Amphidinium gibbosum*, and *Alexandrium ostenfeldii* were grown in three replicates in both photobioreactors and static system (carboys). Mass-spectrometry based toxin analyses of the harvested cells was performed to assess the impact of photobioreactor culturing on production. Microalgae growth rates and maximal cell concentrations from photobioreactors were on average 3-fold greater than the best attainable value in the static system. To confirm the robustness of the system, 10 other toxic microalgal species were additionally successfully grown in the photobioreactors with similar results: *Alexandrium molinatum*, *A. minutum*, *Azadinium dexteroporum*, *Karenia brevis*, *Karenia selliformis*, *Karlodinium veneficum*, *Margalefidinium polykrikoides*, *Microcystis aeruginosa*, *Prymnesium parvum, Pseudochattonella verruculosa*). The IKA Algaemaster 10 control photobioreactor is flexible and easily adaptable. It reduces the footprint for growing high densities of algae and can be used directly as a low-cost experimental instrument for researchers, teachers, and industry to determine optimal growth conditions for a large variety of algae.

Disclosure of Interest: None Declared

Biotechnology or adding value to toxic and harmful algae

O-191

Enhanced accumulation of radioactive cesium (Cs) by a novel microalga with nanomagnetic composite

B.-G. Ryu [1,*]

[1]Applied Bioresources Research Division, Nakdonggang National Institute of Biological Resources (NNIBR), 137, Donam 2-gil, Sangju-si, Gyeongsangbuk-do, Republic of Korea

Abstract: Among the radioactive nuclides released by controlled methods or accidentally, there are serious concerns about 137-cesium (^{137}Cs) from nuclear fission due to its long half-life (approximately 30 years), its strong emission of gamma rays, its high water solubility, and its high bioavailability. The radiation stability and high ion-exchange capacity of Cs^+ can lead to bioaccumulation, with many terrestrial and aquatic organisms mistaking it for potassium ion (K^+). This has heightened the concern about radioactive Cs. Thus, there is increasing interest in developing effective and environmental-friendly methods for removal of radioactive Cs from contaminated aquatic ecosystems and radionuclide-containing wastewater. Conventional separation methods involving chemical precipitation and/or ion exchange have been widely studied, but most such methods are costly or ineffective. This is because Cs, as a weak Lewis acid, interacts very weakly with ligands due to its very low charge/radius ratio and low polarizing power. Therefore, an effective and low cost approach for removal of radioactive Cs is still needed.

This study investigated the feasibility of using a novel strain of the microalga, *Desmodesmus armatus* SCK, for removal of cesium ions (Cs^+) and radioactive $^{137}Cs^+$ from aqueous solutions, and examined the effects of temperature, potassium (K^+) starvation, and addition of organic substrates, including volatile fatty acids (VFAs), on the accumulation of Cs^+. The results indicated that *D. armatus* SCK accumulated 2.08 nmol Cs^+ per 10^6 cells at 25°C under constant illumination, much more than two other green microalgae (*Chlorella* and *Chlamydomonas*). At 10°C, Cs^+ accumulation was 3.7-fold greater than at 25°C. *D. armatus* SCK grown in conditions of K^+-depletion accumulated 26% more Cs^+ than cells grown in K^+-sufficient conditions. Addition of VFAs also enhanced Cs^+ uptake by *D. armatus* SCK; maximum yield was achieved by acetic acid, followed by butyric acid and propionic acid. The Cs^+ taken up by this microalga was easily recovered by use of magnetic separation with poly(diallyldimethylammonium) (PDDA)-FeO_3. In addition, a radionuclide experiment indicated that this strain can eliminate more than 99% of radioactive ^{137}Cs ranging from 10 Bq mL^{-1} to 1000 Bq mL^{-1}. Collectively, use of this microalga to remove radioactive Cs^+ from the environment or liquid waste has promise as a bioremediation technique.

[This work was supported by the Nakdonggang National Institute of Biological Resources granted by the Ministry of Environment, Republic of Korea]

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-192

Response of bacterial community during succession of a *Mesodinium rubrum* bloom to a *Dinophysis acuminata* bloom in laboratory microcosm

H. Gao [1], C. Hua [1], T. Mengmeng [1,*]

[1]Ocean College, Zhejiang University, Zhoushan, China

Abstract: Prevalence of the diarrhetic shellfish toxins caused by *Dinophysis* spp. is always attributed to both abiotic and biotic factors in the water column. An important process is mixotrophy in which *Dinophysis* spp. intensively feed on *Mesodinium* spp. for nutrients and kleptochloroplasts. During this process, nutritional characteristics could also be changed due to their preference of different nutrients, prey cell debris generated by sloppy feeding and in turn, degradation by microorganisms. However, insights of the role of bacterial community during this succession and how they directly or indirectly interact with the mixotrophs have rarely investigated. Here, lab experiments were performed to characterize the bacterial communities and ambient dissolved nutrients changes during the succession of *M. rubrum* and *D. acuminata* bloom. Dissolved nutrient, especially ammonium was significantly increased during the feeding process, probably linked to the rapid collapse of *M. rubrum* population. Both UniFrac and Bray-Curtis distances of bacterial communities increased along with the succession which could be mainly explained by changes of nutrients status and activities of *Dinophysis* cells. Presence of *Dinophysis* cells also accelerated the natural decline of the *M. rubrum* bloom and contaminated the cultures by toxins, but their influence on prokaryotic communities was limited onto the fraction in low abundance (relative abundance <0.1%), indicating that the interaction between *D. acuminata* and bacteria may be species-specific and only happen intracellularly or in phycosphere. Moreover, a majority of dominant bacterial taxa in our cultures may also exhibit metabolism flexibility and thus unaffected taxonomically by changes within the succession.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-193

STRUCTURE AND FUNCTION OF THE MICROBIOME OF THE TOXIC DINOFLAGELLATE ALEXANDRIUM TAMARENSE

D. Erdner [1], C. Jauzein [2,*]

[1]University of Texas Marine Science Institute, Port Aransas, USA, [2]Centre de Brest, IFREMER, Plouzane, France

Abstract: Like all organisms, algae harbor specific microbiomes that can have both positive and negative effects on the algal partner. Our previous work with the toxic dinoflagellate *Alexandrium tamarense* has shown that the presence of bacteria can be associated with faster growth, larger cell size, higher photosynthetic efficiency, and greater cellular chlorophyll and toxin content. To characterize interactions between *A. tamarense* and its microbiome, we used two sub-cultures of a single genetic strain of this species, one bacterized (xenic) and one axenic, which show significant differences in morphology and physiology. Bacteria from the xenic strain were re-inoculated into the axenic strain, producing replicate “rexenic” strains. At 9 and 15 months, we characterized algal physiology and the community composition of both the algal-attached bacteria and those that were free in the medium. Parental microbiomes were stable over a long time scale (6 years). Despite differences in physiology between the two rexenic strains, their free bacterial communities were similar to each other, and distinct from the xenic parent. The attached bacterial communities of the rexenic strains were similar to the parent at 9 months, but had diverged by 15 months. Microbiome differences were driven primarily by variations in the abundance of the dominant species, which were shared between strains. These results suggest that the algal microbiome composition is not resilient to severe disturbance. Nonetheless, structurally dissimilar communities can be functionally equivalent in terms of impact on the algal partner.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

O-194

Co-occurrence pattern of phytoplankton and microbiota in coastal water of Korea based on recurrent association network analysis

S.-J. Chun [1 2,*], Y. Cui [1], S. H. Baek [3], H.-M. Oh [1 2], C.-Y. Ahn [1 2]

[1]Korea Research Institute of Bioscience and Biotechnology, [2]Korea University of Science and Technology (UST), Daejeon, [3]Korea Institute of Ocean Science & Technology, Geoje, Republic of Korea

Abstract: Microorganisms form various ecological relationships, ranging from mutualism to competition, and associate with succession of phytoplankton populations. Recently, network techniques have frequently been applied to microbial abundance data to detect significant patterns of mutual exclusion between taxa and to represent them as a network. The sampling was carried out every month from July 2016 through July 2017 in the South Sea, Korea. Surface water was filtrated for three size fractions of microorganism (free-living (FL), nano-particle associated (NP), micro-particle associated (MP) fractions). For bacterial and archaeal communities analysis, about 180 samples were sequenced using high-throughput paired-end Illumina sequencing. *Alphaproteobacteria* and Marine group II dominated in a FL fraction, while *Flavobacteriia* and *Woesearcheota* dominated MP fraction. Combined with microscopic analysis, several different types of phytoplankton blooms, including harmful algal blooms (HABs), were observed, which accompanied with significant changes in microbial communities in each fraction (e.g., *Flavobacteriia* in FL, *Verrucomicrobiae* in MP). We constructed recurrence association network and performed topological property analysis to reveal significant relationships and their network function among microbiota, phytoplankton, and environmental parameters. Several nodes were assigned as a “module hubs” or “connectors”. In conclusion, microbiota could reshape and interact closely with phytoplankton bloom as well as environmental parameters, forming diverse networks.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-195

Effects of repeated dosing with bacterial algicide IRI-160AA on microbial community composition in the Delaware Inland Bays

C. Grasso [1], D. Nanjappa [1], Y. Wang [1], K. Coyne [1,*]
[1]University of Delaware, Lewes, USA

Abstract: Global increases in harmful algal blooms (HABs) have spurred interest in measures to control these blooms. Prevention, control, and mitigation of HABs are of human interest, and there are naturally-occurring compounds that may directly influence these processes. Use of these compounds appears promising, but effects to non-target organisms are also of concern. The Delaware Inland Bay (DIB; Delaware, United States) system is host to the algicidal marine bacterium *Shewanella* sp. IRI-160, which secretes a compound(s) having allelopathic effects to dinoflagellate algae. Here, the effects of the algicide, designated IRI-160AA, on microbial communities in the DIB were evaluated. Natural community experiments were conducted to assess proactive application efficiency of the algicide to communities as a preventative measure. Here, repeated dosing was performed under non-bloom conditions with mixed dinoflagellate communities at EC05, EC50, and EC95 concentrations of the algicide, and changes in the microbial community were evaluated via molecular fingerprinting methodology. Repeated dosing saw dose-dependent decreases in dinoflagellate abundance, yet overall increases in photosynthetic biomass. Dissolved organic carbon (DOC) analysis initially showed a dose-dependent increase with higher concentrations of algicide exhibiting higher levels of measured DOC. A second experimental iteration on a separate community showed a small, treatment-independent decrease in DOC suggesting that community structure strongly influences these results. Next Generation Sequencing (NGS) was performed on the 18S rRNA gene to track eukaryotic community changes within one of the repeated dosing experiments. Overall increases in abundance included diatoms (class Coscinodiscophyceae, Bacillariophyceae), cryptophytes (Cryptophyta), and ciliates (Oligohymenophorea). Decreases in abundance were observed for raphidophytes (Raphidophyceae), chlorophytes (Chlorophyta), and dinoflagellates (Dinophyceae). Shifts in the eukaryotic community were found to be dose-dependent based on both sequencing data and community fingerprinting. The results of this investigation demonstrate the efficacy of IRI-160AA as a dinoflagellate-specific algicidal agent without negatively impacting the greater microalgal community in the Delaware Inland Bays.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-196

Associated bacterial flora and toxicity of cultured benthic isolates from the genus *Prorocentrum* from Mexican coasts

L. M. Duran-Riveroll [1,*], S. Pajares-Moreno [2], Y. B. Okolodkov [3], M. D. C. Pelayo-Zárate [2], A. D. Cembella [4]

[1]Unidad Académica de Ecología y Biodiversidad Acuática, CONACYT - UNAM, [2]Unidad Académica de Ecología y Biodiversidad Acuática, Universidad Nacional Autónoma de México, Mexico, [3]Instituto de Ciencias Marinas y Pesquerías, Universidad Veracruzana, Boca del Río, Veracruz, Mexico, [4]Ecological Chemistry, Alfred Wegener Institute, Bremerhaven, Germany

Abstract: Marine benthic dinoflagellates produce a bewildering array of secondary metabolites, and many of these compounds possess high biological activity. These bioactive secondary metabolites may play diverse roles (currently unknown) in intracellular regulation of cell growth and metabolism as well as in extracellular regulation of population growth via allelochemical interactions. Diverse ecological functions for phycotoxins have been proposed, and most of the information on the importance of chemical signaling processes in marine environments has been obtained from benthic organisms, especially on secondary metabolites acting as chemical defenses. Little is known about the chemical signaling processes and interactions in benthic communities of which dinoflagellates are often prominent members. Nevertheless, recent research suggests that the associated bacteria can play a crucial role in the growth, physiology and modulation of the toxin biosynthesis in dinoflagellates. Many *Prorocentrum* species colonize the surfaces of the benthos, as epiphytes or epizooites or are found upon or within the interstices of sandy or silty sediments or attached to nets or floating macroalgae or detritus. Benthic species of this genus frequently produce toxins associated with diarrhetic shellfish poisoning (DSP) – linear polyethers with high potency as protein phosphatase inhibitors, whereas these compounds are typically absent from primarily pelagic species. In this study, we focus on the chemical and ecological interactions between the relatively sessile benthic toxic dinoflagellates in laboratory cultures and the potentially symbiotic, mutualistic or algicidal bacteria on biofilms and in culture media. In particular, the cell toxin content and composition of multiple benthic isolates of *Prorocentrum* species and their associated bacteria from the Veracruz Reef System and the Mexican Caribbean coast were analyzed. The dinoflagellate-associated bacterial assemblages were subjected to taxonomic and phylogenomic analysis of 16S rDNA to establish patterns of biodiversity. Among associated bacteria, *Roseobacter* and *Marinobacter* were predominant, suggesting that these clades are intimately associated with the host dinoflagellate. This coupled approach provides the basis for testing hypotheses regarding the possible role(s) of benthic dinoflagellate toxins in spatial competition for substrate and other allelochemical interactions within the microphytobenthos.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-197

Specific microbiome members are associated with toxic Pseudo-nitzschia blooms from a 2-year time series at a coastal Pacific site

M. Sison-Mangus [1,*], M. Kempnich [1], S. Mehic [2]

[1]Ocean Sciences, [2]METX, University of California Santa Cruz, Santa Cruz, USA

Abstract: The production of domoic acid (DA) by toxigenic *Pseudo-nitzschia* is influenced by many environmental factors including marine bacteria. Our previous study suggests that the DA production of toxigenic *Pseudo-nitzschia* becomes absent or minimal in the absence of bacteria. DA production, however, is rescued by co-culturing with single bacteria, and DA is consistently and significantly enhanced by the microbiota from *Bacteroidetes* group *and Planococcus (Firmicutes),* as compared to other bacterial groups. We asked whether these DA-enhancing microbiome members are also present and associated with toxic *Pseudo-nitzschia* blooms in an ecological setting. We carried out an opportunistic microbiome sampling during *Pseudo-nitzschia* blooms in 2010-2011 (11 time points) and a weekly sampling of microbiome populations along with environmental and biological metadata from 2014 to 2015 (55 weekly time points) in Monterey Bay, and sequenced the 16s rDNA gene using next generation sequencing. In 2010-2011, *Planococcus* bacteria is the most abundant microbiome member that is associated with four independent toxic *P. australis* bloom events in 2011, but is not seen during a month-long semi-toxic *P. fraudulenta* bloom event in 2010. In 2014-2015, on the other hand, *Bacteroidetes* bacteria are the only abundant microbiome members in the community that are significantly correlated with six toxic *Pseudo-nitzschia* bloom events. Notably, the toxic *Pseudo-nitzschia* blooms in 2014 and 2015 have been massive (i.e., Spring blooms lasted for 3 months) and were the highly-toxic blooms ever recorded in a decade in Monterey Bay. Based from these physiological, genomic and ecological measurements, we conclude that specific microbiome members are highly- associated with toxic *Pseudo-nitzschia* blooms and may even be required by the diatom for the successful expression of this specific evolutionary trait.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

O-198

Elucidation of bacterial composition throughout the toxic dinoflagellate *Gambierdiscus balechii* growth cycle

K. C. Yip [1 2,*], W. H. Lee [1 2], Y. L. Mak [1], Y. Sun [3], J. Wu [1], L. L. Chan [1 2]

[1]State Key Laboratory in Marine Pollution, [2]Department of Biomedical Sciences, City University of Hong Kong, Hong Kong, Hong Kong, [3]Department of Computer Science, Michigan State University, East Lansing, USA

Abstract: Benthic dinoflagellates are capable of producing various kinds of marine biotoxins. These marine biotoxins can accumulate along food chains and pose threat to seafood consumers. For example, *Gambierdiscus* spp. can produce potent gambiertoxins (GTXs) and ciguatoxins (CTXs) which can induce ciguatera fish poisoning (CRF). Reports indicated that there were associations between climate change, ocean acidification and expansion of distribution of toxigenix benthic dinoflagellates. However, little is known about the mechanisms of dinoflagellates to produce biotoxins. Recently, studies indicated that bacteria may play a role to govern the rate of toxin production and toxin composition. In this study, 16S amplicon sequencing has been used to elucidate the bacteria community composition of benthic dinoflagellate *Gambierdiscus balechii* at different growth phases. V3 - V4 region of the 16S rDNA was used to analyze the taxonomic composition of bacteria isolated from the culture of *G. balechii*. The microbial communities in the cultures were further identified by comparing their relative frequency of operational taxonomic units (OTUs). Mouse neuroblastoma assay (MNA) was used to determine toxicity of *G. balechii* at its late stationary phase. Based on our data, different composition of bacteria has been spotted in the cultures of *G. balechii* and we argued that different composition of bacteria community would resulted in different toxicity of *G. balechii*.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-199

Highly Sensitive and Practical Fluorescent Sandwich ELISA for Ciguatoxins

T. Tsumuraya [1,*], T. Sato [2], N. Oshiro [3], M. Hirama [1], I. Fujii [1]

[1]Department of Biological Science, Graduate School of Science, Osaka Prefecture University, Sakai, [2]Cell Science Inc., Sendai, [3]Division of Biomedical Food Research, National Institute of Health Sciences, Kawasaki, Japan

Abstract: Ciguatera fish poisoning (CFP) caused by the consumption of fish that have accumulated ciguatoxins (CTXs) affects more than 50,000 people annually. The spread of CFP causes tremendous damage to public health, fishery resources, and the economics of tropical and subtropical endemic regions. The difficulty in avoiding CFP arises from the lack of sensitive and reliable analytical methods for detection and quantification of ciguatoxins in ciguatoxic fish, along with the normal appearance, smell, and taste of fish contaminated with the causative toxins. Thus, an accurate, sensitive, routine, and portable detection method for CTXs has been highly desirable to be available. We have successfully developed a highly sensitive fluorescent sandwich ELISA, which can detect, differentiate, and quantify four major CTX congeners, CTX1B, CTX3C, 51-hydroxyCTX3C, and 54-deoxyCTX1B with a detection limit of less than 1 pg/mL. The ELISA protocol using one microtiter plate coated with two mAbs, 10C9 and 3G8, and ALP-linked 8H4 can detect any congeners of the four CTXs by a single operation. CTX1B spiked into fish at the FDA guidance level (0.01 ppb) was also proven to be reliably detected by this ELISA. Furthermore, the efficiency of extraction/purification procedures and the matrix effect of contaminants in fish were evaluated in detail, since the pretreatment and matrix effect are critical for ELISA analysis,

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-200

A single validation study on matrices-insensitive test procedure for quantitative analysis of the Pacific type ciguatoxins in fish

M. Nagae[1], T. Igarashi[1,*], K. Kuniyoshi[2], N. Oshiro[2], T. Yasumoto[1]

[1]Applied Science, Japan Food Research Laboratories, Tama, [2]Biomedical Food Research, National Institute of Health Sciences, Kawasaki, Japan

Abstract: Ciguatera fish poisoning (CFP) is an endemic illness in tropical and subtropical regions worldwide. To advance relevant studies, the liquid-chromatography coupled to mass spectrometer (LC-MS) can be regarded as the most potent tool for its high sensitivity, selectivity, and accuracy. Although the high potential of an LC-MS method was demonstrated by Yogi et al. on ciguateric fish in the Pacific, a validation study to put the method for wide use could not be carried out, for two reasons: first the lack of calibrated pure toxins and secondly large fluctuation due to matrices effect in some fish. Since the calibrated toxins are now available, upon request, from JFRL to identify and quantify toxins occurring in the Pacific, we tackled in this study the second problem, the matrices interference. To remove, or reduce, the species dependent effect of matrices, we carried out spiking tests using flesh (2g) of *Lutjanus bohar*, *Variola louti*, *Seriora quinqueradiae* and *Pagrus major*. The first two*, L. bohar* and *V. louti* were the major causative fish in Okinawa. *L. bohar* was found to be the most problematic. The following points were considered critical for change: aqueous methanol for the initial extraction, heating of the extract, partitions with alkali and acid solutions, use of ion exchanges cartridges, change formic acid to acetic acid in the mobile phase of LC separation, proper intervals with methanol washing between sample injections. In the course of the validation study, CTX1B and epiCTX3C were used for spiking. The instrument was an Agilent 1200 Series LC coupled to Agilent 6460 Triple Quad MS with a Zobax Eclips Plus C18 column (2.1 x 50mm id, 1.8 micro-m particle size). At an intermediary step, the average recovery (AVE) and relative standard deviation(RSD) were as follows; CTX1B *S. quinqueradiae* 0.5 ppb (AVE 73%, RSD 3.1%)， 0.1 ppb (89%, 9.8%)， *P. major* 0.5 ppb (78%， 22.7%), 0.1 ppb (82%, 3.9%); 49-CTX3C *S. quinqueradiae* 0.5 ppb (68%, 5.2%), 0.1 ppb (83%, 8.4%), *P. major* 0.5 ppb (82%, 23.5%), 0.1 ppb (97%, 26.8%). At 0.5ppb and 0.1ppb spike levels, AVEs above 80% with less than 15% RSD were so far achieved on *L. bohar and V. louti*. Future inter laboratory study are to follow after fixing final conditions.

Yogi *et al.* , *Analytical Chemistry*, 83, 8886-8891 (2011).

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-201

A contribution for establishing a specific, simple, fast and sensitive cell-based assay for ciguatera toxin screening

R. A. F. Neves [1 2,*], M. A Pardal [2], S. M Nascimento [1], P. J Oliveira [3], E. T Rodrigues [2]
[1]Laboratory of Marine Microalgae, Ecology and Marine Resources, Federal University of the State of Rio de Janeiro, Rio de Janeiro, Brazil, [2]CFE - Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Coimbra, [3]CNC - Centre for Neuroscience and Cell Biology, University of Coimbra, UC Biotech Building, Cantanhede, Portugal

Abstract: Ciguatera Fish Poisoning (CFP), which is caused by the consumption of fish contaminated by toxins produced by the benthic dinoflagellate *Gambierdiscus*, is the most frequent reported non-bacterial syndrome in the world. *Gambierdiscus* mainly produces ciguatoxins (CTXs) and maitotoxins (MTXs), possibly two of the most potent marine toxins identified to date. Gastrointestinal, neurological and cardiovascular symptoms may persist from a few days to several weeks or months. Some of the challenges for the detection of *Gambierdiscus* toxins in biological samples include the presence of low concentrations of these toxins in fish samples, and the great diversity of toxin congeners within a single sample. Therefore, the main goal of the present study was to develop a specific, simple, fast and sensitive cell-based assay for *Gambierdiscus* toxin screening. To achieve that, a series of cell based-assays with mammalian cardiomyoblast H9c2(2-1) cells were performed using seven exposure times (22.5, 45, 90, 180, 360, 720 and 1440 min). Lipophilic toxins from *Gambierdiscus excentricus* (UNR-8) lyophilized cells were extracted in DMSO, and this crude extract was used to prepare the H9c2(2-1) exposure medium. The biological response was evaluated by determining decrease in cell mass as an endpoint using the sulforhodamine-B colorimetric assay. *Gambierdiscus excentricus* toxins induced a time-response effect on H9c2(2-1) cells with significant reduction of cell mass in all tested incubation periods. Since the time-response results of H9c2(2-1) cells treated with crude extracts from other toxic benthic dinoflagellates (*Coolia malayensis*, *Ostreopsis* cf. *ovata*, *Prorocentrum hoffmaniannum*, *Prorocentrum lima*) showed discriminatory responses, a specific ciguatera toxin cell-based assay has possibly been found. Only H9c2(2-1) cells treated with *G. excentricus* extract allowed the determination of IC_{50} results in less than 720 min of exposure. Moreover, the very low IC_{50} value obtained for *G. excentricus* toxins compared to the other tested toxin extracts from different genera indicates that H9c2(2-1) cells are very sensitive to these toxins. Despite the promising results, further studies should be conducted in order to confirm the applicability of the H9c2(2-1) cell-based assay developed in the present study: the test of extracts from different *Gambierdiscus* species and strains originated from worldwide geographical regions, as well as from contaminated biological samples (e.g., fish). To demonstrate specificity, extracts from other toxic dinoflagellate species should also be tested (e.g., *Alexandrium*, *Dinophysis*, *Karenia*). The present study aimed to promote environmental and human safety by contributing to the development of a specific, simple, fast and sensitive cell-based assay for *Gambierdiscus* toxins to be used in routine screening.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-202

Solid Phase Adsorption Toxin Tracking (SPATT) technology as a useful tool for the passive field-monitoring of *Gambierdiscus* toxins

M. Roué [1,*], H. T. Darius [2], J. Viallon [2], A. Ung [2], C. Gatti [2], M. Sibat [3], T. Harwood [4], P. Hess [3], M. Chinain [2]

[1]Institut de Recherche pour le Développement - UMR 241 EIO, [2]Institut Louis Malardé - UMR 241 EIO, Papeete, French Polynesia, [3]IFREMER, Nantes, France, [4]Cawthron Institute, Nelson, New Zealand

Abstract: Ciguatera poisoning is a food-borne illness caused by the consumption of tropical coral reef fish or marine invertebrates contaminated with polyether neurotoxins, i.e. ciguatoxins (CTXs) that are produced by dinoflagellates in the genus *Gambierdiscus*. Most surveillance programs currently rely on the survey of *Gambierdiscus* cell densities and species composition and/or the monitoring of CTXs in material intended for human consumption. However, such methods are time consuming and expensive, thus emphasizing the need for supplementary tools, e.g. those based on the time and spatially integrated sampling of algal toxins directly in marine environments.

Solid Phase Adsorption Toxin Tracking (SPATT) technology uses porous synthetic resins capable of adsorbing dissolved toxins from the water column. Since its first introduction more than a decade ago, SPATT technology has proven to be effective for the detection of a wide range of both lipophilic and hydrophilic toxins produced by micro-algae or cyanobacteria. Among the numerous adsorbent substrates tested, Diaion® HP20 resin appears to be the most versatile substrate. Regarding the monitoring of toxins associated with ciguatera poisoning, one laboratory study has demonstrated the efficacy of HP20 resin for the detection of dissolved CTXs and maitotoxins (MTXs) in *Gambierdiscus* cultures. However, validation of this method for the field-monitoring of *Gambierdiscus* toxins was still pending.

In order to confirm the ability of SPATT technology to detect *Gambierdiscus* compounds in ciguateric biotopes, SPATT devices filled with 2.5 to 10 g of HP20 resins were deployed during 1 to 7 days in several islands of French Polynesia. *Gambierdiscus* toxins, including several CTXs congeners, were successfully detected, using both the mouse neuroblastoma cell-based assay (CBA-N2a) and liquid chromatography - tandem mass spectrometry analyses (LC-MS/MS), in SPATT extracts deployed in areas with high risk of ciguatera. These results emphasize the relevance of this toxin-based technique as a useful supplementary tool when assessing the risks associated with *Gambierdiscus* proliferation. Moreover, given the risk of simultaneous accumulation of multiple biotoxins in seafood products in tropical environments, the use of SPATT technology when combined with downstream analyses such as LC–MS/MS multi-toxin screenings, also offers promising prospects in the framework of monitoring programs aiming at the surveillance of emerging toxins in locations prone to HABs.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-203

Maitotoxin-3: Structural insights and production by co-habitating marine dinoflagellates

J. S. Murray [1,*], D. T. Harwood [1], L. Rhodes [1], A. Selwood [1], M. Boundy [1], R. Munday [2]
[1]Cawthron Institute, Nelson, [2]AgResearch , Hamilton, New Zealand

Abstract: Over the last three decades the benthic dinoflagellate *Gambierdiscus* has been demonstrated to produce an array of toxins including ciguatoxins, gambierol, gambieric acid, gambieroxide, gambierone, and maitotoxins 1-4 (MTX 1-4). The structural characteristics of MTX-3 have eluded scientists since it was first identified by Lewis and Holmes in 1994. It was first demonstrated to be produced by *G. toxicus* and has since been shown to be ubiquitous to all *Gambierdiscus* species tested to date, but in the case of *G. carpenteri* not all strains. More recently it was discovered that isolates of co-habitating benthic dinoflagellates of the genus *Coolia* and *Fukuyoa* also produce MTX-3, adding to the importance of research and characterization of the structure of this putatively assigned toxin. Toxicological assessments of algal cultures that produce MTX-3 showed relatively low oral and ip toxicity despite high toxin production. MTX-3 has been shown to bioaccumulate in the viscera of herbivorous reef fish species and therefore it is important to determine the toxicity of MTX-3 on purified material to ascertain the risk it poses to human health when consumed.

There is currently only one known published method for the chemical analysis of MTX-3, which was developed at the Cawthron Institute using liquid chromatography-tandem mass spectrometry (LC-MS/MS). It has been used to screen 89 isolates, from seven *Gambierdiscus* species, to select the best producers of MTX-3 for bulk culturing purposes to enable isolation and purification. Negative ESI mode is used to monitor the production of MTX-3, with a transition generated from the singly deprotonated $[M–H]^-$ ion (*m/z* 1,037.6>96.8). This allowed comparison of MTX-3 production between the different isolates. Our assignment of the $[M–H]^-$ ion differs from that proposed by those who discovered MTX-3. Lewis *et al*., used positive ESI mode and assigned the *m/z* 1,039.5 signal as the $[M+Na]^+$ ion, although this was performed on the assumption that MTX-3 was a disulphated molecule with a disodium salt MW of 1060.5 Da. Our assignment is based on interpretation of the original spectrum, and LC-MS/MS analysis of algal cultures and semi-purified material.

This presentation will focus on recent isolation efforts and structural insights into MTX-3; including partitioning trials, orthogonal preparative columns and LC-MS/MS analysis.

Disclosure of Interest: None Declared

Epidemiology, reports of human outbreak cases

O-204

Ciguatera Fish Poisoning: A 25-year experience of the Marseille Poison Centre.

L. De Haro [1,*], M. Glaizal [1], C. Schmitt [1], R. Torrents [1], N. Simon [1]
[1]Toxicovigilance, Marseille Poison Centre, Marseille, France

Abstract: Introduction: Ciguatera Fish Poisoning (CFP) is a common intoxication in the tropical countries. Since 1993 the Marseille Poison Centre (MPC) is working on the topic of marine toxicology including seafood poisoning. The authors present the experience of this unit of clinical toxicology from 1993 to 2017 concerning CFP. Method: Every year, around 25 000 poisonings are managed by the MPC (childhood accidents, suicide attempts, addictions…). All cases are included in a national databank which was explored to extract CFP observations managed by the MPC during 25 years. Results: 49 poisonings were studied concerning 175 patients (25 cases with one patient; 24 collective cases with 2 to 41 patients). 174 patients were tourists poisoned during vacation. The remaining patient was a sailor who was brought back to France after having consumed contaminated seafood while on shore leave. The fish species was identified for 70% of the poisonings, mainly belonging to 3 fish families: 10 groupers (Serranidae), 5 barracudas (Sphyraenidae), 5 snappers (Lutjanidae).The poisonings took place in the Caribbean Sea for 23 cases (98 patients), in the Indian Ocean for 12 cases (53 patients) and in the Pacific Ocean for 14 cases (24 patients). 61% of the CFP (30/49 poisonings) were managed during the 10 last years, proving that this disease is an emerging health problem in the non-endemic countries. Symptoms appeared from 2 to 12 hours after the meal and varied slightly according to location. All poisonings occurring in the Atlantic Ocean areas began with gastrointestinal effects while in more than 50% of cases occurring in the Pacific Ocean areas, the patients had no gastrointestinal effects, and the onset was characterized by neurological symptoms such as paresthesia and dysesthesia. One deadly poisoning was observed in 1999: a 73-year-old man died of respiratory paralysis after eating a barracuda in a local restaurant in Cuba (his family has been repatriated back to France due to severe CFP). The evolution of the 174 surviving patients has been classic for CFP with persistent symptoms for several weeks (63% of the patients) or even months (18% of the patients). 36% of the patients reported exacerbation of neurological signs (paresthesia, dysesthesia, cold allodynia, itching) several months after poisoning following consumption of alcoholic beverages or seafood. Such clinical feature resurgence has been reported previously but the mechanism is unclear. Conclusion: CFP must be considered as a travel-related risk as well as the risk of contracting parasitic, bacterial or viral diseases in the tropical zones. There is currently no treatment with proven efficacy in improving patient comfort. Further research is needed to enhance management of patients presenting with this seafood poisoning, heretofore rarely observed in Europe.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

O-205

Ciguatera Fish Poisoning: Healthcare workers knowledge and practice assessment in an endemic region.

M. Tranchet [1], C. M. I. Gatti [2,*], M. Giard [3], M. Chinain [2]

[1]Service des urgences, Centre Hospitalier de Polynésie française, [2]Laboratoire des Microalgues Toxiques, Institut Louis Malardé - UMR 241 EIO, [3]Bureau de Veille Sanitaire, Direction de la Santé de Polynésie française, Papeete, French Polynesia

Abstract: Ciguatera Fish Poisoning (CFP) is a non-bacterial seafood poisoning resulting from the consumption of coral reef seafood. It is a frequent cause for consultation in French Polynesia, an area known as a long-standing CFP hotspot. In the absence of biological diagnostic tools and specific treatments, the disease diagnosis and the medical care provided to patients may vary from one practitioner to another, depending on his knowledge of the pathology, his previous experience with CFP patients (acute and chronic) and his personal evaluation of treatments efficacy over the years (allopathic and traditional).

A questionnaire-based survey was conducted to assess the level of knowledge of CFP, and the disparities regarding CFP patients' management (diagnosis, treatments, advices, *etc*) among healthcare workers (HCW) practicing in French Polynesia. Their degree of involvement in the CFP epidemiological surveillance program conducted since 2007 was also assessed. Groups of HCW were targeted according to different criteria: 1) public health professionals (doctors and nurses) *versus* private and emergency practitioners; and 2) from urban *versus* rural hospitals, dispensaries, infirmaries or private structures.

Information collected in the framework of the present study will be helpful in tuning the communication strategy targeted at HCW in order to (i) increase awareness about CFP, ii) maintain or ensure a good level of participation in the CFP epidemiological surveillance program, and (iii) improve/standardize CFP patient's management in French Polynesia.

Disclosure of Interest: None Declared

Risk assessment for algal and cyanobacterial toxins

O-206

Toxic cyanobacteria in a water reservoir in Sicily: Considerations for monitoring programs and risk assessment.

M. Manganelli [1], P. Zuccarello [2], S. Vichi [1], M. Stefanelli [3], O. G. Conti [2], M. Ferrante [2], E. Testai [1,*]
[1]Environment and Health Dept., Istituto Superiore di Sanità, Roma, [2]Laboratorio di Igiene Ambientale e degli Alimenti (LIAA), Dip. “G.F. Ingrassia", Università Di Catania, Catania, [3]Dip. Innovazione tecnologiche e sicurezza degli impianti, Prodotti e Insediamenti Antropici, Laboratorio VIII – Biotecnologie, INAIL, Roma, Italy

Abstract: During the last century several water reservoirs have been built for drinking and irrigation purposes in Sicily, the largest island in the South of Italy. Over the years some of them have been reported to be affected by cyanobacteria blooms, but monitoring programs have been discontinuous. A quarterly two years survey of cyanobacteria (CB) and cyanotoxins (CTX) in the main reservoirs started in 2016, in order to assess the possible risks for the exposed population. All lakes but one have a CB community and CTX concentrations within acceptable ranges. Here we present the preliminary results of the critic one, Lake Disueri, with a surface of 1,85 km2 and a 31 and 15.2 m maximum/average depth, which is strongly reduced during persistent dryness. Blooms of CB species have been detected for the first time during summer 2017 (characterized by very high temperature in a very dry period). Microcystis sp. and Cylindrospermopsis raciborskii were detected in July (108 and 107 cell/L, respectively) and by mid-Aug were replaced by Anabaenopsis sp. and Plankthotrix rubescens, still growing in mid-Sept were (107 and 106 cell/L, respectively). MCs concentrations were low, in agreement with the lack of qualitative amplification of the mcyE gene (demonstrated by a 370 bp product in the two positive controls), indicating that the MC-producing fraction within CB population was low. The amplification of both cyrJ (~ between 550 and 600 bp) and stxB (~ 270-300 bp + a high mw variable band) occurred in all samples, concurrently with high density of C. raciborskii and Anabaenopsis sp.. Therefore, a potential ability to produce CYN and STX could be assumed for these samples, even if at the moment no positive control was available. The detection of MC levels allowed to assess a very low risk for the population and livestock using the lake’s water, while by the number of cell the risk would have been considered much higher. The use of a simple qualitative PCR is a good tool to investigate the presence of potentially toxic CB, and to check for possible new toxin production population. The presence of cyrJ and stxB, once confirmed, could indicate the presence of a Cylindrospermopsis strain with potential production of CYN and STX, for the first time in Italy and Europe, and of a Anabaenopsis strain producing CYN and STX.

Disclosure of Interest: None Declared

Risk assessment for algal and cyanobacterial toxins

O-207

Environmental assessment of cyanobacteria and cyanotoxins in European temperate freshwater systems – The Portuguese situation

C. Moreira [1,*], C. Gomes [1], V. Vasconcelos [1 2], A. Antunes [1 2]

[1]CIIMAR, Matosinhos, [2]FCUP - Faculdade de Ciências da Universidade do Porto, Porto, Portugal

Abstract: CyanoBlooms are a natural phenomenon well reported in the northern hemisphere temperate freshwater systems with well-known associated health risks. In Portugal these risks are poorly understood and monitoring is essential to assess the negative impacts that CyanoBlooms can represent to humans, animals and plant life. Under the scope of the CYANOTOX project we aim at monitoring relevant freshwater ecosystems in Portugal combined with the application of PCR-based methods and immunosorbent assays (ELISA). To accomplish this seven freshwater ecosystems are being annually surveyed from May to September where water samples are being collected along with bloom occurrence and strain isolation. The data retrieved from our project shows that for the first sampling year all four main cyanotoxins including microcystins, cylindrospermopsins, anatoxin-a and saxitoxins genes are present in Portuguese freshwater systems. In regards to the presence of cyanobacteria taxa PCR-based methods, strain isolation and microscopic observation revealed that *Microcystis aeruginosa*, *Cylindrospermopsis raciborskii*, *Planktothrix agardhii*, *Microcystis* sp., *Dolichospermum* sp. and *Chrysosporum* sp. are a part of the cyanobacterial community. Bloom occurrence was observed in all the sampled ecosystems with the exception of two. Finally ELISA assays and DNA sequencing are awaiting results for the cyanotoxin enumeration and for the identification of the toxic genera associated with the biosynthesis of each cyanotoxin detected. This integrated approach will improve the knowledge on cyanobacteria and cyanotoxins occurrence in Portugal and provides a better tool in the assessment of the associated risks of CyanoBlooms in Portugal and in temperate freshwater systems.

Disclosure of Interest: None Declared

Risk assessment for algal and cyanobacterial toxins

O-208

Interest of bivalves to evaluate the transfer of cyanobacteria and cyanotoxins from fresh to estuarine waters

E. Lance [1,*], Z. Amzil [2], M. Bormans [3], L. Brient [3], A. Lepoutre [1], V. Savar [2], D. Reveillon [2], E. Robert [2]
[1]UMR_I 02 SEBIO, University Reims Champagne Ardennes, Reims Cedex 2, [2]Laboratoire Phycotoxines, Ifremer, Nantes, [3]UMR 6553 ECOBIO, University Rennes 1, Rennes, France

Abstract: The frequency of cyanobacterial proliferations is increasing worldwide in fresh waters and the cyanotoxins represents a threat for ecosystems and human health. However, the risk of cyanobacteria and cyanotoxin transfer from fresh to estuarine waters has been rarely investigated. The objective of this project was to evaluate the potent transfer of cyanobacteria and cyanotoxins [microcystins (MCs), nodularins (NOD), cylindrospermopsins (CYN) and anatoxins-a (ATX-a)] along a river continuum from withholding freshwaters (2 stations) to interconnecting estuarine (2 stations) and coastal (1 station) areas. We monthly evaluated, during two years, the phytoplanktonic communities and the cyanotoxin concentrations in phytoplankton (intracellular) and in water (dissolved toxins integrated in Solid Phase Adsorption Toxin Tracking, SPATT). In addition, mollusks bivalves were caged in freshwater (*Anodonta anatina*) and estuarine (*Mytilus edulis*) sites, and free and protein-bound MCs were measured in their tissues in order to assess their use as potent sentinel organisms integrating the water contamination between sampling dates.

First results show that cyanobacteria dominated phytoplankton with high densities (up to 2 270 000 cells/mL) in freshwater sites during the summer and autumn periods, with a cell transfer in estuarine (up to 206 000 cells/mL) and coastal (up to 1200 cells/mL) sites during the two years. Cyanobacteria were dominated by the genus *Microcystis* exhibiting up to 7 species in fresh waters. A progressive species selection was observed along the continuum reducing the cyanobacteria diversity from 42 to 7 species from fresh to estuarine waters, highlighting species resistance to the salinity gradient. MCs were continuously detected in phytoplankton samples (up to 165 µg/L), and in tissues of the bivalve *A. anatina* (up to 9 µg/g FW) in freshwater sites. In the first estuarine site, MCs were detected on 8 out of 17 sampling dates of the 2-year investigation in phytoplankton (up to 1.5 µg/L), and in *M. edulis* tissues (up to 3.5 µg/g FW). In the port and the costal sites, MCs were detected three times in phytoplankton samples, and at low concentration (respectively < 0.16 µg/L and < 0.04 µg/L). However, in these sites MCs were often detected in bivalves (up to 7.5 µg/g FW in the port and up to 0.25 µg/g FW in the costal site), as well as in the water via the SPATTs. The cyanobacteria and MC transfer from fresh to estuarine waters, threatening marine species, was highlighted by the presence of: i) cyanobacteria and MCs in water and in phytoplankton samples of estuarine sites, and ii) MCs (free and protein-bound forms) in marine bivalve tissues. Bivalves integrated the MC contamination of waters between two sampling dates, which reinforce their pertinence to be used as sentinel species.

Disclosure of Interest: None Declared

HumanToxicology

O-209

ANSES opinion on acute and chronic toxicity of BMAA

N. ARNICH[1,*], Z. AMZIL[2], J. BELEGAUD[3], C. CASELLAS[4], T. CLAUDEPIERRE[5], N. DELCOURT[6], E. LAGRANGE[7], E. LANCE[8], C. MATTEI[9], V. PICHON[10], J.-P. VERNOUX[11], T. MAIGNIEN[12], J.-M. FREMY[13]

[1]Risk Assessment Department, Anses, Maisons-Alfort, [2]IFREMER, Nantes, [3]Retired, Paris, [4]University, Montpellier, [5]Lorraine university, Nancy, [6]University, Toulouse, [7]Hospital, Grenoble, [8]University, Reims, [9]University, Angers, [10]ESPCI, Paris, [11]Retired, Caen, [12]Anses, Maisons-Alfort, [13]Retired, Antony, France

Abstract: Anses received a mandate by the French Ministries on Food and on Health regarding BMAA (β-N-methylamino-L-alanine), a neurotoxin produced by cyanobacteria and other organisms such as diatoms. Anses was requested to assess the link between dietary exposure to BMAA and neurodegenerative diseases (especially Amyotrophic Lateral Sclerosis, ALS), and perform a risk assessment if possible. A working group (WG) was created for reviewing all available data on the toxicity and occurrence of BMAA. The report and the opinion were published in May 2017. The main results are presented here.

Firstly the WG evaluated the analytical methods used to identify and quantify BMAA in biological matrices, because there has been controversy in the literature about the reliability of different methods/results. Based on their performance parameters, the methods were ranked in different categories of reliability. Only studies using reliable methods were taken into account.

The toxicological data (*in vivo* and *in vitro*) demonstrate the neurotoxicity of BMAA. Anses concluded that BMAA is hazardous to humans. However, data are insufficient for establishing either a dose-effect relationship or a health based guidance value (acute or chronic). It is therefore not possible to characterize the hazard represented by BMAA.

The link between BMAA and ALS was analyzed according to the Hill criteria (temporality, strength of association, consistency, biological gradient, experimental evidence, specificity, plausibility, biological coherence, analogy). The WG concluded that the causal link between exposure to BMAA and ALS is not proven based on the current state of knowledge. However, the hypothesis that exposure to BMAA is a factor favoring neurotoxicity is highly probable, mainly via its ability to activate neurotoxic mechanisms leading to neurodegeneration (excitotoxicity, hyperphosphorylation of Tau protein, overexpression of TDP-43, protein incorporation, interaction with neuromelanin).

Exposure through consumption of shellfish, fish and crustacean from sea and freshwater was estimated in both acute and chronic scenarios, taking into account only data from studies with a reliable analytical method to quantify BMAA. Exposure was in the range of below 0.1 µg up to 5 µg/kg bw/day for total BMAA (free and bound). Exposure was also estimated for BMAA analogs: DAB (2,4-diaminobutyric acid), AEG (N-(2-aminoéthyl)glycine) and BAMA (β–amino-N-méthylalanine).

Data were too limited to conclude on the level of concern for public health.

Regarding the recommendations on future research, the priority should be given to case-control epidemiological studies and on occurrence data using a reliable analytical method in order to better assess the dietary exposure of consumers of aquatic products.

Disclosure of Interest: None Declared

Risk assessment for algal and cyanobacterial toxins

O-210

Probabilistic risk assessment model of potential PSP intoxication during a bloom of *Alexandrium catenella*: monitoring's application

A. Thebault [1,*], M. Richard [2], G.-A. Rovillon [3], P. Legall [2], C. Chiantella [2], E. Abadie [2], S. Mortreux [2], M. Lunghi [4], H. Hégaret [5], N. Arnich [4], Z. Amzil [3]

[1]ANSES, Maisons-Alfort Cedex, [2]IFREMER , Sète, [3]IFREMER , Nantes, [4]ANSES, Maisons-Alfort, [5]LEMAR, CNRS, Plouzané, France

Abstract: The Thau lagoon is the main production site for shellfish culture in French Mediterranean. Blooms of the harmful *Alexandrium catenella,* producer of paralytic toxins*,* induced regularly shellfish area closures since 1998 to protect consumers vs. Paralytic Shellfish Poisoning (PSP). The phytoplankton and phycotoxins network (REPHY) of IFREMER permits to follow *A. catenella* blooms and control PSP contamination in shellfish. Above the sanitary threshold of 800 µg eq. STX.Kg^{-1}, all the production zone is closed. Re-opening necessitates two successive negative controls. In order to check the monitoring efficiency and to highlight its usefulness, we planned to establish the number of avoided cases, and their severity during the 2016 *Alexandrium* bloom. As part of the REPHY, a weekly sampling was organized at the sentinel site (Crique de l'Angle) and at 2000 m, at the Bouzigues production site, in order to survey the beginning of bloom. When abundance reached 1,000 cells per liter, sampling of oysters (n=20) was weekly triggered at production site. As part of the REPHY, PSP concentrations were determined, using the regulatory AOAC mouse bioassay for PSP, from acid extract done from a whole oyster pool (100g). Concentration of PSP were in parallel analyzed by HPLC/FLD in a sub-sample of the acid extract, of the oyster pool (20g) and in five oysters per sampling date. Data of contamination with time were fitted with a Generalized Additive model. Data of consumption of coastal consumers were acquired in 2,413 respondents, giving the distribution of size of meals. A probabilistic model was built to achieve quantitative risk assessment approach. An ordinal dose-response, already established by meta-analyses of human outbreaks was used. We can estimate the number of cases and their severity for 10,000 contaminated meals we avoid by applications of regulations. For all the bloom duration, the cumulative risk of death is 0.01%. It can be estimated that the increasing and decreasing part of contamination is critical, with lower doses of PSP that can generate mild symptoms of short durations. The use of dose-response model with HPLC/FLD can give quite realistic estimates of the human safety risk. Making cases prediction for different blooms, can be a way for optimizing better management strategies, and can create better understanding for producers. Perspectives would be to explore spatial variability of PSP contamination in order to achieve realistic estimates at the scale of shellfish area, ie. sampling oysters at Marseillan site located at 15 km of Crique de l'Angle.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods
O-211

Trends in the analysis of marine biotoxins: Confirmatory methods and analytical challenges

A. Gago-Martinez [1,*], J. M. Leao [1], P. Estevez [1], J. Giraldez [2]
[1]Analytical and Food Chemistry , University of Vigo and EU Reference Laboratory for marine biotoxins , [2]Analytical and Food Chemistry, University of Vigo , Vigo , Spain

Abstract: The transition from animal tests to alternative methods has represented a significant and challenging change in the control of marine biotoxins. Remarkable efforts are being made on the development of screening and confirmatory methods for a reliable and efficient control of existing and emerging toxins and advances in the field have been clearly identified in the last few years. One of the most critical challenges has been the use of mass spectrometry analyzers for the detection and confirmation of these toxins, the use of these analyzers coupled to chromatographic techniques have been widely applied for years as powerful analytical tools to a number of food and environmental contaminants, while the application in the marine biotoxins field is more recent and, in some cases, even not yet fully resolved. One of the main limitations on the application of these techniques is the lack of reference materials for these natural compounds with multiple congeners of different toxicity, which makes difficult to progress on the identification of the different toxin analogues involved in the contamination. Also the nature of these compounds and the complex biological matrix in which they are present are critical challenges hampering the advances on the implementation of these methods, to be considered as analytical tools for their establishment as reference methods for the control of the different marine biotoxin groups, or even as alternative methods for their screening in routine. Examples on the application of confirmatory methods to the control of existing and emerging marine biotoxins and the main challenges of their application are going to be presented and discussed in this work.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-212

LC-High resolution MS and LC-tandem MS as complementary tools for a comprehensive toxin analysis in environmental and food matrices

L. Tartaglione [1,*], F. Varriale [1], S. Casabianca [2], S. Capellacci [2], A. Penna [2], M. G. Giacobbe [3], A. Turner [4], A. Hiskia [5], T. Kaloudis [6], D. Carmela [1]

[1]Pharmacy, University of Naples Federico II , Naples, [2]Biomolecular Sciences, University of Urbino, Urbino, [3]IAMC CNR, Messina, Italy, [4]Centre for Environment, Fisheries and Aquaculture Science, Weymouth, United Kingdom, [5]NCSR Demokritos, [6]EYDAP SA, Athens, Greece

Abstract: Efficient strategies are highly required to detect toxins in seafood, environment and food supply with the final aim to protect human health and to guarantee seafood safety and quality. Authorities responsible for safeguarding public health often indicated Liquid Chromatography coupled to Mass Spectrometry (LC-MS) as the more promising technique able to guarantee accurate monitoring of both environmental and food samples. Several MS based approaches have been developed to disclose the presence of toxins and even to elucidate their structures based on the interpretation of their fragmentation patterns.

Although LC tandem MS on TQ MS analyzers surely offers the highest sensitivity, selectivity and reproducibility of results, sometimes it is not able to guarantee a comprehensive overview of the actual toxic scenario of a real sample: the presence of unknown toxin analogues may not emerge in a targeted analysis. So, High Resolution MS (HRMS) is often the key technique to identify the complete toxin profile of a sample. The analysis of marine and freshwater algal and mussel samples contaminated by palytoxins, azaspiracids, and microcystins will be presented as case studies. In some cases, an approach combining TQ MS and HRMS appears to be the most desirable in order to avoid an underestimation of sample toxicity.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-213

Targeted and non-targeted analysis of marine toxins in shellfish by LC-HRMS

I. Dom [1 2,*], M. Nicolas [1], G. Lavison-Bompard [1], R. Biré [1], T. Guérin [1]

[1]Université Paris-Est, ANSES, Laboratory for Food Safety, Maisons-Alfort, [2]Agreenium, the French Agricultural, Veterinary and Forestry Institute, Paris, France

Abstract: Current detection methods for marine toxins rely on *a priori* defined target lists of a relatively small number of toxins, and are generally inappropriate for the detection and identification of emerging compounds. The ability to search for emerging toxins is an important issue when considering the geographical expansion of marine toxins, the emergence of new toxins and the associated risk for human health.

The study described here was carried out to assess the performance of a detailed strategy for simultaneous quantitative screening of known toxins, and the qualitative detection of non-target analytes in shellfish by liquid chromatography coupled to high resolution mass spectrometry. The method developed relies on a workflow combining both target and non-target analysis composed of three approaches; (i) the target screening similar to low resolution MS where reference standards are used to search for the compounds of interest; (ii) the suspects screening which consists in querying a database/library including an exhaustive list of suspect compounds for which reference standards may not be available; (iii) the non-target screening performed without *a priori*, i.e. without reference standards or suspects to identify unexpected compounds. The first focus was made on the assessment of performance characteristics (specificity, repeatability, intermediate precision and the accuracy) of the target quantitative analysis proving its efficiency for the analysis of 14 known toxins in shellfish samples, as a prerequisite for the non-target analysis. The second step consisted in a critical evaluation of the workflow for suspects screening and without *a priori* analysis by analyzing blind samples spiked with marine toxins. A multistep specific data filtering strategy has been established to optimize the identification of features of interest by reducing the size of search space. In both approaches (suspects screening and without *a priori* analysis), the toxins spiked in the blind samples were identified.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-214

A fiber passive sampling device (fPSD) with nanopore sorbents for monitoring microcystins in water

D. Shea [1,*], X. Xia [1], X. Kong [1], W. Wang [2], R. Hardison [3]

[1]Statera Environmental, Raleigh, USA, [2]Yunnan University, Kunming, China, [3]NOAA, Beaufort, USA

Abstract: A major challenge to the quantitative risk assessment of microcystins produced from cyanobacteria blooms is determining chronic exposure to the toxins. The USEPA recently issued 10-day exposure health advisories for microcystins, but concentrations in water can vary several orders of magnitude over a 10-day time period due to the complexities of bloom formation and persistence, toxin production, extracellular release, transport and fate. We have developed a fiber passive sampling device (fPSD) to measure the time-integrated concentrations of microcystins in water that overcomes the challenge of measuring the chronic exposure over a 10-day period. The fPSD contains nanopore sorbent particles packed in a porous hollow fiber that allows the microcystins to pass through the membrane and be sorbed and preserved within the nanoporous particles. Pilot fPSDs were fabricated using Oasis HLB sorbent and polyethersulfone hollow fiber with a surface area to sorbent mass ratio of 157 cm^2/g. The nanopore sorbent prevented the accumulated microcystin-LR (MC-LR) from degradation for 25 days, while MC-LR in lake water with native microorganisms was completely degraded within 20 days. The fPSD remains in a linear uptake phase for at least 15 days, exceeding the USEPA 10-day exposure requirement. The sampling rate of the pilot fPSD was 0.017 $L/day/cm^2$ at an exposure concentration of 0.1 µg/L MC-LR. The fiber format is self-supported with a low fabrication cost; a higher sampling rate can be achieved simply by using multiple fibers. The fPSD can be used for environmental monitoring of microcystins well below regulatory thresholds (sub nanogram-per-liter). The time-integrated concentration of MC-LR provided by fPSD field deployment was in excellent agreement with the mean dissolved MC-LR concentration taken from repeated daily grab sampling over a 10-day period in Dianchi Lake, China.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-215

On-line detection of algal toxins in sea water

A. Porchetta[1], S. Bodini[2,*], L. Micheli[1], G. Volpe[1], L. Fabiani[1], E. Moscetta[2], L. Sanfilippo[2], P. Moscetta[3], K. Petroupoulos[1], G. Palleschi[1]

[1]Department of Chemistry, University of Rome Tor Vergata, Rome, [2]Researcher, Systea SpA, Anagni, [3]Systea SpA, Rome, Italy

Abstract: Surveillance programs have to be adapted to integrate the monitoring of traditional chemical parameters with the investigation of “contaminants of emerging concern”. Among these, marine biotoxins represent a hazard because they can accumulate in fish and mussels and enter the food chain. The detection of marine biotoxins released by algae has mostly been directed on the analysis of fish/shellfish homogenate rather than seawater samples. To fill this gap, we developed and tested a novel automated networked system (ASMAT - Analytical System for Marine Algal Toxins) based on the micro Loop Flow Reactor (µLFR) technology to achieve on-line monitoring of marine toxins in coastal areas. Specifically, we designed and built an on-line analyzer hosting three sub-module sensors based on Enzyme–Linked Immuno–Magnetic Colorimetry (ELIMC) technique that allowed the measurement of three algal toxins: Domoic Acid (DA), Saxitoxin (STX) and Okadaic Acid (OA). The competitive immunoassay allows the detection of algal toxins as a function of the rate of a color production that decreases proportionally to the concentration of the toxin. This methodology combines the selectivity of the antibody, the convenience of a separation step through the use of magnetic beads (MBs), and the straightforwardness of the colorimetric detection. To provide full automation of single manual operations, the flow cell was designed to alternatively act also as a reactor, incubator and magnetic chamber. ELIMC assays were thus integrated within a fully automated system able to recognize and quantify sub-ppb levels of the target toxins. The main variables studied to optimize the ASMAT system were the amount of magnetic beads, flow cell, number of washings, reagent container positions and reagent volumes. In particular, the system architecture, including reagent dilution and circuit washings, was optimized to solve problems associated with carryover and affinity to plastic of the antibodies.

Calibration curves for the three analytes were consistent with the strict requirements limiting the presence of the toxins in environmental waters. On-line suitability of ASMAT was also demonstrated by a field installation within a floating platform in the port of La Spezia (Italy). After a general testing cycle, performed for checking the instrument, a full cycle of algal toxins analysis (calibration and seawater sample analysis) was executed and it confirms the applicability of the automated analysis for toxin detection *in situ*.

Although further work should be performed to develop additional specific antibodies to extend the application on other natural pollutants released by plants, algae and microorganisms, with a particular attention on freshwater cyanotoxins, results demonstrated that this automated monitoring system is robust and sensitive enough to allow the on-line monitoring of marine toxins in coastal areas and aquaculture plants.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

O-216

Exploring growth dynamics and ichthyotoxicity of the Chilean *Pseudochattonella verruculosa*

J. I. Mardones [1,*], K. Zenteno [2], G. Fuenzalida [1], L. Norambuena [1], L. Guzmán [1], A. Astuya [3], J. J. Dorantes-Aranda [4], C. Alves-de-Souza [5], G. Hallegraeff [4]

[1]Centro de Estudios de Algas Nocivas (CREAN), Instituto de Fomento Pesquero (IFOP), Puerto Montt, [2]Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, [3]Laboratory of Cell Culture and Marine Genomics, Marine Biotechnology Unit, Universidad de Concepción, Concepción, Chile, [4]Institute for Marine and Antarctic Studies (IMAS), University of Tasmania, Hobart, Australia, [5]Algal Resources Collection, MARBIONC, University of North Carolina Wilmington, Wilmington, USA

Abstract: Despite the large economic losses suffered by the Chilean salmon industry in 2016 (US$800M) due to a massive outbreak of the dictyochophyte *Pseudochattonella verruculosa* (~7000 cells ml^{-1}), the effect of environmental drivers and the potency of lytic toxins produced by the local clones of this species remain still largely unexplored. Monoclonal cultures of *P. verruculosa* ARC498 strain (ribosomal and mitochondrial sequencing ID) have shown a very narrow niche window for cell growth with growth rates enhanced at 30 salinity, 17°C and an irradiance of 20 µmol photon m^{-2} s^{-1}. The rainbow trout cell line RTgill-W1 assay was used to i) investigate the ichthyotoxicity of the ARC498 strain under natural estuarine salinity levels (25 and 35), and ii) differences in toxicity of lysed cells and supernatant at 5 different cell concentrations (from 10 to 100,000 cells ml^{-1}). No significant differences were observed between lysed cells and supernatant treatments in the two salinity levels, suggesting a potent extra-cellular lytic activity. Cytotoxicity was correlated to cell abundance, reducing gill cell viability down to 80 and 65% of controls at 10,000 and 100,000 cells ml^{-1}, respectively at 25 in salinity and only after 1h exposure. Unexpectedly, lytic compounds from ARC498 strain at 35 in salinity showed to be less toxic. Lytic compounds are highly unstable and rapidly degraded in the light after 4 and 8 days storage at 15°C. Research on the possible role of PUFA and ROS in ichthyotoxicity of *P. verruculosa* is pursued.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

O-217

Prymnesium parvum blooms and DMSP: production, metabolism and its impact to the environment

P. P. L. Rivera [1,*], J. Todd [1]
[1]School of Biological Sciences, University of East Anglia, Nowich, United Kingdom

Abstract: Major blooms of Prymnesium parvum often result in large-scale fish kills that have huge economic and ecological implications. P. parvum produces toxins called prymnesins, which are highly toxic to fish and other gill-breathing organisms. Apart from toxins, they also produce an organosulfur compound dimethylsulfoniopropionate (DMSP) and its enzymatic cleavage product dimethylsulfide (DMS). DMSP/DMS production by phytoplankton may have links to global climate and plays a pivotal role in marine sulfur biogeochemistry. DMSP may serve as a compatible solute, cryoprotectant, grazing deterrent, antioxidant and in the methionine cycle in marine DMSP-producing organisms. However, less is known about its production, metabolism, and impacts in the marine/aquatic environment at the cellular level. Here we examined the production of DMSP and its by-products by Prymnesium in relation to different environmental conditions. We isolated a strain of P. parvum in the Norfolk Broads, a series of interconnected rivers and lakes (Broads) found in the East of England, established cultures and subjected them to different environmental conditions. Results show that DMSP production in Prymnesium was not only stage-specific but also dependent on nutrient availability and varying salinity. Blooms of P. parvum may cause deleterious effects in the environment but they might also play a crucial role in the global sulfur cycle.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

O-218

FEEDING RESPONSES OF PERNA VIRIDIS & MAGALLANA BILINEATA WHEN EXPOSED TO HARMFUL ALGAE, PYRODINIUM BAHAMENSE

J. Maister [1,*], A. Yniguez [1], G. Wikfors [2], J. K. Andres [1], J. Mendoza [1], R. Azanza [1]

[1]University of the Philippines Diliman - The Marine Science Institute, Quezon City, Philippines, [2]Northeast Fisheries Science Center, National Oceanic and Atmospheric Administration (NOAA), Milford, USA

Abstract: Harmful Algal Blooms (HABs) and PSP (Paralytic Shellfish Poisoning) events are recurrent problems in many parts of the Philippines. *Pyrodinium bahamense* has been causing major PSP events through commercially-important shellfish such as *Perna viridis* (Asian green-lipped mussel) and *Megallana bilineata* (Philippine cupped oyster). Information on the feeding responses of Philippine bivalves to *P. bahamense* is limited, and understanding feeding behaviors could help improve current management policies and tools. This study aimed to understand the feeding responses of *P. viridis* and *M. bilineata* by assessing clearance rate, toxin uptake, and toxin removal of the bivalves when exposed to toxic and non-toxic phytoplankton. The bivalves were exposed to *P. bahamense* (toxic species), *Chattonella subsalsa* (non-toxic raphidophyte), or *Chaetoceros sp.* (non-toxic diatom) as mono-species treatments (*i.e., P. bahamense* “PbcM”*, C. subsalsa* “CsM,” or *Chaetoceros sp.*”CM”) and also exposed to combination-species treatments *(i.e,. P. bahamense* & *C. subsalsa* “PCs” or *P. bahamense* & *Chaetoceros sp*. “PC”). Clearance rate was estimated by counting cells (microscopy) remaining in the water at pre-determined time intervals (0, 5, 20, 50, 80, 115, 180, 245 minutes). Similarly, toxin uptake and removal was estimated every 4-5 hours (total of 24-36 hours) by dissecting and separating organs (1) gills, gonads & mantle, 2) digestive organs and 3) muscles and foot, and testing for toxicity using high-performance liquid chromatography (HPLC). Microscopy revealed increasing cell & bivalve fragments over time and occasional presence of bivalve eggs. Fragments were particularly discernable in CsM and PCs treatments. Clearance rates differed between the two bivalve species. *M. bilineata* displayed a smaller range in clearance values (all oyster treatments) as compared to *P. viridis,* wherein clearance values between the mussel treatments were very different. Highest and lowest clearance values were observed for *P. viridis*, with highest clearance values in the PbcM treatment and lowest clearance in the CsM treatment. Although *M. bilineata* clearance values did not differ as much as *P. viridis*, the same pattern of higher clearance values in PbcM treatment and lowest in CsM treatment was observed. All combination-species treatments displayed clearance values lower than those observed in PbcM. STX was the dominant toxin signature of *P.bahamense*. The mantle, gills and gonad displayed the highest microgram saxitoxin equivalent per 100g of meat. A shift in toxin burden was observed from the mantle, gills and gonad to the digestive organs near the end of the experiment. Low toxin concentrations were observed in the foot and muscles.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

O-219

Diversity and occurrence of harmful algal blooms in Kuwait's marine environment, NW Arabian Gulf

I. Polikarpov [1,*], M. Saburova [1], F. Al-Yamani [1]

[1]Ecosystem-Based Management of Marine Resources Program, Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research, Al-Kuwait, Kuwait

Abstract: Microalgal blooms occur in Kuwait's waters in the north-western area of the Arabian (Persian) Gulf year round and are caused by microalgae, cyanobacteria, and photosynthetic ciliates. The diverse assemblage of potentially toxic microalgae known elsewhere to cause syndromes of human poisoning was revealed in Kuwait's marine environment, including *Pseudo-nitzschia* (amnesic shellfish poisoning), *Dinophysis*, *Phalacroma* (diarrhetic shellfish poisoning), *Karenia* (neurotoxic shellfish poisoning), *Alexandrium*, *Pyrodinium bahamense*, *Gymnodinium catenatum* (paralytic shellfish poisoning), and *Fukuyoa*, *Ostreopsis*, *Coolia* (ciguatera fish poisoning). A wide range of potentially harmful microalgae that can be associated with massive mortality of marine biota, including known ichthyotoxic dinoflagellates (*Akashiwo*, *Amphidinium*, *Karenia*, *Karlodinium*, *Margalefidinium*, *Prorocentrum*, *Protoceratium*, *Takayama*), raphidophycean (*Chattonella*, *Fibrocapsa*, *Heterosigma*) and haptophyte (*Chrysochromulina*, *Prymnesium*) flagellates, were observed routinely in Kuwait's waters. The chronological overview of recorded algal bloom events indicates that Kuwait's marine environment has increasingly affected by HABs. Over the past decade, the frequency of algal blooms has enlarged significantly. Both toxic and high biomass algal blooms were associated with fish mortality in Kuwait's coastal waters. The most of algal blooms occurred during warm period in spring and autumn, whereas fish mortality events linked to high biomass phytoplankton blooms mainly coincided with hot season. The presence of listed above microalgal species may indicate a potential risk for Kuwait's marine environment, and necessitates conducting comprehensive studies on microalgal taxonomy, ecology and toxicology in the north-western Gulf area.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

O-220

Abalone farms suffer devastating mortalities attributed to yessotoxin-producing dinoflagellates

G. C. Pitcher [1,*], C. Foord [1], B. Macey [1], L. Mansfield [1], A. Mouton [2], M. Smith [3], S. Osmond [2], L. van der Molen [4]
[1]Fisheries Research and Development, Cape Town, [2]Amanzi Biosecurity, Sandbaai, [3]CSIR, [4]Aspirata, Cape Town, South Africa

Abstract: In January 2017 the development in Walker Bay on the South African coast of large dinoflagellate blooms impacted three land-based abalone farms (*Abagold, HIK Abalone,* and *Aqunion Whale Rock*) with devastating results. The death of several million abalone served to demonstrate the vulnerability of these farms to harmful algal blooms on the South African coast. This paper details various aspects of this bloom and its impact on abalone and abalone farming. Bloom development monitored remotely through satellite derived observations of ocean colour is described in conjunction with the collection and enumeration of phytoplankton samples collected at farm intakes. The dominant dinoflagellate species *Gonyaulax spinifera* and *Lingulodinium polyedrum* were identified by means of light microscopy and genetic sequencing data. The presence of yessotoxins (YTXs) was established in both phytoplankton and abalone, with the appearance of YTXs in abalone clearly coinciding with increases in cell concentrations of *G. spinifera* and *L. polyedrum.* The effect of these toxins on abalone was examined by histological analysis. The most significant pathology occurred in the gills which showed severe, generalized disruption of the gill epithelium characterized by degeneration and necrosis of epithelial cells accompanied by a modest inflammatory response. The impact on abalone farming in the region is documented and potential mitigation measures for future bloom events of a similar nature are discussed.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

O-221

Acute effects on Danio rerio exposed to extracts of *Sphaerospermopsis torques-reginae* (Cyanobacteria) (ITEP-24) producing anatoxin-a(s)

E. Pinto[1,*], K. Fernandes[1 2], T. Henry[2]

[1]School of Pharmacy, University of Sao Paulo, Sao Paulo, Brazil, [2]Institute of Life and Earth Sciences, Heriot-Watt University, Edinburgh, United Kingdom

Abstract: Anatoxin-a(s) [atx-a(s)] is a neurotoxin produced by some species of cyanobacteria, mainly of the genus *Anabaena* and *Sphaerospermopsis*. This neurotoxin is known as the only natural organosphosphate capable to inhibit acetylcholinesterase. Little information is available on the agonist and cholinergic effects of atx-a(s) on aquatic organisms, especially for the fish group. The aim of this study is to obtain a semi-purified atx-a(s) fraction of *S. torques-reginae* (strain ITEP-24) and investigate the adverse effects of this toxin as well as other components present in extracts of this cyanobacterium on *Danio rerio*. For this, LD50 experiments, histopathological and morphological analyses were performed. ITEP-24 strain has been cultivated in the laboratory under controlled conditions, aiming to obtain biomass for the semi-isolation experiments. Six extraction protocols were tested using lyophilized cells from the ITEP-24 strain with different solvents, to evaluate which was better to extract atx-a(s). Samples were analysed by liquid chromatography coupled to the mass spectrometer (LC/MS-QqQ) with ZIC-HILIC chromatographic column. From the extraction protocols tested, the combination of methanol/water (20:80 v/v) in 0.1M acetic acid was better to extract the ant-a(s) from the crude ITEP-24 extract. The same samples for the extraction protocols with crude extract and fresh culture were also analysed in the LC/MS-Q-TOF with C18 column, which allowed the identification of other compounds besides the atx-(s) itself, such as Namalides A and B, Spumigin K, Shinorine (an amino acid of the mycosporine type). After the selection of the extraction and identification method of the atx-a(s), Solid Phase Extraction (SPE) techniques were optimized using reverse-phase and weak polymer-cation exchange (StrataTM-X-CW) -isolation of the antx-a(s). The use of the StrataTM-X-CW column allowed the elimination of several interferences present in the crude extract of the ITEP-24 strain, at the end, fractions enriched with atx-a(s) were obtained. As there is no commercial standard of atx-a(s), the techniques adopted in the present study can contribute substantially to the identification and the semi-isolation of atx-a(s) in water bodies in greater quantity for analytical and toxicological studies. The acute exposure tests were conducted with *Danio rerio* embryos and larvae at concentrations below 1 mg/mL. The results showed LD50 rate of embryos and larvae in concentrations higher than 0.08 mg/mL and 0.31 mg/mL, respectively, for 96 hours of exposure. Other in vivo exposure tests were performed throughout this study, and aimed to assess not only mortality, but other sub-chronic and chronic effects during the life cycle of this species.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

O-222

Towards consensus reporting on the Global Status of Harmful Algal Blooms

G. M. Hallegraeff [1,*], H. Enevoldsen [2], L. Schweibold [3], A. Zingone [4]

[1]Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Australia, [2]IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Copenhagen, Denmark, [3]Institut Universitaire Europeen de la Mer, Plouzane, France, [4]Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Napoli, Italy

Abstract: Following the lead of IPCC consensus reporting, IPHAB expressed the need for a *Global HAB Status Report* compiling a worldwide overview of Harmful Algal Bloom events and their societal impacts. This effort aims to settle questions on the probability of change in HAB frequencies, intensities, and range resulting from environmental changes at local and global scales. The status as of mid 2018 of the *Harmful Algae Event Data Base* (HAEDAT) and *Ocean Biogeographic Information System* (OBIS) on the global distribution of HAB species are summarized. We collated historic HAB reports and analyse the status and probability of change in the period 1970-2018, interpreted against the key drivers of increased aquaculture, scientific awareness and monitoring efforts. Different regions are dominated by different seafood toxins (PSP in North America, DSP in Northern Europe, CFP in Oceania), but countries with finfish aquaculture are also significantly impacted by gill-damaging HABs. Some HAB problems flare up and dissipate (*Pyrodinium* in Phillipines), while changes in monitoring (closure of fisheries) and regulatory approaches also drive patterns. A first formal analysis of global HAB trends is attempted. Examples from the Australia-New Zealand region are compared with North European (ICES) and North-American (PICES) events.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

O-223

Overview of HABs in the Mediterranean Sea: a contribution to the Global HAB Status Report

A. Zingone [1,*], K. Aligizaki [2], L. Escalera [3], M. Fernández-Tejedor [4], A. Ismael [5], M. Montresor [1], S. Tas [6]

[1]Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Naples, Italy, [2]Laboratory Unit on Harmful Marine Microalgae, Biology Dept., Aristotle University of Thessaloniki, Thessaloniki, Greece, [3]Monitoring and Data Management Unit, Stazione Zoologica Anton Dohrn, Naples, Italy, [4]Institut de Recerca i Tecnologia Agroalimentaries (IRTA), - Sant Carles de la Ràpita, Tarragona, Spain, [5]Faculty of Science, Alexandria University, Moharram Bey, Egypt, [6]Institute of Marine Sciences and Management, University of Istanbul, Istanbul, Turkey

Abstract: The Mediterranean Sea (MS) is a temperate-subtropical basin characterized by oligotrophic offshore waters and coastal waters ranging from almost pristine to heavily impacted. Main resources are tourism and fisheries, whereas aquaculture is more developed in the western MS and continental Greek coasts, which are also the most intensively monitored.

Information on MS HABS date back to the beginning of the 1800, with reports of 'mare sporco' (mucilages) in the Adriatic Sea. Since then, mucilage events have been reported at several sites, at times concerning the benthic environment. Discolourations have been recorded since the midst of the last century (e.g., *Chattonella subsalsa, Prorocentrum cordatum, Noctiluca scintillans* and *Alexandrium minutum*) often occurring in restricted areas or harbors (e.g., *A. taylorii*). Mucilage and discolourations are a major issue in touristic areas in summer.

Although more than 70 potentially toxic species are detected in the MS, toxicity is less frequently reported and is mainly related to *Dinophysis* (e.g., *D. sacculus* and *D. fortii*) and *Alexandrium* (*A. minutum* and *A. pacificum*), with an important impact on aquaculture. *Pseudo-nitzschia* blooms are widespread, but domoic acid in shellfish rarely exceeds regulatory levels. In the southwestern Spanish coasts, closures in shellfish growing areas are also related to blooms of *Gymnodinium catenatum* and *Pseudo-nitzschia australis*. Fish kills are probably less sporadic than reported, representing a problem e.g., along the southern MS coasts and in the Ebro river delta. In the easternmost MS, blooms mainly affect the Golden Horn Estuary in the Sea of Marmara, sometimes associated to discolourations.

More recent are the blooms of the benthic dinoflagellates *Ostreopsis*, which occur all along the northern coasts, while the species is also found in the southern MS. Several new records of *Gambierdiscus* and *Fukuyoa* in the western and eastern MS raise concerns about the possible risk of Ciguatera. *Vulcanodinium rugosum* in different lagoons of the French MS is responsible for the accumulation of pinnatoxins in shellfish. New entries are also *Azadinium* species. While more harmful species and increasing trends are noticed, possibly related to increased monitoring activities, some decreasing trends are also seen, such as *Alexandrium minutum* disappearing from its type locality, the Harbour of Alexandria.

Disclosure of Interest: None Declared

O-224

Development of an inter-agency Global Ciguatera Strategy: FAO, IAEA, IOC-UNESCO and WHO joint initiative to address Ciguatera Poisoning

M.-Y. Dechraoui Bottein [1,*], M. Chinain [2], P. Hess [3], P. Tester [4], C. Gatti [2], E. Garrido Gamarro [5], A. Tritscher [6], H. Enevoldsen [7]

[1]Research scientist, International Atomic Energy Agency, Environment Laboratories (IAEA-EL), 4a Quai Antoine Ier, MC-98000, Monaco, Monaco, [2]Laboratory of Toxic Microalgae, Institut Louis Malardé UMR-EIO 241, PO Box 30, 98713, Papeete, French Polynesia, [3]Ifremer, Laboratoire Phycotoxines, 44311 Nantes, France, [4]Ocean Tester, LLC, 381 Gillikin Road, Beaufort, North Carolina 28516 , Beaufort, USA, [5]Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome, Italy, [6]Food Safety and Zoonoses Department, World Health Organization, Via Appia 20, Geneva, Switzerland, [7]Intergovernmental Oceanographic Commission of UNESCO, IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, 2100 , Copenhagen , Denmark

Abstract: Important progress limiting adverse impacts of harmful algal blooms (HABs) has been achieved by implementing rigorous monitoring programs addressing HABs species in the environment, marine toxins in seafood, and associated human diseases. However, the situation is very different for benthic HABs (BHABs), especially for *Gambierdiscus* species responsible of ciguatera poisoning (CP), the most common non-bacterial seafood poisoning globally. Small island states in tropical regions are particularly vulnerable to the consequences of CP, and global changes in climate may exacerbate the incidence rates, and impacts on natural and economic resources of ciguatera endemic populations.

To mitigate or prevent the adverse effects of CP on human health, including on food safety, nutrition, food security, and on the environment, an interagency initiative was proposed building on the strength and complementarity of four International Organizations and their respective mandates: the IAEA International Atomic Energy Agency (IAEA) aims to assist Member States meet their development needs through nuclear science, technology and innovation, the World Health Organization (WHO) for improving public health through reduction of food poisoning, the Food and Agriculture Organization (FAO) to ensure seafood safety and security, and the Intergovernmental Oceanographic Commission of the United Nations Educational, Scientific and Cultural Organization (IOC-UNESCO) to promote ocean research and education. Such a multidisciplinary initiative seeks to gain the necessary visibility and resource allocation to build-up resilience of affected populations and contribute to achieve their sustainable development goals (SDGs), via sustainable and safe seafood production, and improved monitoring strategies to reduce human health impacts due to CP.

The Goal will be achieved though implementation of a joint work plan including the development of 1) local guidance through compilation and sharing of global, regional and national data on ciguatera poisoning; 2) technical guidance for authorities on CP monitoring and management (including development of curriculum and training material, case definition and treatment options); 3) Codex Guidance and 4) recommendation and prioritization of research and development activities to overcome knowledge and data gaps. For the success of the Inter-Agency Global Ciguatera Strategy, commitment of laboratories worldwide to support and contribute to the tasks involved in the proposed workplan will strongly be encouraged.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

O-225

Harmful Algae Networking with ICES Working Groups on Introduction /Transfer of Marine Organisms and Ballast Water / Other Ship Vectors

C. H. Mckenzie [1,*] and ICES WGITMO ICES WGBOSV
[1]Northwest Atlantic Fisheries Centre, Fisheries and Oceans Canada, St. John's, Canada

Abstract: The Working Group on Introduction and Transfers of Marine Organisms (WGITMO) addresses aquatic non-native species that have an influence on and/or occur in the marine environment. Established in 1969, WGITMO addresses multiple research and applied issues related to bioinvasions, such as the early detection of harmful algal species and alerts to their potential spread, population dynamics and distribution, ecological impacts, and risk assessments. Of particular interest to the harmful algae network is WGITMO's work on the effect of climate change on the introduction and spread of non-native marine organisms (including phytoplankton and harmful species), especially in the Arctic environment. The group maintains annual records on new invasions in the ICES area and provides management advice upon request. The expert group has global vision, and it has established effective links with scientists representing other research organisations, such as the Mediterranean Science Commission (CIESM) and North Pacific Marine Science Organisation (PICES). The Working Group on Ballast Water and Other Ship Vectors (WGBOSV) has a focus on shipping activities, and it provides scientific support to the development of international measures aimed at reducing the risk of transporting non-native species in this manner. Shipping sub-vectors - such as ballast water, ballast sediments, in-tank biofouling and fouled hulls - are recognized globally as primary mechanisms for introducing aquatic non-native species to new habitats. As a joint working group, WGBOSV follows and supports the work of its three umbrella organizations: the International Council for the Exploration of the Seas (ICES), the Intergovernmental Oceanographic Commission of UNESCO (IOC), and the International Maritime Organization (IMO). Activities that are particularly relevant to the harmful algae network include: consideration of the appropriate discharge standards of organisms in ballast water, risk assessment methods, testing of ballast water treatment systems, and recommendations on port ballast water sampling programmes. Both working groups have collaborated with the ICES Working Group on Harmful Algal Bloom Dynamics (WGHABD) in refining sampling and analysis protocols for ballast water and, more recently, in the introduction and transfer of harmful algal species to the Arctic environment.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

O-226

The Global HAB Status Report: the FANSA update based on OBIS and HAEDAT maps and databases.

I. Sunensen [1 1,*], S. M. Mendez [2], J. Mardones [3], G. Pizarro [4], L. Proenca [5], S. Sanchez [6], G. Torres [7]

[1]Ficología, CONICET and División Ficología, FCNyM, UNLP, La Plata, Argentina, [2]Phytoplankton Laboratory, National Direction of Aquatic Resources, Montevideo, Uruguay, [3]Centro de Estudios de Algas Nocivas, Instituto de Fomento Pesquero, Puerto Montt, [4]Instituto de Fomento Pesquero, Punta Arenas, Chile, [5]Instituto Federal de Santa Catarina, Florianopolis, Brazil, [6]Instituto del Mar del Perú IMARPE, Lima, Peru, [7]Instituto Oceanogáfico de la Armada INOCAR, Guayaquil, Ecuador

Abstract: The Global HAB Status Report was implemented in 2016 continuing the compilations gathered by the HABMap project, facilitated by the regionalization of the world. The South American "FANSA" region is integrated by Brazil, Uruguay, Argentina, Chile, Peru and Ecuador. The trends observed in this region in the last years include: a) new toxic species in the region, b) the geographical expansion of already known species in the area, c) production of new toxins and d) HAEDAT events increasing in duration and/or impacts.

New HAB species records for the region comprise: Ciguatoxins producing species of *Gambierdiscus, Coolia* and *Ostreopsis* in Brazil, domoic acid producing *Pseudo-nitzschia sub-pacifica* from Peru, *Karenia brevis* and *Margalefidinium polykrydoides* from Ecuador, *Lingulodinium polyedrum* and the ichthyotoxic *Pseudo-chattonella* and *Karenia* from Chile and *Pseudo-nitzschia brasiliana* and *Azadinium* species from Argentina. To date, the most significant and notorious cases involving both geographical expansion and production of new toxins have developed in Chile. In addition to the significant expansion of *Alexandrium catenella* further north to coastal areas that negatively affected human health and the local salmon industry, two unprecedented world records have been registered in the Aysén region in summer 2018: a) PSP accumulation as high as 143,130 µg STXeq./100g and b) *Dinophysis acuta* cell concentrations of 118,700 cell/L. Most of the HAEDAT events reported in the FANSA region correspond to dinoflagellates of the genera *Alexandrium* (46 %) and *Dinophysis* (33 %) followed by *Gymnodinium catenatum* (20 %), species of *Pseudo-nitzschia* and *Pseudo-chattonella* (1 %). In the last decade DSP events have increased in relation to the PSP (previous to 2000: PSP/DSP events 80/20 %, after 2000: 62/36%, with the addition of ASP events 2%), especially in the Southwestern Atlantic Ocean, related to the long lasting blooms produced by *Dinophysis acuminata/ovum complex,* with events of over a year of duration in coastal waters in front of Uruguay, Brazil and Argentina.

The relevance that HAB topics are taking in the FANSA region due to the serious economic losses, the potential risk to seafood consumers and the ecological impacts, imply the need to expand the monitoring programs in all the countries also encompassing the benthic HAB species.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

O-227

International phytoplankton intercomparison (IPI) exercises in the abundance and composition of marine microalgae 2011-2017

R. Salas [1,*], J. Larsen [2], D. Walsh [3], H. O. Enevoldsen [2]

[1]Scientific and Technical Officer, Marine Institute, Oranmore, Ireland, [2]Biology, IOC Science and Communication center on harmful algae, Copenhagen, Denmark, [3]Phytoplankton, Marine Institute, Galway, Ireland

Abstract: This study presents data from a series of Phytoplankton intercomparison exercises between laboratories across the world over a seven year period (2011-2017) in microalgae abundance and composition as part of the IPI (International Phytoplankton Intercomparison) testing scheme.The purpose of this exercise is to compare and evaluate the performance of testing laboratories and to monitor the laboratories continuing performance over time.

Regular quality control assessments are crucial to ensure a high quality output of phytoplankton data. Combined performance scores over successive rounds provide a better representation of the ongoing competency of laboratories and analysts than single round scores. The data shows that phytoplankton laboratories are highly competent at the identification and enumeration of microalgae which are important oceanographic biological observations. However, patterns of performance can be observed with a small number of laboratories tending to over or under estimate systematically. This may suggest methodological and/or technical difficulties that need improvement. The scores are based on the consensus values of up to 90 analysts from over 40 laboratories across the world.

The data shows that analytical methods equivalence based on counting strategies and volume analysed is unresolved, while most analysts use the Utermöhl cell counting technique for analysis, the preferred volumes analysed are 25 and 10ml and these do not show equivalence. There are also significant differences due to the counting strategies used, with whole or half chamber cell counts very different with results from transect or field of view cell counts. analysis of same sample volumes and using similar counting strategies minimises the lack of reproducibility and repeatability between and within laboratories.

The re-scaled Sum of Z-scores (RSZ) versus the Relative Laboratory Performance (RLP) charts were used to investigate counting effects between analysts over 7 ring tests. The results show that the performance of a small number of analysts is not of the standard required. In some cases, the performance can be seen to improve over several rounds, which suggest that there is a learning process and an improvement in the technical and methodological areas. Other analysts do not improve over several rounds indicating that training is required. Some individual may be bias towards certain species.

The results show that analysts are better at identifying 'toxic' than 'non-toxic' species suggesting that laboratories put more emphasis in the learning and identification of these species. In relation to qualitative results, all analysts are given a correct, incorrect, not identified flag for each of the measurands in the samples, so in order to pass the test at least 80% correct abundance and composition results are needed to pass the round.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-228

Boronate techniques for clean-up and concentration of diol-containing algal toxins

C. O. Miles [1,*], D. G. Beach [1], E. Kerrin [1], P. McCarron [1], J. Kilcoyne [2], S. D. Giddings [1], T. Waaler [3], T. Rundberget [4], I. A. Samdal [3], K. E. Løvberg [3]

[1]Measurement Science and Standards, National Research Council Canada, Halifax, Canada, [2]Marine Institute, Galway, Ireland, [3]Norwegian Veterinary Institute, [4]Norwegian Institute for Water Research, Oslo, Norway

Abstract: Although boronates bind reversibly to many vic-diols, and vic-diols are a common structural feature of many algal toxins, this boronate-diol interaction has not been exploited for clean-up and concentration of algal toxins. We have developed methodology for cleaning up and concentrating azaspiracids from mussel extracts using a polymer-bound boronic acid. The method removed the colour from the extract, as well as the majority of interferences and matrix effects normally seen in LC-MS analyses of azaspiracids in mussels. We then developed a modified approach using aryl boronates to selectively capture tetrodotoxin, which also contains a vic-diol, and many of its congeners. The tetrodotoxin analogues were selectively released from the boronate material to yield very clean extracts containing tetrodotoxin analogues and very little else. Potential interferences in LC-MS analyses, such as arginine and other amino acids, were completely eliminated, resulting in a simplified analysis. Boronate technology may also be useful for preparative purification of larger amounts of algal toxins for the preparation of reference materials and toxicological studies. We are now examining the applicability of boronate techniques to the analysis and purification of a range of other algal toxins that contain vic-diols, including ciguatoxins, palytoxins, pectenotoxins and the okadaic acid group.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-229

Simultaneous monitoring of maitotoxin and ciguatoxins in algal cultures and fish extracts

D. T. Harwood [1,*], M. Boundy [1], A. Selwood [1], J. S. Murray [1]
[1]Cawthron Institute, Nelson, New Zealand

Abstract: Ciguatera fish poisoning is a serious human health issue caused by the consumption of reef fish contaminated with ciguatoxins and is reported as the most common cause of non-bacterial food poisoning globally. The causative toxins bioaccumulate and biotransform up the food chain, from small herbivorous reef fish that graze on microalgae of the genus *Gambierdiscus* into the higher trophic level omnivorous and carnivorous fish that predate on them. The number of *Gambierdiscus* species being described is increasing rapidly and the role of other toxins produced by this benthic dinoflagellate genus in ciguatera intoxications, such as maitotoxin, remains unclear. Ciguatoxins and maitotoxin are among the most potent marine toxins known and there are currently no methods of analysis that can simultaneously monitor these toxins with a high degree of specificity.

To address this, a rapid and selective ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method has been developed to rapidly screen *Gambierdiscus* cultures for the production of ciguatoxins and maitotoxin. A fast, simple extraction method has also been developed and validated for fish samples to allow sensitive quantification of the potent ciguatoxin fish metabolite P-CTX-1B. Novel aspects of this analytical method include the use of alkaline mobile phase for chromatographic separation and specific monitoring of the various toxins through use of both quantitative and confirmation transitions. This method has good potential to help evaluate ciguatera risk associated with *Gambierdiscus* and related microalgal species, and to help reinvigorate method development activities for this important and analytically challenging toxin class.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-230

Characterising the performance of the ciguatoxin-radioligand receptor binding assay using a brevetoxin as standard

M. L. Ranada [1,*], K. J. Estorque [1], R. Clausing [2], M.-Y. D. Bottein [2], R. S. Tabbada [1]

[1]IAEA Collaborating Center on Harmful Algae, Chemistry Research Section, Philippine Nuclear Research Institute - Department of Science and Technology (DOST-PNRI), Diliman, Quezon City, Philippines, [2]Environment Laboratories (IAEA-EL), International Atomic Energy Agency, 4a Quai Antoine Ier, Monaco

Abstract: Ciguatera poisoning (CP), is one of the most common food poisoning globally due to the consumption of seafood that have accumulated ciguatoxins (CTXs) in their tissues. CTXs are fat soluble polyether neurotoxins targeting the voltage-gated sodium channels (Na_v) leading to neurological, gastroinstestinal and cardiovascular symptoms. The methods developed to analyze this family of toxins in seafood and algal samples include LC-MS/MS, N2A cell-based assays, and radioligand and fluorescent receptor binding assays (RBA). The radioligand RBA measures relative toxin potencies of CTX using a tritium-labelled brevetoxin ($[^3H]$-PbTx-3) as standard, which competes with CTX for specific binding to Na_v. As a result, a relative potency of 1:5 for both Pacific P-CTX-2 and P-CTX-3C are determined comparing these to PbTx-3 standard curve. The RBA showed high sensitivity at IC_{50} = 6.718 nM PbTx-3 ± 1.063 (n = 11) and within a calibration range of 0-6000 ng/mL, and is now being adapted for marine research applications. This endeavour will be essential to CTX-detection in fish to strengthen the seafood monitoring program.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-231

The zebrafish animal model as a tool for detecting marine unidentified biotoxins and toxins 'metabolites

S. Rainieri [1,*], A. Ereño [1], A. Barranco [1]

[1]Department of Food Quality Safety and Identity, AZTI, Derio, Spain

Abstract: Several methods based on functional, biochemical and chemical tests for detecting marine biotoxins are currently available. When the toxic effect is produced by a mixture of toxins, and /or by toxins' metabolites or by unidentified toxins, the available methodology could present some limitations. To overcome this problem, we have developed a procedure based on the evaluation of the effect of toxins on the model vertebrate zebrafish (*Danio rerio*). The procedure is an adaptation of a method employed for evaluating the effect of other chemical contaminants. It consists of extracting toxins from contaminated shellfish using standard methods and to expose zebrafish embryos to the extracted solution during 24-48h. After the exposure, acute toxicity is detected by evaluating embryos' death and malformations. In addition, *in vitro* activity of enzymes involved in the xenobiotic metabolism and the differential expression of the genes encoding these enzymes was also assessed. This procedure is based on a detection-by-effect method; therefore, it cannot identify the toxin itself. However, it can be useful as a preventive tool to alert for the presence of biotoxin material of unidentified nature that could be overlooked in periodical monitoring.

So far, the procedure has been tested on contaminated mussels and cockles and has proved to be effective for detecting lipophilic toxins related to the okadaic acid group as well as their metabolites. Zebrafish embryos are not considered laboratory animals up to 5 days of life, therefore the procedure can be considered alternative to the use of animals. In spite of this, embryos provide the response of a fully formed organisms that share a very high degree of functional homology with all vertebrates including humans. This model has been used before for testing the developmental toxicity of marine and freshwater toxins (eg. domoic acid); nevertheless, it has not used before as a living sensor for their detection.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-232

Enzymatic biosensor for the detection of paralytic shellfish poisoning toxins

M. I. Raposo [1], M. T. S.R. Gomes [1], M. J. Botelho [2 3], S. T. Costa [2], C. Vale [3], A. Rudnitskaya [1,*]
[1]Chemistry Department and CESAM, Aveiro University, Aveiro, [2]IPMA, Lisbon, [3]CIIMAR, University of Porto, Porto, Portugal

Abstract: Proliferation of microalgae, also called harmful algal blooms (HABs), affect bivalve harvesting and bivalve production in the coastal waters. Some phytoplankton species are producers of paralytic shellfish toxins (PSTs), which are of particular concern due to the life-threatening neurological symptoms they cause. To protect consumers, routine monitoring programs for PSTs in commercial bivalves are established in most coastal countries. Several assays and biosensors, mostly based on antibodies, sodium channels and neural cells has been proposed for rapid and low cost detection of PSTs. However, they aim mostly to detect saxitoxin (STX), which is the most common PSTs worldwide. As PST profile detected in bivalves from Portuguese coast during *Gymnodinium catenatum* events differs due to the predominance of N-sulfocarbamoyl and decarbamoyl toxins, usefulness of these biosensors is quite limited.

In this work enzymatic assay employing PSTs' transforming enzyme, carbamoylase, was developed for the determination of one of the most abundant PSTs in Portuguese waters - gonyautoxin GTX5. Carbamoylase was extracted and purified from the surf clam *S. Solida*. Carbamoylase action consists in the cleavage of the carbamate moiety of STX producing dcSTX or one sulfocarbamoyl moiety of GTX5 and C1&C2, producing dcSTX and dcGTX2+3. Carbamoylase extracted from *S. Solida* was capable to hydrolyze both carbamate (STX) and N-sulfocarbamate toxins (GTX5 and C1&2) though it is specificity towards STX was lower. Specificity and molecular weight (95 kDa determined by SDS-PAGE) indicate that enzyme extracted from *S. Solida* is similar to carbamoylase extracted from Japanese bivalve *M. chinensis* [1]. After experimental conditions optimization, such as temperature, pH and buffer concentration, carbamoylase was used for bioassay development. Bioassay consisted in incubation of enzyme solutions with samples containing GTX5 toxin and quantification of the product of enzymatic reaction, dcSTX, using potentiometric chemical sensor. Potentiometric sensor with plasticized PVC membrane with sensitivity to dcSTX and STX and no sensitivity to GTX5 and C1&2 was selected [2]. Enzymatic assay showed response to GTX5 of 38mV/pX in the concentration range from 0.54 to 2.2 µmolL^{-1}. Both STX and GTX5 give dcSTX as a product of enzymatic reaction. As STX is typically absent in contaminated bivalves during *G. catenatum* events, GTX5 can be determined selectively using developed biosensor.

References

1. Cho, Y., Ogawa, N., Takahashi, M., Lin, H.-P., Oshima, Y., 2008. Biochim. Biophys. Acta, 1784, 1277–1285.
2. Ferreira, N.S., Cruz, M.G.N., Gomes, M.T.S.R., Rudnitskaya, A. 2018. Talanta, 181, 380-384.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-233

First insights into the biosynthesis of ovatoxins, palytoxin analogues from the dinoflagellate *Ostreopsis* cf. *ovata*.

E. Ternon [1,*], M. Peralez [1], M.-Y. Bottein Dechraoui [2], O. P. Thomas [1 3]
[1]CNRS Géoazur, University of Nice, Nice, France, [2]IAEA, Monaco, Monaco, [3]NUI Galway, Galway, Ireland

Abstract: Ovatoxins present in the dinoflagellate *Ostreopsis* cf. *ovata* are structural analogs of the potent palytoxin. Like most toxins found in dinoflagellates, ovatoxins are supposed to originate from a polyketide biosynthetic pathway. Polyketides are assembled by the incorporation of acetic, propionic or butyric acid units and can further be enriched by the incorporation of amino acids. The biosynthesis of dinoflagellate polyketides is far to be fully understood mainly because of the lack of experimental data. While gene clusters seem highly complex and very difficult to identify, preliminary feeding experiments are also challenging due to the large volumes of culture needed with labelled precursors. First results obtained from ^{13}C incorporation into some dinoflagellate polyketides have concluded on the intertwined involvement of simple carboxylic acids with frequent rearrangements therefore highlighting the need for additional data.

In the context of the project OCEAN-15 funded by the French ANR, the biosynthesis of the ovatoxins was investigated experimentally on Mediterranean dinoflagellate *Ostreopsis* cf. *ovata*. A preliminary feeding experiment was conducted using ^{14}C radio-labelled precursors to identify the putative precursors of ovatoxins. The following ^{14}C labelled precursors were selected for our experiments: (i) acetate and propionate as key building block units, (ii) *S*-adenosin-methionine (SAM) as the precursor of the branched methyls, and (iii) beta-alanine for its involvement in the formation of the amides. Ovatoxins were purified two consecutive times on different solid phases to avoid any radioactive contaminant. The specific activity of each fraction was measured on TLC by the highly sensitive Beta-imager and it showed a significant incorporation of the two acids acetate and propionate but also beta-alanine, and a very low incorporation of SAM. Further experiment using stable isotopes labelling (^{13}C and ^{15}N) are planned to help defining the incorporation sites of the two acids and the beta-alanine.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

O-234

Synergistic and antagonistic effects of multi-specific HAB on reproduction of Japanese pearl oyster

L. Basti [1,*], J. Go [2], S. Okano [2], K. Higuchi [2], K. Nagai [2]

[1]Deaprtment of Ocean Sciences, Tokyo University of Marine Science and Technology, Tokyo, [2]Pearl Research Laboratory, K. Mikimoto & Co. LtD., Mie, Japan

Abstract: Several aspects of the effects of Harmful Algal Blooms (HAB) on the reproduction of aquatic organisms remain unclear. In the present paper, the impacts of HAB on the reproduction of bivalve molluscs are reviewed. In addition, the results of recent experiments on the effects of several HAB species on the reproduction of Japanese pearl oyster are presented. The selected HAB species are witnessing range expansion in some coastal areas of Japan and are forming multi-specific blooms.

Fertilized eggs and three larval stages were exposed to mono-specific and multi-specific blooms of *Heterocapsa circularisquama*, *Karenia mikimotoi*, *K. papilionacea*, and *Chattonella marina* (mixed blooms: *H. circularisquama*+*C. marina* (*HCm*); *K. mikimotoi*+*K. papilionacea* (*KmKp*); *K. mikimotoi* + *C. marina* (*KCm*)). Embryo anomaly was used as a measure of the effects on embryogenesis, following 7 h exposures. Mortalities, activity rates, and activity of the anti-oxidants enzymes super-oxidase dismutase (SOD), Catalse (CAT), Gluthatione-S-transferase (GST), and Gluthatione peroxidase (GPx) were assessed in larvae following 24, 48 and 72h of exposure.

Embryogenesis was affected mainly by mono-specific exposure to *H. circularisquama*. In the multi-specific exposures, embryogenesis was affected by exposures to mixed blooms of the HAB species. Larval survivorship was not affected by any of the exposures. The activity was, however, affected as well as the activity of the anti-oxidant enzymes. For *KCm* and *KmKp* experiments, larval activity decreased and anti-oxidant enzymes activity increased, especially following exposures to mixed blooms where *K. mikimotoi* was dominant. For *HCm* experiments, larval activity decreased and anti-oxidant enzymes activity increased, especially following exposures to mixed blooms where *C. marina* was dominant. But these effects were less pronounced than in the control mono-specific exposures.

The results of the study highlight the implication of reactive oxygen species (ROS) in the negative effects of the HAB species on reproduction of Japanese pearl oyster. In addition, differential synergistic effects in mixed blooms of the HAB species were found (*K. mikimotoi* and *K. papilionacea; K. mikimotoi* and *C. marina*), whereas antagonistic effects were shown in others (*H. circularisquama* and *C. marina*). These effects should be further studied to understand both the behavior of HAB in mixed blooms and the impacts on the reproduction of commercial shellfish.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

O-235

Bioactive extracellular compounds produced by *Alexandrium minutum* affect oyster gametes

J. Castrec [1,*], M. Boulais [2], P. Soudant [3], N. Le Goïc [3], C. Fabioux [3], H. Hégaret [3]

[1]LEMAR, University of Western Brittany, Plouzané, France, [2]Center for Marine Science, University of North Carolina, Wilmington, USA, [3]LEMAR UMR 6539 CNRS/UBO/IRD/Ifremer, Plouzané, France

Abstract: Dinoflagellates from the globally distributed genus *Alexandrium* are known to produce both Paralytic Shellfish Toxins (PSTs) and uncharacterized Bioactive Extracellular Compounds (BECs) with allelopathic and cytotoxic activities. In France, blooms of *Alexandrium minutum* appear generally during the spawning period of most bivalves. These blooms could therefore impact gamete quality and/or larval development of bivalves, causing real issues for economically important species, such as the Pacific oyster *Crassostrea gigas* (gen. nov. *Magallana gigas*).

The aim of this work was to test the effects of three strains of *Alexandrium minutum* producing either only PSTs, only BECs, or both PSTs and BECs upon oyster gametes. Previous study using a cellular bioassay has showed that the BEC-producing strain is more cytotoxic than the strain producing both PSTs and BECs for the diatom *Chaetoceros* sp. Oocytes and spermatozoa were exposed *in vitro* during 4 hours to a range of *A. minutum* concentrations (10 to 2.5×10^4 cell mL^{-1}). Cellular characteristics of gametes (mortality, Reactive Oxygen Species (ROS) production, sperm Mitochondrial Membrane Potential (MMP)) were assessed by flow cytometry, and oocyte morphology and spermatozoa motility were analysed by microscopy.

The *A. minutum* BEC-producing strain increased male and female gamete mortality, spermatozoa ROS production, and decreased spermatozoa MMP and motility. The strain producing both PSTs and BECs increased oocyte mortality, whereas the PST-producing strain had no apparent effect on gamete cellular parameters. These results suggest that a direct exposure of oyster gametes to *A. minutum*, reducing gamete survival and “quality”, can affect oyster fertility and reproduction success. This study highlights the significant toxicity of the BECs produced by *A. minutum* on oyster gametes and the need to characterize these unknown substances.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

O-236

The Paralytic Shellfish Saxitoxin induces apoptosis of oyster immune cells via a caspases-dependent pathway

J.-L. Rolland [1,*]

[1]UMR 5244 IHPE, IFREMER, Monptellier, France

Abstract: Oyster exposure to the toxic dinoflagellate *Alexandrium pacificum (catenella)* was previously shown to result in apoptosis of *Crassostrea gigas* immune cells, the hemocytes. We investigated whether hemocytes were directly targeted by paralytic shellfish toxins (PSTs), and found that *in vitro*, coumarin-labeled saxitoxin localized in cytoplasmic granules of hemocytes, as determined by multiphoton microscopy. We also demonstrated that PSTs, including saxitoxin, were directly responsible for the apoptosis of hemocytes. Indeed, *in vitro,* STX and derivatives induced nuclear condensation, phosphatidylserine exposure, membrane permeability and DNA fragmentation of hemocytes, as determined by a series of labeling and microscopy experiments. The apoptosis pathway involved was dependent on caspase activation and independent of ROS production. Collectively, our results show that PSTs produced by toxic dinoflagellates enter the cytoplasm and induce apoptosis of oyster immune cells through a caspase-dependent pathway.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

O-237

Tropical waters: Cyanobacterial dynamics and impact of their toxins over 3 generations on the tropical *Daphnia lumholtzi*

T.-S. Dao[1], J. Nimptsch[2], K. Dinh[3], T.-M.-C. Vo[1], T.-L. Pham[1], C. Wiegand[4,*]

[1]Faculty of Environment and Natural Resources, Hochiminh City University of Technology, Hochiminh, Viet Nam, [2]Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile, [3]National Institute of Aquatic Resources, Technical University of Denmark, Lyngby, Denmark, [4]UMR CNRS 6553 EcoBio, Université Rennes 1 , Rennes, France

Abstract: Climate change and human activities including eutrophication of water bodies and changing of water regimes induce an increased frequency and intensity of cyanobacterial blooms, which are potentially toxic. Compared to temperate zones, in tropical water reservoirs such as Tri An and Dau Tieng Reservoirs, Vietnam, the annual higher temperature additionally favours cyanobacterial development and their toxin production. Intracellular concentrations of MC reached 2.50 and 2.13 ▫▫g MC.L^{-1}in September and February with total phosphor as the primary abiotic factor influencing cyanobacterial biomass and MC concentrations.

Zooplankton communities belong to the first affected group, which is well investigated for temperate species, but less in tropical freshwater ecosystems. As zooplankton needs to sustain exposure during their life span or for multiple generations, we focussed on chronic and multigenerational impacts of cyanobacterial extracts and pure microcystin-LR in environmental realistic concentrations on the temperate species, *Daphnia magna* and the tropical species *Daphnia lumholtzi*. Fitness-related traits including survival, maturity age, body length, fecundity, were determined in both daphnid species for 1-3 generations.

Both species' life traits were slightly affected in the 'parent' generation (F0), and survivorship decreased during chronic exposure with increasing microcystin concentration. Depending on the exposure scenario, effects were more pronounced in offspring, or offspring developed tolerance: If *D. magna*parent generation was continuously exposed, their offspring took longer to reach maturity and their survival decreased, even if they were raised in control medium. Besides, cessation of the eggs/embryos and malformation of neonates was caused by cyanobacterial toxins. In *D. magna* offspring of pulse exposed parent generation, development of tolerance in terms of better survival was mediated by detoxification enzymes.

D. lumholtzi suffered strong direct, accumulated and carried-over impacts of continuous exposure to the toxins on life history traits on the F1 and F2, including delayed maturation, reductions of survival, growth and reproduction, and consequently reduced intrinsic rate. *D. lumholtzi* needed at least 2 consecutively exposed generations before signs of tolerance development appeared. Chronic exposure to long lasting blooms, even at low density, evidently impaired population density of *D. lumholtzi* in tropical lakes and reservoirs.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

O-238

Can the blue diatom *Haslea ostrearia* with strong biological activity be considered as a harmful alga?

C. Falaise [1,*], R. Tremblay [2], P. Cormier [3], C. François [4], A. James [3], A. Seger [5], A. Hayashi [5], G. Hallegraeff [5], J.-L. Mouget [1]
[1]Laboratoire Mer Molécule Santé (EA 2160, FR CNRS 3473 IUML), Le Mans Université, Le Mans, France, [2]Institut des Sciences de la Mer (ISMER), Université du Québec à Rimouski, Rimouski, Canada, [3]Integrative Biology of Marine Models, Sorbonne Universités (UPMC Paris 06, CNRS, UMR 8227) Station Biologique de Roscoff, Roscoff, [4]Laboratoire de Génétique et de Pathologie des Mollusques Marins (SG2M-LGPM), Ifremer, La Tremblade, France, [5]Institute for Marine and Antarctic Studies (IMAS), University of Tasmania, Hobart, Australia

Abstract: Marennine is the water soluble blue pigment produced by the marine diatom *Haslea ostrearia*. Blooms in oyster ponds are not associated with mortality events and oyster lovers have been eating “green” oysters for centuries without any record of harmful impacts.

Although the blue diatom and its pigment have been described centuries ago little is known about the ecological significance of marennine. This compound has previously been shown to display allelopathical, antioxidant or antibacterial activities *in vitro*, but no evidence has emerged for its possible ecological advantage. The application of marennine has been considered in aquaculture to limit bacterial growth or improve physiological condition of farmed shellfish. A prophylactic effect has been observed at low concentration, improving mussel larval survival when exposed to pathogenic *Vibrio*. Other studies showed larval mortalities at high concentrations or modification of shellfish behavior.

To assess if marennine is a biologically active substance or can be classified as a “natural toxin”, we investigated its activity against marine bacteria, echinoderms, bivalves, human gut epithelial and fish gill cell lines, with acute and sublethal effects sometimes observed depending on the target organism, and marennine concentration. Marennine can cause mortality or embryonic developmental delays in various marine organisms. The biological activity of marennine is compared with that of various algal toxins and similarities and differences are pointed out.

Disclosure of Interest: None Declared

O-239

GlobalHAB (IOC-UNESCO and SCOR): International coordination for sound knowledge of HABs to manage their impacts

E. Berdalet [1,*], N. Banas [2], E. Bresnan [3], M. Burford [4], K. Davidson [5], C. Gobler [6], B. Karlson [7], R. Kudela [8], P. T. Lim [9], L. Mackenzie [10], M. Montresor [11], V. Trainer [12], G. Usup [13], K. Yin [14], H. Enevoldsen [15], E. Urban [16]

[1]Marine Biology and Oceanography, Institute of Marine Sciences (ICM-CSIC), Barcelona, Spain, [2]University of Strathclyde, Glasgow, [3]Marine Scotland Science, Aberdeen, United Kingdom, [4]Australian Rivers Institute, Griffith University, Nathan, Queensland, Australia, [5]Scottish Marine Institute, The Scottish Association for Marine Science, Oban, PA37 1QA, United Kingdom, [6]Stony Brook University, Southampton, New York, NY, USA, [7]Swedish Meteorological and Hydrological Institute, Västra Frölunda, Sweden, [8]University of California, Santa Cruz, CA, USA, [9]Institute of Ocean and Earth Sciences, University of Malaya, Kuala Lumpur, Malaysia, [10]Cawthron Institute, Nelson, New Zealand, [11]Stazione Zoologica Anton Dohrn, Napoli, Italy, [12]Northwest Fisheries Science CenterNational Marine Fisheries Service , National Oceanic and Atmospheric Administration, Seattle, WA, USA, [13]School of Environmental and Resource Science, Faculty of Science and Technology , Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia, [14]Sun Yat-Sen (Zhongshan) University, Guangzhou, China, [15]IOC UNESCO/ University of Copenhagen, Copenhagen, Denmark, [16]Scientific Committee on Oceanic Research, SCOR, Newark, Delaware, USA

Abstract: The Global Harmful Algal Blooms (GlobalHAB, www.globalhab.info) Programme is aimed at fostering and promoting international cooperative research directed toward improving the understanding and prediction of HAB events in aquatic ecosystems, and providing sound knowledge for policy- and decision-making to manage and mitigate HAB impacts in a changing planet. GlobalHAB is sponsored by the Intergovernmental Oceanographic Commission (IOC) of UNESCO and the Scientific Committee on Oceanic Research (SCOR). GlobalHAB was launched in 2016 and will last for 10 years.

This presentation will summarize the scientific objectives included in the GlobalHAB Themes, which range from small-scale (e.g., cellular) subjects (e.g., biodiversity, adaptive strategies) to studies at ecosystem scale and climate change-related processes. GlobalHAB's perspective is multidisciplinary, integrating physics, chemistry and biology, and addressing the impacts of HABs on the environment and human societies (including Health, Economy and Socio-cultural aspects). Improved observation within and across the different themes should result in better predictions of HAB events and contribute to the management of their impacts.

Ongoing activities to implement these objectives sponsored or co-sponsored by GlobalHAB will also be presented. For instance, a "Best Practice Guidelines for the Study of HABs in the context of Climate Change" is been elaborated as a toolbox to enhance the ability to study the specific effects that climate change may have on the character, frequency and intensity of HABs. GlobalHAB is also participating in the intercomparison exercise on methods to sample harmful benthic dinoflagellates in coordination with IAEA, RAMOGE and NOAA. This illustrates the international nature of GlobalHAB with interaction with other entities (e.g. ICES, PICES, ISSHA, FAO, WHO) and programmes (e.g. GOOS, GO2NE, IPCC) that share objectives on HABs research, management and mitigation.

GlobalHAB encourages the participation of the international community. Thus, Scientists are invited to participate in GlobalHAB by designing scientific activities in keeping with the goals and objectives of GlobalHAB, by applying for endorsement of such activities, and by participating in framework activities.

Disclosure of Interest: None Declared

O-240

INCORPORATION OF STATE AND LOCAL FISHERY AND HEALTH MANAGERS IN THE FOMULATION OF NATIONAL HAB POLICY

D. Ayres [1,*]

[1]Coastal Shellfish Manager, Washington State Department of Fish and Wildlife, Montesano, USA

Abstract: Federal scientists and policy makers working on harmful algal bloom (HAB) issues in the United States (U.S) have long sought to include state and local fishery and human health managers who are involved with natural resources impacted by HABs. This inclusion has ranged from inviting such managers to join informal discussions all the way to including state managers in proving oral testimony before Congressional Committees considering HAB related legislation and also asking managers to join external peer review panels of federal agency HAB portfolios. In addition, the U.S. National HAB Office, has long used federal funds to provide travel support for state and local managers to attend the U.S. HAB Symposium held bi-annually in various locations around the nation. This inclusion has resulted in strong federal-state-local relationships that have built a vibrant and successful HAB “community” throughout the nation and greatly helped to address HAB issues in the U.S. This talk will discuss the author’s long experience with such state/federal interactions and that of other managers with the purpose of encouraging federal scientists and policy makers from other nations to begin to build the same relationships with their state and local fishery and human health management communities.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

O-241

Looking for HABs along the East Coast of the USA

E. Fensin [1,*], C. Alves-de-Souza [2], N. Hall [3], C. Holland [4]

[1]North Carolina Division of Water Resources, Raleigh, [2]Algal Resources Collection, MARBIONC at CREST Research Park, Wilmington, [3]Institute of Marine Sciences, University of North Carolina-Chapel Hill, Morehead City, [4]National Oceanic and Atmospheric Administration, Center for Coastal Fisheries and Habitat Research, Beaufort, USA

Abstract: As part of the Global HAB Status Report for Region I, a literature search was conducted to find records of harmful algal bloom taxa from coastal regions along the Eastern US Coast (Georgia, USA through Maine, USA). Findings were submitted to OBIS (Ocean Biogeographic Information System) during February 2018. The majority of the harmful algal bloom literature in this region focuses on the Chesapeake Bay (Virginia and Maryland), the Pamlico Sound (North Carolina), the South Carolina coast, and the Gulf of Maine and was published from the mid-1900s to present. While many of the region's harmful algal bloom taxa produce toxins, most impact humans indirectly through fish kills and tourism (for example, stinking and discolored water). Notably, harmful algal blooms in the region have shifted from dinoflagellates (*Karenia, Pfiesteria*) in the 1980s and 1990s to cyanobacteria (*Dolichospermum, Microcystis*) during the last several years.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

O-242

Intercalibration activity of the Italian Regional Environmental Protection Agencies network for Ostreopsis bloom managements

R. Bertolotto [1], V. Giussani [1,*], F. Sbrana [2], N. Melchiorre [3], M. Vassalli [4], E. Ottaviani [5], M. Chiantore [6], V. Asnaghi [6], E. Spada [7], P. Borrello [7]

[1]ARPAL Liguria - Regional Environmental Protection Agency, [2]Schaefer South-East Europe Srl, Genoa, [3]ARPAL Liguria - Regional Environmental Protection Agency, La Spezia, [4]IBF-CNR , [5]OnAir Srl, [6]University of Genova, Genoa, [7]Italian Institute for Environmental Protection and Research (ISPRA), Rome, Italy

Abstract: The benthic dinoflagellates *Ostreopsis* cf. *ovata* is reported in almost all Italian coastal waters and often causes blooms which can affect both humans and marine organisms. A noteworthy event took place in Genoa (Ligurian Sea, Italy), where 209 people required medical attention care following exposure to marine aerosols likely contaminated by this toxic dinoflagellate.

Given the increasing concern regarding this species, since 2006 many Italian Regional Environmental Protection Agencies, coordinated by the National Institute for Environmental Protection and Research (ISPRA), started collaborating with Academia and Research Institutions in order to improve standardized monitoring programs and produce effective tools for the management of HABs. After many years of monitoring, in 2016, coastal Regional Environmental Protection Agencies with the coordination of ISPRA have been in charge to assess and validate an innovative counting technique, represented by an automated opto-electronic digital microscope (called OvMeter), which was developed in the framework of the ENPI-CBCMED M3-HABs and the POR-FAS OvMeter projects. This system includes a pattern recognition software trained to recognize *O.* cf. *ovata* cells inside the microscope images.

Samples of sea water (# 74) and macrophytes (# 48) collected in 16 sampling stations along Italian coasts have been processed and counted using both traditional counting methods (performed by 20 trained operators from different Agencies) and the automatic opto-electronic system. Outcomes from this inter-calibration study showed significant correlation between the two counting methods, especially within the concentration range of 10^3 – 10^5 cell/l that well covers the alarm thresholds currently in use in Italy. Ineffective estimates were calculated in case of critical samples (e.g. extreme high cells concentrations, presence of sediment).

Overall, the automated opto-electronic system proved a very good agreement in *O.* cf. *ovata* alert detection and, consequently, has been added as an alternative and innovative method in the official Italian protocol for the monitoring of *Ostreopsis* blooms. Both OvMeter and the inter-calibration systems can be easily extended to count different species, providing a dataset of training images labeled by an experienced operator, also in the framework of possible spread of other toxic microalgae in Mediterranean waters.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

O-243

UniEuk: time to speak a common language in protistology

C. Berney[1], R. Siano[2,*], M. Montresor[3], E. Berdalet[4], L. Guillou[1], J. del Campo[4], C. Quast[5], P. Yilmaz[5], C. de Vargas[1]
[1]Station Biologique de Roscoff, Roscoff, [2]Ifremer Centre de Brest DYNECO PELAGOS, Plouzané, France, [3]Stazione Zoologica Anton Dohrn, Napoli, Italy, [4]Institut de Ciències del Mar, Barcelona, Spain, [5]Max Planck Institute for Marine Microbiology, Bremen, Germany

Abstract: Universal taxonomic frameworks have been critical tools to structure the fields of botany, zoology, and bacteriology, as well as their large research communities. Animals and plants have relatively solid, stable morpho-taxonomies built over the last three centuries, while bacteria have been classified for three decades under coherent molecular taxonomic frameworks. By contrast, no such common language exists yet for microbial eukaryotes / protists, even though environmental 'omics' surveys suggest that they make up most of the genetic complexity of our planet's ecosystems. With the current deluge of eukaryotic molecular data, we urgently need a universal eukaryotic taxonomy bridging the 'protist-omics' age to the fragile, centuries-old body of classical knowledge linking protist taxa to morphological, physiological, and ecological information.

UniEuk (www.unieuk.org) is an open, community-based and expert-driven international initiative to build a flexible, adaptive universal taxonomic framework for eukaryotes, focused primarily on protists. The UniEuk system comprises 3 complementary modules allowing direct community input:

- *EukRef*, a standardized, open-source bioinformatics pipeline that allows taxonomic curation of publicly available phylogenetic marker sequences (starting with 18S rDNA), generating homogeneous sets of curated, aligned sequences and phylogenetic trees.
- *EukBank*, a public repository of high-throughput metabarcoding datasets (starting with the V4 region of 18S rDNA) that allows monitoring of total eukaryotic diversity (e.g. saturation, phylogeny) across biomes, and identification of ecologically relevant new lineages.
- *EukMap*, a user-friendly representation of the taxonomic framework in the form of a publicly navigable tree, fully editable by registered users, where each node/taxon is associated with standardized features (name, contextual data, links to pictures and literature, etc.).

As a whole, the *UniEuk* system represents a community hub to centralize, safeguard and promote our current global knowledge on eukaryotic diversity and evolution, integrating expert knowledge on morphology and ecology with key molecular information from phylogenetic markers and environmental metabarcoding surveys. The resulting taxonomy, which will include toxic microalgae, will be implemented into the European Nucleotide Archive at EMBL-EBI and propagated to NCBI, and useable as a stand-alone resource for scientific, educational, or public outreach purposes. In this communication, we will present the project in more detail, and explain how members of the HAB community can get involved, including a *EukRef* workshop focusing on dinoflagellates (5-9 Nov 2018, Roscoff, France). Overall, *UniEuk* will contribute to advances on key taxonomic challenges crucial to ascertain the biogeographic distribution, range of expansion, ecology and links with toxin production of relevant harmful species.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-244

Preparation of qNMR calibrated standards for metabolite ciguatoxins and brevetoxins

T. Yasumoto [1,*], T. Kato [1], M. Saito [1], T. Igarashi [1]

[1]Applied Science, Japan Food Research Laboratories, Tama, Japan

Abstract: Ciguatera fish poisoning (CFP) and neurotoxic shellfish poisoning (NSP) are the two important seafood-borne poisoning which share characteristic features in common. The causative toxins, ciguatoxins (CTXs) in CFP and brevetoxins (BTXs) in NSP originate from marine dinoflagellates, possess ladder-shape polycyclic-ether skeletons, undergo biochemical modification in the vectors to produce a variety of metabolite toxins, and bind the same site of the voltage-dependent sodium ion channel. To prevent occurrence of food poisoning and elucidate the dynamics of toxins during the food chain, the liquid chromatography coupled to a mass spectrometer (LC-MS) or a fluorescence detector best suit for use. To achieve the maximum sensitivity and high accuracy, use of standard toxins is the prerequisite. All the toxins are the metabolites of the fish or shellfish and thus unavailable by culturing the dinoflagellates. After a great effort made, five CTXs representative in the Pacific were isolated in pure form from fish and the absolute amounts were determined by qNMR: ciguatoxin-1B, ciguatoxin-3C, 51-hydroxyCTX3C, 52-*epi*-54-deoxyCTX1B, and CTX4A. Brevetoxin-B2 and -B4, the major toxins in green lipped mussels, were isolated from samples collected in New Zealand and quantified by qNMR. Although propanol-containing mobile phase has successfully led to the LC isolation, the low solubility in conventional solvents is the hindrance to apply LC-MS analysis. Promising results are being produced to improve their solubility by modifying their amino and/or carboxyl function by simple chemical reactions. Fluorescence labelling of the two BTXs was also performed with ease.

*T. Kato and T. Yasumoto: *Marine Drugs* **2017**, 15, 309.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-245

Reference Materials and Analytical Methods for Analysis of Tetrodotoxin in Seafood

P. McCarron [1,*], K. Thomas [1], K. Reeves [1], R. Perez Calderon [1], S. Crain [1], E. Kerrin [1], D. Beach [1]
[1]National Research Council Canada, Halifax, Canada

Abstract: Recent studies have shown the presence of tetrodotoxin (TTX) in bivalve shellfish from temperate European waters. Recent FAO/WHO and EU reports consider monitoring programs for TTX in shellfish and establishment of regulatory limits. To address current and future method needs in Canada and abroad calibration solutions and pilot scale matrix reference materials have been developed for analysis of TTX in seafood.

Research for a TTX calibration solution CRM included studies on the pH and temperature stability of TTX in solution. This was followed by production of a CRM (NRC CRM-TTX) for TTX at 6.8 µg/g in 1 mM acetic acid, which was value assigned by quantitative ^{1}H-nuclear magnetic resonance spectroscopy using external calibration. The stability of CRM-TTX was assessed in both short- and long-term isochronous studies. Feasibility studies were then carried out for a multi-class mussel tissue matrix reference materials for polar marine toxins including TTX, paralytic shellfish toxins (PSTs) and domoic acid (DA). This was done by blending control and DA contaminated mussel tissue with a small amount of TTX contaminated sea slug and PST contaminated dinoflagellate biomass. Analyte stability during the processing of the material was assessed, and accelerated and long-term stability studies were conducted on equivalent wet homogenate and freeze-dried materials at a range of temperature conditions.

The availability of these materials is important for development of analytical methods for the trace analysis of TTX in seafood. They will be used for in-house validation of a reverse phase liquid chromatography-post column hydrolysis-fluorescence method and a HILIC-MS/MS method for metrological applications. The experience gained during the development of these initial TTX reference materials, along with the method advances facilitated by their availability, will establish the necessary capabilities at NRC to produce fully certified shellfish matrix CRMs for TTXs and other polar toxins.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-246

Portable System for Early Detection of Harmful Algal Bloom Toxins

M. Lochhead [1], S. Bickman [1,*]
[1]MBio Diagnostics, Inc., Boulder, USA

Abstract: Harmful Algal Blooms in freshwater and marine environments are increasing in frequency and duration and constitute a growing public health threat while also carrying substantial economic, ecologic, and food supply implications. Recent development of guidelines for microcystin (MC) and cylindrospermopsin (CYN) in drinking water has increased the need for rapid onsite testing for these toxins. MBio Diagnostics had developed a simple yet powerful portable system that enables users in the field to perform laboratory-quality, cost-effective cyanotoxin testing that will help protect drinking water, monitor commercial food resources, and provide critical data for ecosystems management. MBio's cartridge and reader system provides an unprecedented combination of speed, ease-of-use, quantitation and results multiplexing in a field-portable device. Here, we present results from a one-step, 10-minute assay that simultaneously measures microcystin and cylindrospermopsin toxin, with quantitative output centered at the WHO guideline value of 1 microgram/L for MC-LR. Data are presented for 40 natural lake water samples with and without known *Microcystis* and *Planktothrix* blooms; MBio data show concordance with laboratory immunoassay methods. Data are also presented for water samples fortified with eight different MC congeners, demonstrating broad performance for total microcystin. A one-step, 10-minute, field-portable mechanical cell lysis method will also be described in this presentation. By adding the portable lysis module, a fully integrated, field-portable kit is enabled.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-247

Micro-distribution of tetrodotoxin in the New Zealand clam *Paphies australis*

L. Biessy [1 2 3,*], S. Wood [3], K. Smith [3], T. Harwood [3], M. Boundy [3], R. Lesley [3], I. Hawes [1]

[1]Faculty of Science and Engineering, University of Waikato, Hamilton, [2]New Zealand Food Safety Science & Research Centre , Palmerston North, [3]Cawthron Institute, Nelson, New Zealand

Abstract: Tetrodotoxin (TTX) is a highly potent neurotoxin. It has long been known as the causative agent in puffer fish poisoning and was originally thought to only occur in puffer fish (family Tetraodontidae). It has now been identified in 12 different classes of marine, freshwater and terrestrial vertebrate and invertebrate organisms, including marine bivalves. Despite being one of the most studied biotoxins in the world, the origin of TTX remains a mystery with both exogenous and endogenous sources proposed. The present study focuses on *Paphies australis*, a TTX-containing clam species that is endemic to New Zealand. Fifty wild specimens were collected: ten *P. australis* were homogenised whole, and thirty were dissected with the organs and tissues pooled into five groups (siphons, digestive gland, adductor muscles and the 'rest'). These samples were analysed for TTX using liquid chromatography and mass spectrometry (LC-MS). The micro-distribution of TTX was visualised using immunohistochemistry techniques incorporating a TTX-specific monoclonal antibody in section taken from the remaining ten individuals. The LC-MS analysis revealed that TTX concentrations were highest in the siphons. This result was confirmed by the immunohistochemistry analysis which showed that TTX was localised in the interior cells of the siphons and inside the digestive track. Improving knowledge on the location of TTX in marine bivalves will provide new insights into the ecological function and sources of the neurotoxin in these organisms.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-248

Microcystins Toxicity Equivalent

D. Y. Huang [1,*], V. Charoensuk [1], D. Donnelly [1], X. Zhang [1], D. El-Mougi [1], D. Kinniburgh [1]
[1]Alberta Centre for Toxicology, University of Calgary, Calgary, Canada

Abstract: Several species of freshwater cyanobacteria produce a group of hepatotoxins, the microcystins (MCs). Depending on the amino acids present at the second and fourth position of the ring structure, there have been over 200 members identified in this peptide family. The MC congeners share a similar mechanism of action. After being transported across the cytoplasmic membrane by the organic-anion-transporting polypeptides, they covalently bind to and inhibit protein phosphatase 1 and 2A. One of the direct consequences is cytoskeleton change, which will lead to cell death.

Since MC-LR used to be the most prevalent and the most studied congener with significant toxicological data, the WHO set guidelines based on MC-LR concentration. However, with the availability of more congener reference materials and analytical methods, researchers have shown that the MCs congener composition can vary temporally and geographically. MC-LR is not always the most prevalent congener in different parts of the world. Therefore, it's important to collect more congener toxicity information in order to revisit the guidelines.

In this study, nine microcystin congeners (LR, YR, RR, LY, LF, LW, LA, HtyR, dMeLR and dMeRR) and three naturally occurring protein phosphatase inhibitors (nodularin, tautomycin and okadaic acid) were tested using two effect-based assays. The cytotoxicity assay, using the real time cell electronic sensing technology, assesses the congener toxicity at tissue level. The protein phosphatase inhibition assay detects the congeners by their toxicity potential at the molecular level. Both assays demonstrated different toxicity potentials for the MC congeners. By introducing a conversion factor (the toxicity equivalent index) based on the individual congener IC50 relative to MC-LR IC50, the concentration of MC congeners in a mixture can be reported as MC-LR toxicity equivalents. The TEI was applied to the Abraxis MCs proficiency test samples and there was good agreement between effect-based assays and the accurate quantification by LC-HRMS. Our study suggests a potential application of MCs toxicity equivalent for regulatory decisions.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

O-249

Spirolides from *Alexandrium ostenfeldii* Isolated from Greenland

J. Nieva [1,*], U. Tillmann [1], B. Krock [1], J. Tebben [1]
[1]Ecological Chemistry, AWI, Bremerhaven, Germany

Abstract: Spirolides are a group of phycotoxins produced by Alexandrium ostenfeldii. Composed of an imine moiety and a spiroketal ring system, spirolides are highly diverse with toxin profiles that vary among different strains collected from different regions. This study aims to characterize the spirolide composition from the A. ostenfeldii isolates collected from Gothaab Fjord and Disko Bay, Greenland. A total of 11 cultures of A. ostenfeldii isolated from both sites (Gothaab Fjord: n = 5; Disko Bay n = 6) were grown in the laboratory. Cell pellets were prepared from the aliquots of the cultures and extracted with MeOH prior to spirolide analysis by tandem mass spectrometry (MS/MS). Results have shown that A. ostenfeldii from the given sites are producing varying known and unknown spirolides. Gothaab Fjord and Disko Bay isolates produced known spirolides SPX-1, Spirolide C and 20-Methyl Spirolide G and Spirolide H. In addition, isolates from both embayments produced nine unknown spirolides, having mass transitions of m/z 678 > 164, 686 > 164 (two compounds), 708 > 164, 710 > 164, 718 > 164, 720 > 164, 722 > 164 abd 722 > 164. Only the unknown spirolide m/z 694 > 164 was found in Disko Bay isolates. In qualitative terms, A. ostenfeldii isolates from Disko Bay were found to be more diverse in overall spirolide composition compared to Gothaab Fjord. Total spirolide cell quotas of isoltaes from both fjords ranged between 6 and 24 pg cell^{-1}. The structural characterization of unknown spirolides from each site, as determined by high-resolution mass spectrometry (HRMS) and collision induced dissociation (CID) spectra will be further discussed.

Disclosure of Interest: None Declared

Mitigation of HABs

O-250

Feasibly successful trials of sediment perturbations as prevention strategies for *Chattonella* red tides in the Seto Inland Sea, Japan

I. Imai [1,*], A. Nakano [2], N. Ako [3], A. Kakumu [3], K. Koike [4], S. Ohara [4], Y. Nakajima [4], R. Yano [5], E. Hagiwara [6], K. Ogawa [6], H. Yoneyama [6]

[1]Professor emeritus, Hokkaido University, Kyoto, [2]Graduate School of Marine Technology, Tokyo University of Marine Science and Technology, Tokyo, [3]Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, [4]Graduate School of Biosphere Science, [5]Faculty of Applied Biological Science, Hiroshima University, Higashi-Hiroshima, [6]Hiroshima Prefectural Agriculture, Forestry and Fisheries Bureau, Hiroshima, Japan

Abstract: It is empirically known that diatom cells are sparse in water during the course of red tides of harmful flagellates such as dinoflagellates and raphidophytes. A promising strategy to prevent the occurrences of red tides by harmful flagellates is to artificially induce diatom blooms just before the development of red tides of harmful flagellates. There usually exist more than 10^5 cells of diatom resting stage cells or more in bottom sediments of coastal water areas, but they need light for germination and rejuvenation to become planktonic form. Sediment perturbations are expected to convey diatom resting stage cells in sediments from bottom to euphotic zone and to induce germination and rejuvenation. Resultant vegetative cells are also expected to proliferate in the surface water and to overwhelm harmful flagellate populations through the exhaustion of inorganic nutrients (N and P) by diatoms. Trials of sediment perturbations were made using dragnet fishing boats in coastal sea area of Tomono-Ura, Fukuyama City, the Seto Inland Sea, in July 2016 (ca. 1 km^2) and June 2017 (ca. 1.5 km^2). The densities of diatom resting stage cells were higher than 10^5 g^{-1} wet sediment at the both sites inside and outside of the dragnet-trawling zone. The densities of planktonic diatoms increased in the water column at the station inside the zone just after the -trawling, but not at the station outside the trawling zone, and totally increased thereafter (maximum value of 1383 cells mL^{-1}) in the 2016 trial. The cell densities of the raphidophyte *Chattonella* spp. decreased at the both stations. The water sample after sediment perturbation was collected at 2m deep at the inside station, and incubation experiments were conducted for the bottles in combination with the inoculation of *Chattonella antiqua* (200 cells mL^{-1}) and the strengthening of SWM-3 culture medium (1/100) in 2016. Diatoms always increased and *Chattonella* cells decreased in all the experimental bottles. In the trial of June 2017, the cell densities of diatoms increased from ca.1500 cells mL^{-1} to 3983 cells mL^{-1} in surface water after the sediment perturbation, and *Chattonella* spp. consistently decreased from 91 cells mL^{-1} to disappearance level three days after the perturbation. In the bottle incubation experiments, diatoms always showed predominance over *Chattonella* spp. The present studies demonstrated a possible feasibility of sediment perturbation as an environment-friendly and effective prevention strategy for red tides of harmful flagellates in coastal sea areas with the artificial induction of phytoplankton flora to desirable diatom dominant communities.

Disclosure of Interest: None Declared

Mitigation of HABs

O-251

The mechanism of modified clay controlling harmful algal blooms revealed by physio-biochemistry and transcriptome analysis

Z. Yu [1 2 3,*], S. Liu [1 2], J. Zhu [1 2], X. Song [1 2 3], X. Cao [2 3], L. He [2 3]

[1]University of Chinese Academy of Sciences, Beijing, [2]CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, [3] Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China

Abstract: In recent years, harmful algal blooms (HABs) have developed into a global marine disaster. As an effective method for HAB controlling, the modified clay (MC) technology is used widely in coastal waters of China. The previous studies have shown that flocculation between algal cells and MC particles were the main principle for MC to control HABs. Further research has found that after modified clay treatment, the bloom organisms could not keep bloom state or grow to form blooms again even if they were still suspended in waters. Therefore, this studies were conducted on the effects of MC on these residual cells viewing from consideration of physio-biochemistry and transcriptome. The changes were observed in the residual cells after treatment using MC: 1) antioxidant systems were enhanced, not only the antioxidase activity increased, such as superoxide dismutase (SOD)， catalase (CAT) and malondialdehyde (MDA), but also some stress adaptation and detoxification related genes were overexpressed; 2) photosynthetic systems were damaged, pigment contents and net photosynthetic rate decreased, electron transport chain blocked, the photosynthetic light reaction related genes were significantly up-regulated ($p<0.05$) and several transcripts increased by as much as six-fold than normal, however, the expression of dark reaction related genes was not changed; 3) energy metabolic rate was accelerated, genes associated with carbon metabolism and lipid synthesis were up-regulated accompanied by lipid accumulation; 4) the growth rate was obviously inhibited, DNA replication and transcription related genes were down-regulated, while the cell cycle regulatory genes was up-regulated. Based on the results above, it could be concluded that collisions and electrical effects between MC particles and algal cells lead to the accumulation of reactive oxygen species (ROS) in residual cells, which could damage the photosynthetic systems resulting in disordered energy metabolism. Ultimately, MC disrupts physiological algal cell processes resulting in inhibition of cell growth and proliferation. Thus, this study elucidated why blooms could not be continued or form again after treatment with MC in the level of physio-biochemistry and transcriptome, which supplement and consummate the mechanism of MC controlling HAB.

Disclosure of Interest: None Declared

Mitigation of HABs

O-252

Impacts and transcriptomic effects of algicidal compounds produced by Shewanella sp. IRI-160 on *Karlodnium veneficum*

Y. Wang [1,*], E. Ternon [2], K. Coyne [1]

[1]College of Earth, Ocean, and Environment, University of Delaware, Lewes, USA, [2]University of Nice Sophia Antipolis, Nice, France

Abstract: Previous research identified a bacterium *Shewanella* sp. IRI-160, which secretes water-soluble algicidal compound(s). The algicidal exudate, called IRI-160AA, inhibits the growth of harmful dinoflagellates while having no significant impacts on other phytoplankton species tested. Ammonium and three other amines, putrescine, diethylamine, and n-butylamine, were identified in IRI-160AA. In this study, the algicidal effects of each amine were evaluated separately and in combination when applied to the harmful dinoflagellate, *Karlodinium veneficum*. Results showed both dose-dependent and synergistic effects, for example where putrescine in combination with ammonium resulted in greater cell death than each amine alone. It is known that internal concentrations of polyamines (e.g. putrescine) in phytoplankton are responsive to environmental stress, and are important for the growth and cell division of these organisms, but little is known about the adverse impacts of exogenous amines or their synergistic effects on algal species. To assess the response of dinoflagellates to the algicidal components at a cellular level, transcriptomic analysis was conducted on *K. veneficum* treated with putrescine and ammonium separately or together at the level where a synergistic effect was observed. Cultures treated with the bacterial exudate, IRI-160AA, were also subjected to transcriptomic analysis to better understand the contribution of amines to the algicidal effects of IRI-160AA. The results of this study will not only shine a light on the cellular mechanisms behind the algicidal effect by IRI-160AA, but also contribute to our knowledge of adverse impacts of exogenous amines and their synergistic effects on the physiology and gene transcription of dinoflagellates.

Disclosure of Interest: None Declared

Mitigation of HABs

O-253

Effects of a bacteria-produced algicide on non-target marine animal species

V. E. Simons [1,*], T. Targett [1], K. Coyne [1], D. Nanjappa [1], J. Cohen [1]
[1]School of Marine Science and Policy, University of Delaware, Lewes, USA

Abstract: Harmful algal blooms (HABs) are a topic of concern in both freshwater and marine systems. In the Delaware Inland Bays (USA), blooms of dinoflagellates such as *Karlodinium veneficum* can cause fish kills and render waters unsafe for recreational use. Laboratory experiments suggest an algicidal exudate produced by a local isolate of the bacterium *Shewanella* could be used at ~1% concentrations (v/v) to combat blooms. Before this exudate can be tested in a real-world scenario, its effects on non-target organisms need to be determined. In this study, the algicide (IRI-160AA) was tested on the copepod *Acartia tonsa* and fish *Fundulus heteroclitus*. Mortality experiments with *A. tonsa* revealed that the 12h LC50 was 16.5% (95% CI: 12.5 – 19.2) and the 24hr LC50 was 20.8% (95% CI: 18.4 – 25.2). Respiration rate for *A. tonsa* was measured in a microrespirometer and found to be unaffected by most sublethal concentrations of algicide tested (3%, 6%, and 9%), but did increase in the 12% (v/v) concentration by a factor of 2. Activity levels were determined using locomotor activity monitors (LAMs) over a 24h light:dark cycle. Levels were not affected in the 1% and 13% treatments but were reduced in the dark phase in the 17% treatment. Fish were exposed to five treatments at 25 and 30 degrees Celsius to test for a primary stress response, indicated by increased cortisol production. Treatments were: a) algicide (1%), b) algicide-free control, c) algicide-free media control (1%), d) diel-cycling hypoxia as a potential secondary stressor, and e) algicide with diel-cycling hypoxia. Blood cortisol levels did not differ among the five treatments at either temperature. Cortisol increased at the higher temperature. Results demonstrate that *A. tonsa* and *F. heteroclitus* are tolerant to the bacterial algicide at concentrations required for dinoflagellate mortality. Thus, IRI-160AA remains a promising tool for mitigating HABs in the Delaware Inland Bays and elsewhere in the region.

Disclosure of Interest: None Declared

Mitigation of HABs

O-254

Can macroalgae be used to mitigate harmful effects of *Cochlodinium polykrikoides* and other HABs on coastal marine aquaculture?

P. Sylvers [1,*], C. Gobler [1]

[1]School of Marine and Atmospheric Sciences, Stony Brook University, Southampton, USA

Abstract: Harmful algal blooms (HABs) are increasing in range, frequency, and intensity throughout the world due in part to anthropogenic forcing. These HABs have already had devastating effects on coastal resources such as aquaculture and fisheries; for example, *Cochlodinium polykrikoides*, a dinoflagellate HAB species, is highly ichthyotoxic and has resulted in enormous economic losses to caged fish aquaculture in East Asia. Therefore, there is a need to develop effective strategies to mitigate the effects of *C. polykrikoides* blooms on vulnerable coastal aquaculture species. Many genera of macroalgae have broad inhibitory allelopathic effects on phytoplankton, including HAB dinoflagellates such as *C. polykrikoides*. Furthermore, the inclusion of macroalgae in coastal integrated multi-trophic aquaculture systems is attractive for other reasons, including macroalgae's role as an assimilative biofilter for nutrients, its buffering capability against ocean acidification, and its high economic value. In this study we quantify the protective effect of macroalgae against toxic *C. polykrikoides* blooms on coastal forage fish. First, we conduct 24-48h toxicity assays of lab-cultured *C. polykrikoides* on larval forage fish *Menidia beryllina* and *Cyprinodon variegatus* with and without macroalgae. We found that adding *Ulva* significantly prolonged larval fish survival at bloom concentrations of *C. polykrikoides* (10^3 -10^4 cells ml^{-1}) with the mitigating effect being more pronounced at lower concentrations of *C. polykrikoides* (5 x 10^3 cells ml^{-1}). Experiments further demonstrated that the protective effect of *Ulva* scaled with its concentration, more significantly mitigating the toxicity of *C. polykrikoides* at higher macroalgae densities. These findings suggest that high-density macroalgae culturing approaches may make integrated aquaculture systems more resilient to *C. polykrikoides* and potentially other HABs. Results on the ability of macroalgae to mitigate the toxic effects of HABs on economically valuable aquacultured bay scallops will also be presented.

Disclosure of Interest: None Declared

Mitigation of HABs

O-255

One model to help them all? Challenges facing harmful algal bloom prediction in the coastal zone

C. Anderson [1,*], R. Kudela [2]

[1]Southern California Coastal Ocean Observing System, Scripp Institution of Oceanography, La Jolla, [2]Ocean Sciences , University of California, Santa Cruz, Santa Cruz, USA

Abstract: With the explosion of myriad modeling and remote sensing techniques to estimate the extent and timing of harmful algal blooms, we are now faced with assessing the successes and failures associated with reducing complex biology into a relatively constrained state-space. The practical matter of mitigating the impacts of harmful algal blooms with an early warning system is complicated by the spatial and temporal discontinuities associated with the multiple scales of variability inherent in available model and earth observation data products. The impacts are widespread across these many scales and affect nearshore aquaculture, public health, and offshore fish and mammal populations. Regional modeling efforts have been successful at reaching stakeholders from a variety of these sectors but fail to be a comprehensive mitigation tool for the full suite of vulnerable systems. In this talk, we will address the lessons learned from an operational harmful algal bloom forecasting system in coastal California and efforts to extend the reach of the model to predict higher trophic level impacts in nearshore environments. With the introduction of the California Harmful Algae Risk Mapping (C-HARM) System, coastal conditions conducive to toxic algae can be monitored in real-time. This kind of advanced warning has proven valuable to marine mammal resource managers given the tight connection between offshore toxins and animal stranding events. Where C-HARM provides less information is in major inlets such as the San Francisco Bay-Estuary and Humboldt Bay. The latter is the site of some of the highest commercial oyster production in the nation and is very rarely affected by toxin bioaccumulation even when the coastal region is experiencing a major bloom event. While this breakdown in the model-observation relationship is in part due to the limitations of C-HARM's spatial resolution, there are also knowledge gaps when translating in-water properties to shellfish toxicity, particularly in semi-enclosed systems. Using a combination of particle tracking simulations and paired measurements of shellfish and water toxin levels, we show the potential for toxic particles to enter the bay from the coastal boundary and, in fact, lead to some of the highest toxin levels on record in an area rarely affected by commercial shellfish closures. We will end with how the use of mechanistic approaches could enhance forecasting systems while at the same time offer a powerful research tool for understanding the dynamical changes that lead to harmful algal blooms in the coastal zone.

Disclosure of Interest: None Declared

POSTER COMMUNICATIONS

(*) Poster also presented during an Ignite Talk Session

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-001

Adaptation of phycocyanin and phycoerythrin concentration in *Microcystis* spp. under low and elevated light conditions

D. S. Derminio [1,*], E. Kilbourn [1], G. Boyer [1]

[1]Department of Chemistry, State University of New York College of Environmental Science and Forestry, Syracuse, NY, USA

Abstract: Cyanobacterial harmful algal blooms (cHABS) are a common occurrence in many lakes across the world, which is important to public health because some blooms occur in drinking water reservoirs. When blooms occur, cells often self-shade and must adapt to acquire light under low light conditions. To survive, cyanobacteria gather light energy for growth within photosystem II using a phycobilisome. Phycobilisomes contain two major pigment-proteins complexes in their light-gathering antenna complex: phycocyanin (PC) and phycoerythrin (PE). These pigment-proteins complexes can undergo chromatic adaptation under different light intensities by lengthening to gather light or shorten to stop photoinhibition. Quantification of PC and PE on a cellular basis was measured in three *Microcystis* spp. to determine how quickly adaptation can occur under changing light conditions. Cultures were sampled every two hours over a 24-hour period. Photosynthetic efficiency, photochemical and non-photochemical events, and electron transport rate were measured on the Walz PhytoPAM. Cyanobacteria specific chlorophyll was measured using a bbe Fluoroprobe. Cultures were filtered at each time point to obtain extracted PC and PE concentrations. This study intends to improve the fundamental understanding of how *Microcystis* pigment composition responds to an ever-changing light field and has important implications for the use of fluorescent techniques in monitoring cHABs.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-002

Paralytic shellfish toxin content and sxtA gene copy number of *Gymnodinium catenatum* in different growth phases

A. Mendoza-Flores [1,*], I. Leyva-Valencia [1], C. Band-Schmidt [1], C. Galindo-Sánchez [2], F. Hernández-Sandoval [3], J. Bustillos-Guzmán [3]

[1]Departamento de Plancton y Ecología Marina , Instituto Politecnico Nacional - Centro Interdisciplinario de Ciencias Marinas, La Paz, [2]Departamento de Biotecnología Marina, Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, [3]Centro de Investigaciones Biológicas del Noroeste, La Paz, Mexico

Abstract: Dinoflagellates are characterized by having large genomes and most of the genes are present in multiple copies. The gene *sxtA* is involved in the biosynthesis of paralytic shellfish toxins (PST), catalyzing one of the initial steps of the PST synthesis pathway and is present in multiple copies in the genome. In some species of *Alexandrium* the gene copy number (GCN) of *sxtA* and PST content are related, but the variation in the different growth phases has not been documented. In this study, we determined the relation of *sxtA* gene (domains *sxtA1* and *sxtA4*) GCN and the PST content in *Gymnodinium catenatum* strain L62*,* and how these contents varied along the different growth phases. *G. catenatum* was grown in modified GSe medium at 24ºC, salinity of 34, and light dark cycle 12:12. Cells were harvested by centrifugation for DNA extractions and by filtration for toxin analysis, in the lag, early exponential, late exponential and stationary phases. The GCN was calculated with qPCR assays, and the PST content and profile were determined by HPLC-FLD. A relationship was found between PST content and GNC of *sxtA4* domain along the growth phases; being higher in the initial phase and decreasing during the following growth phases. The GCN of the domain *sxA1* did not show a relation with the PST content. There are differences in the GCN among domains, *sxtA4* has a higher GCN than *sxtA1*. This is the first time that the GCN of domain *sxtA1* has been determined in dinoflagellates. The differences in the relation with *sxtA1* and *sxtA4* in GCN to PST content suggest that the domain *sxtA1* is involved in other metabolic pathways. It is important to compare these results with other dinoflagellates PST-producers to gain a better understanding of how PST content is related with the GCN of the genes involved in saxitoxin biosynthesis.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-003

An effective method for detecting prey DNA from the genera *Dinophysis* and *Phalacroma* by using two specific primer sets

G. Nishitani [1,*], A. Ishikawa [2]

[1]Graduate School of Agricultural Science, Tohoku University, Sendai, [2]Graduate School of Bioresources, Mie University, Tsu, Japan

Abstract: The toxic dinoflagellate species belonging to the genera *Dinophysis* and *Phalacroma* have been reported as mixotrophic or heterotrophic actively feeding on planktonic ciliates. Several *Dinophysis* species have been cultivated with the ciliate prey, *Mesodinium rubrum*, under the laboratory conditions. However, little information is so far available on the prey species in natural sea water. We tried to identify its prey organisms by analyzing the intracellular genes in natural cells of *Phalacroma rotundatum*. Two primer sets were designed: (i) a three base pairs mismatch primer with *Dinophysis* or *Phalacroma* to inhibit the amplification of predator DNA and (ii) a ciliate-specific primer. Four natural cells of *P. rotundatum* were isolated from a Japanese coastal seawater. This isolation was done for the cells possessing food vacuoles in which prey DNA could be preserved. Single cell PCR was conducted using the two primer sets. More than 300 sequences were analyzed by gene cloning. In total, eight different sequences of ciliates were detected from the prey DNA. Moreover, DNAs of other dinoflagellate, diatom, seaweed, bivalve, fungi, and even land plant were also included in the prey DNA. The high diversity of prey DNAs detected from *P. rotundatum* implies the complexity of the marine microbial food web. Further application of this technique to the natural cells of *Dinophysis* might reveal the existence of ciliate prey other than *M. rubrum*.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-004

Chemical factors affecting the growth of *Karenia mikimotoi* in the Bungo Channel, Japan

S. Sakamoto [1,*], K. Naito [2], T. Shikata [1]

[1]National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research and Education Agency, Hatsukaichi, [2]Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima, Shobara, Japan

Abstract: The Bungo Channel, located in the western part of the Seto Inland Sea, is one of the biggest aquaculture area in Japan. However, fisheries damages due to red tides of the dinoflagellate *Karenia mikimotoi* have frequently occurred in summer in this area, and it is urgent to clarify the mechanism of the red tide occurrence. In this study, we aimed to clarify the chemical factors such as nutrients and vitamins affecting the growth of *K. mikimotoi* in the summer by the algal growth potential (AGP) test. The field survey was conducted once a month at two sampling stations (Uwajima Bay and Iwamatsu Bay) in northeastern part of the Bungo Channel from June to August 2015. Seawater samples for the AGP test were collected from surface, 5 m and B-1 m layers, filtered aseptically (<0.2 µm) and then stored in a cool (4°C) and dark conditions. An axenic strain of *K. mikimotoi* was cultured in the seawater samples with a single addition or multiple additions of nitrate (N), phosphate (P), Fe-EDTA (Fe), selenite (Se) and vitamin B mixture (B_1, B_7 and B_{12}). After the start of incubation, *in vivo* fluorescence was measured with a Turner Designs‘ fluorometer every 2 days as a general rule. The maximum cell yield of *K. mikimotoi* was compared with one in the seawater sample without addition of the nutrients. This species grew well when both N and P were added. The growth slightly promoted under single addition of N, whereas it did not promote remarkably under single additions of P and Fe. Se also did not promote the growth of this species. This result was inconsistent with past findings (Ishimaru et al. 1989) which reported that Se was the growth promoting factor of this species. On the other hand, vitamin B promoted the growth of *K. mikimotoi* under coexistence of N and P. Furthermore, we examined the effect of each vitamin B on the growth of *K. mikimotoi* by AGP test using seawater collected from Iwamatsu Bay in July 2016. As a result, the growth was promoted by additions of vitamin B_1 and B_{12}. These results suggest that N may be a primary factor limiting the growth of *K. mikimotoi* in the Bungo Channel and that the supply of both N and P may be necessary for formation of a large-scale of the red tides of this species. Moreover, it is also suggested that vitamin B_1 and B_{12} may be growth promoting factors of this species, but these components in the Bungo Channel are presumed to be insufficient for the growth. Vitamin B is component that is added as nutrient enrichments in the feed for aquaculture. Further investigation will be necessary on the relationship between the distribution and concentration of vitamin B in the environment and the occurrence of the red tide, in the future.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-005

From growth to aging, roles and fates of lysosomes (orange-red bodies) in benthic dinoflagellate cells

C. Jauzein [1,*], P. Roux [2], R. Lemée [2]
[1]ODE/DYNECO, Ifremer, Plouzané, [2]Observatoire Océanographique de Villefranche, Villefranche sur mer, France

Abstract: The presence of orange-red bodies in dinoflagellate cells have been reported for different type of life cycle stages, including vegetative cells, planozygotes and cysts. For benthic species, such organels may represent a significant part of the cell volume and sometimes exhibit an intense red autofluorescence. Considering these morphological characteristics, different hypotheses were formulated in previous works regarding their nature and role in cell metabolism.

For *Ostreopsis* cf. *ovata*, the presence of large red-brownish bodies was first related to growth of microalagal cells, describing them as digestive vacuoles containing ingested preys. Even if phagotrophy can not be excluded as a potential nutrition pathway for this species, these first results were not sufficient for validating such assumption. A second hypothesis recently described these vesicles as accumulation bodies. This definition has only been formulated in a precise context, however, which is based on their morphological resemblance to orange accumulation bodies observed in cysts. Pushing this concept, different studies related the formation of large red autofluorescent bodies in *O*. cf. *ovata* cells to environmental stress, defining them as accumulation bodies linked to encystment processes.

Observations were conducted during *O*. cf. *ovata* blooms that occurred in the Bay of Villefranche-sur-mer (Mediterranean Sea, South of France) in 2016 and 2017. The proportion of cells showing an autofluorescent red body was estimated over the bloom duration. Several types of labeling were tested in order to go further on the characterization of these organels, including Lysosensor Blue, DCFHDA and Sytox Green. Results of the present study allow for going further in defining these bodies as accumulation bodies and lysosomes. A new hypothesis can be detailed regarding their role and fate in cell metabolism. When previous works associated their autofluorescence to an accumulation of photosynthetic pigments (preys or carotenoids), we believe this autofluorescence signal mainly comes from the accumulation of a lipoprotein matrix, also called lipofuscin in animal cells. Following this hypothesis, these large organels may be a sign of disturbance of lysosomal degradation, and a characteristic of slowly dividing vegetative cells.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-006

Carbon and light availability influence microcystin production by PCC 7806

D. J. H. Maxwell [1,*], L. Lawton [2], C. Edwards [3]

[1]pHD Student, Robert Gorden University, [2]Robert Gordon Univesity, [3]Robert Gordons University, Aberdeen, United Kingdom

Abstract: Throughout cyanobacterial microcystin literature a consistent issue is the lack of consensus and on the function of microcystins, and how light and CO_2 affect their production. There are many contradictory studies often with different methodologies, strains and analysis protocols. This has led to an uncertainty over the role of microcystins. We will use the model cyanobacterial species Microcystin *aeruginosa* PCC 7806. This best investigation will attempt to understand if the confounding results are caused by complexly interacting environmental and metabolic systems which are often overlooked. Specifically I will focus on the effects of CO_2, light wavelength, shading factors, and cellular metabolites. In addition to experimental conditions we will investigate how data is related and qualified. Although in early stages the objective of this research is to characterize the culture conditions of cyanobacteria as a complex dynamic systems. In addition the relationship between microcystins and other peptides will be quantified using UPLC MS/MS data mining.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-007

Effects of nutrient deficiency and high light on growth, photosynthesis and motility in a red tide dinoflagellate *Karenia mikimotoi*

K. Yuasa [1,*], T. Shikata [2], Y. Nishiyama [1]

[1]Department of Biochemistry and Molecular Biology, Saitama University, Saitama, [2]National Research Institute of Fisheries and Environment of Inland Sea, Japan Fisheries Research and Education Agency, Hatsukaichi, Japan

Abstract: The dinoflagellate *Karenia mikimotoi* is a red tide-forming alga that causes serious damage to aquaculture in coastal areas around the world. Its eco-physiological characteristics have been investigated for a long time, but conditions associated with declines of red tides of *K. mikimotoi* remain unknown. In the present study, we examined effects of nutrient deficiency and strong light on the growth, photosynthesis and motility of *K. mikimotoi*. When *K. mikimotoi* cells were incubated under normal light at 100 µmol photons m^{-2} s^{-1} in media that were deficient in nitrogen (N) and phosphorus (P), most cells survived for 35 days, similarly to their survival pattern in complete medium. In contrast, when cells were incubated under strong light at 1,000 µmol photons m^{-2} s^{-1} for 4 h during light period every day under N-depleted conditions, the activity of photosystem II (PSII) decreased significantly and most cells died within 10 days. Furthermore, PSII activity was much more sensitive to photoinhibition in N-depleted than in complete medium. In particular, the repair of PSII was inhibited under N-depleted medium, as a result of the suppression of *de novo* synthesis of proteins. The diurnal vertical migration became obscure in N- and P-depleted media, and most cells accumulated in the surface layers all day in N-depleted medium. Moreover, cells accumulating in surface layers in N- and P-depleted media descended very little, even when irradiated with high light. These observations suggest that N-deficiency in *K. mikimotoi* might lose the ability of downward migration of cells and increase their susceptibility to photoinhibition of PSII, possibly leading to cell death. Thus, the combination of N-deficiency and strong light appears to be an important event that determines the decline of red tides of *K. mikimotoi*.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-008

Influence of temperature, irradiance and pH on the growth of Teleaulax amphioxeia, trophic chain member of the toxic genus *Dinophysis*

S. Gaillard [1,*], A. Charrier [2], L. Carpentier [1], F. Malo [1], G. Bougaran [2], H. Hégaret [3], P. Hess [1], V. Séchet [1]

[1]Ifremer-Phycotoxines, [2]Ifremer-Physiologie et Biotechnologies des Algues, Nantes, [3]Laboratoire des sciences de l'environnement marin (LEMAR), UBO-CNRS, Brest, France

Abstract: In view of assessing the potential impact of the principal factors of global change on growth and toxicity of certain toxic species of the genus *Dinophysis*, it will be important to also understand the ecology of members of the trophic chain of this obligate mixotroph. The cryptophyte *Teleaulax amphioxeia* is known as a species forming red tides in coastal areas and as a source organism of plastids in the trophic chain of the harmful genus *Dinophysis*. In this study, optimal growth was determined by a factorial design using a quadratic model for temperature, irradiance and pH in an experimental set-up allowing for 15 combinations of environmental factors. Cell concentrations were measured to assess growth rate of *T. amphioxeia*. The model predicted an optimal growth rate of 0.88 d^{-1} at a temperature of 17.6 °C, an irradiance of 400 µmol photons m^{-2} s^{-1} and a pH of 7.6. This optimum was further verified in triplicate batch cultures (0.87 ± 0.01 d^{-1}). Furthermore, two significant effects of combinations on growth rate were observed: the interactions between temperature and pH on one hand and temperature and irradiance on the other hand. Both temperature and irradiance had a negative impact on growth rate when they were increased to 22.6 °C and 645 µmol photons m^{-2} s^{-1} respectively. These results underline the importance of direct effects and interactions of temperature and light conditions on the optimal growth rate of *T. amphioxeia*. On the contrary, this study found no significant direct influence of pH on growth and physiology of *T. amphioxeia*. The cryptophyte tolerated low and high pH since it grew well in a pH range from 6.5 to 8.6, with an optimum at pH of 7.6. To conclude, knowledge provided by this study should improve *T. amphioxeia* culture for the trophic chain nutrition and help in the modelling of toxin production by *Dinophysis* in future climate conditions.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-009

Effects of strong light and high temperature on photosystem II in red tide-forming algae *Karenia mikimotoi* and *Chattonella antiqua*

Y. Nishiyama [1,*], Y. Kuwahara [1], K. Yuasa [1], T. Shikata [2]

[1]Department of Biochemistry and Molecular Biology, Saitama University, Saitama, [2]National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Agency, Hatsukaichi, Japan

Abstract: Photosystem II (PSII), a protein-pigment complex that converts light energy into chemical energy in the photosynthetic machinery, is susceptible to inactivation by strong light. This phenomenon is referred to as photoinhibition of PSII and often limits the growth of photosynthetic organisms under strong light. The mechanism of photoinhibition of PSII and the effects of other environmental stresses on photoinhibition have been intensively studied in model organisms, such as cyanobacteria and *Arabidopsis*, but little is known in red tide-forming algae. In the present study, we examined the effects of strong light and high temperature on PSII in two species of harmful red tide-forming algae,*Karenia mikimotoi*and *Chattonella antiqua*. In *K. mikimotoi*, moderately high temperatures, such as 30°C, promoted photoinhibition of PSII by accelerating both photodamage to PSII and the inhibition of repair of PSII. By contrast, in *C. antiqua*, such high temperatures alleviated photoinhibition of PSII by enhancing the repair of PSII. These observations suggest that *K. mikimotoi*is sensitive to photoinhibition of PSII at high temperatures, while *C. antiqua*is resistant to photoinhibition of PSII at high temperatures. The results of our laboratory experiments are consistent with field observations that *K. mikimotoi*tends to form red tides on cloudy days or in middle layers of water columns on sunny days, whereas *C. antiqua*can form red tides in the surface layers on sunny days even in the middle of summer.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-010

Differences in xanthophyll de-epoxidase activity in high and low toxic strains of *K. brevis*

R. Colon [1,*], K. Rein [1]
[1]Chemistry and Biochemistry, Florida International University, Miami, Fl, USA

Abstract: The marine dinoflagellate *Karenia brevis* produces a suite of poly-ether ladder neurotoxins known as brevetoxins (PbTx). Large scale blooms of *K. brevis* occur around the coast of Florida and the Gulf of Mexico nearly every year. Millions of dollars in damages to coastal economies have been linked to harmful algal blooms (HABs).

The endogenous function of brevetoxin remains unknown. Hypotheses have included a role as a feeding deterrent or mediator of osmotic regulation. However, not all strains of *K. brevis* are the same; some strains can produce up to 60 pg/cell of PbTx, while others produce less than 1 pg/cell. Comparison of high and low-toxic strains may provide clues to the role brevetoxins play for *K. brevis*. The high and low-toxic strains differ considerably, not only in their production of brevetoxin, but in their ability to perform non-photochemical quenching (NPQ) or the dissipation of excess light energy as heat. Whether the lack of the toxin causes the NPQ deficiency, or whether the NPQ deficiency results in lower toxin production is yet undetermined.

The xanthophyll cycle is central to energy dependent non-photochemical quenching (qE). In dinoflagellates, diadinoxanthin is converted to diatoxanthin by enzymatic de-epoxidation of diadinoxanthin via diadinoxanthin de-epoxidase (DDE). We have shown that the low toxic strain of *K. brevis* is deficient in performing this de-epoxidation. However, the source of this deficiency is not understood. It may be a result of differences in expression of DDE or differences in activity due to the redox status of the cell. The q-RT-PCR studies done in our lab present the differences in relative amounts of DDE genes in high and low toxic strains of *K. brevis.* Comparative analysis of DDE at the protein level shall also be presented and how this may relate to the endogenous function of brevetoxin.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-011

Immunolocalization of microcystins and the photoprotective OCP (orange carotenoid protein) in *Planktothrix* during high-light stress

C. Djediat [1,*], A. Brochard [2], K. Feilke [3], L. Caramelle [2], S. Kim Tiam [2], C. Duval [2], D. Kirilovsky [3], B. Marie [2], C. Bernard [2]
[1]Plateform of electronic microscopy, [2]UMR 7245 Molécules de Communication et Adaptations des Microorganismes, Muséum National d'Histoire Naturelle, Paris, [3]UMR 9198 I2BC, CEA Saclay, Gif sur Yvette, France

Abstract: The ecological and physiological role of toxins remains an open question for scientists working on cyanobacteria and microalgae forming harmful blooms. Cyanobacteria have an intrinsic, fast, light-controlled, highly sensitive and effective regulatory mechanism for self-protection against high and fluctuating light. In blooms, there is a mixture of genotypes producing or not microcystins. Several authors have hypothesized that microcystins may have a role in photoprotection providing a selective benefit to genotypes producing microcystins.

The possible role of microcystins in responses to high-light stress was tested during a 24-hour experiment under laboratory conditions. We also tested the capacity of photosynthesis antenna (the phycobilisomes) to switch off rapidly and reversibly into a light-energy-dissipating state that is induced upon photoactivation of the Orange Carotenoid Protein (OCP).

We analyzed in Planktothrix strains producing or non-producing microcystins: i) the presence/absence of OCP genes in the genomes, ii) the OCP synthesis by western blot, iii) the localization in the cells of OCP and microcystins by immunolocalization, and iv) their possible co-localization within cells.

Data obtained during high-light stress experiments were compared between *Planktothrix* strains.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-012

Salt stress response of brackish and freshwater strains of *Microcystis aeruginosa*.

M. Georges Des Aulnois [1,*], A. Caruana [1], E. Briand [1], M. Bormans [2], Z. Amzil [1]
[1]IFREMER, Nantes, [2]UMR CNRS 6553 ECOBIO , Université de Rennes 1, Rennes, France

Abstract: Many cyanobacterial harmful algal blooms (CyanoHABs) involve the genus *Microcystis* which is known to produce a great diversity of hepatotoxic microcystins. Blooms of *Microcystis* are most often observed in freshwaters but increasing numbers of bloom events have been recorded after a transfer to estuaries presenting moderate salinity. Physical transfer of *Microcystis* from freshwater to marine environment could result in the release of microcystin in the water and accumulation in marine organisms. Along this continuum, salinity represents one of the main abiotic factor controlling the presence and toxicity of this genus. Therefore, understanding the impact of salinity on *Microcystis* physiology and toxin production/release is crucial to assess the potential environmental risk. To date, studies on the salinity tolerance of *Microcystis aeruginosa* show highly variable results, notably for salinity thresholds. Despite the intraspecific variability, differences between strain tolerance remains unclear as do the mechanisms involved during a sudden salt shock. Physiological response of *Microcystis aeruginosa* including toxin production were investigated during five days using two toxic strains (PCC 7806 et PCC 7820) respectively from brackish and freshwater origins. After a sudden salt shock at increasing salinity from a salinity of 2 to 18, *M. aeruginosa* PCC 7806 and PCC 7820 presented similar limits of salinity tolerance. Growth of *M. aeruginosa* was inhibited above a salinity of 10.2 and 9.2 for PCC 7806 and PCC 7820 respectively. For both strains, photosynthetic efficiency (Fv/Fm) was not affected below a salinity of 10.2. Moreover, *M.aeruginosa* PCC 7806 and PCC 7820 produced different microcystin variants. *M.aeruginosa* PCC 7806 produced 2 variants of microcystins (MC-LR,> dmMC-LR), and *M.aeruginosa* PCC 7820 produced 6 variants of microcystins (MC-LR > MC-LW > MC-LF > MC-LY > MC-LA > dmMC-LR). Overall, microcystin concentration represents 35 fg/cell for *M. aeruginosa* PCC 7806 at control salinity and 11.5 fg/cell at a salinity of 18. For *M. aeruginosa* PCC 7820, microcystin concentration was reduced by 50% by the salt shock. Overall for both strains, the sudden increase of salinity did not lead to a modification of the toxin profile but increasing salinity decreased the concentration of microcystins per cell. Salt shock also involved reactive oxygen species production and osmolytes production. Further experiments are being conducted to compare the short term (hours) physiological response to salinity shock in both strains with the quantitative expression of several genes using qPCR analysis. In parallel, osmolyte production is studied using targeted analysis. Comparison of those results will provide a better understanding of the mechanisms involved during a sudden salt shock.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-013

Different strategies of attachment to substrate of the toxic benthic dinoflagellate *Ostreopsis* cf. *ovata*

G. Honsell [1,*], R. Pistocchi [2], L. Pezzolesi [2]

[1]Dept. Agricultural, Food, Environmental & Animal Sciences (DI4A), University of Udine, Udine, [2]Dept. Biological, Geological & Environmental Sciences (BiGeA), University of Bologna, Ravenna, Italy

Abstract: The benthic dinoflagellate *Ostreopsis* cf. *ovata* has been responsible of numerous toxic blooms. This species is able to grow on different benthic substrates (macroalgae, pebbles, rocks, etc.) covering them with a continuous mucilage film embedding many cells. Previous studies have revealed that *Ostreopsis* mucilage has specific features, not observed in other dinoflagellates, being formed by a fibrillar network deriving from the aggregation of trichocysts and by an amorphous polysaccharidic matrix. The mucilage has a key role in the colonization of benthic substrates, allowing the attachment of cells to different surfaces and their aggregation through a network of tiny filaments. Then large aggregates may be detached from the sea bottom by waves and float in the water column, being responsible for the formation of toxic aerosols.

Scanning electron microscopy observations of *O.* cf.*ovata* clones isolated from two different Mediterranean areas revealed that cells can attach to substrate in two different ways. In the first mode, many trichocysts converge at the ventral end of cell, and join together to form a long filament that anchors the cell to substrate, allowing rotatory movements round the point of attachment, and contribute to the formation of a network of filaments connecting numerous cells together. In the second mode, trichocysts discharged from thecal pores along the cell margin join together in different points, forming short filaments which firmly fix the cells to substrate: in this case no movement of cells appears to be possible. Cells presenting this mode of adhesion show by SEM a slimy, less definite surface, suggesting that they are covered by a greater amount of amorphous material. This appears to be confirmed by polysaccharide analyses, which indicate a higher production and release by strains with this type of attachment. Previous TEM observations showed that *Ostreopsis* mucilage amorphous component originates from numerous fibrous mucocysts that discharge their content into a canal connected to the ventral pore and by peripheral flask-shaped mucocysts containing granular material connected to trichocyst pores. The first way of attachment with the formation of a network of filaments appeared to be mainly related to mucocyst material, released from the ventral pore, and discharged trichocysts joining together, while the second way, which provides a firmer attachment to substrate, could be based on the discharge of trichocysts and the release of flask-shaped mucocysts material through thecal pores. The existence of two ways of attachment gives *Ostreopsis* the possibility of two different strategies: a strictly benthic strategy with a firm cell adhesion to substrate and a benthic/planktonic strategy where cells can be more easily detached from substrate by disturbance, floating then in aggregates or swimming in the water column.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-014

Physiological changes induced by salinity in filamentous cyanobacteria producing saxitoxins

E. Houliez [1,*], F. Malo [1], G.-A. Rovillon [1], F. Hervé [1], A. Zykwinska [2], L. Marchand [2], E. Briand [1], A. Caruana [1]

[1]ODE, DYNECO, Laboratoire Phycotoxines, IFREMER, [2]Laboratoire Ecosystèmes Microbiens et Molécules Marine pour les Biotechnologies, Ifremer Centre Atlantique, rue de l'Ile d'Yeu, Nantes, France

Abstract: Cyanobacteria are photosynthetic prokaryotes with an incredible plasticity allowing them to inhabit a wide range of ecological habitats. They are found in cold and hot, alkaline and acidic, marine, freshwater, terrestrial and symbiotic environments. They are also characterized by their production of more than 300 bioactive molecules including diverse cyanotoxins. Among these cyanotoxins, saxitoxins are classified as the most potent neurotoxins. The use of surface reservoirs for recreation or as water supplies constitutes the greatest risk for human and animal exposure to saxitoxins produced in freshwaters. Although cyanobacteria occupy diverse habitats, most of the research has focused on their ecology in freshwater systems due to the frequent cyanobacterial blooms in those water bodies. As a consequence, less is known about the possible transfer of cyanobacteria from freshwaters to estuarine systems. It is expected that future global warming consequences on sea level rise, changes in precipitations and drought frequencies associated with an increased use of freshwater for agricultural irrigation may modify salinity in freshwater ecosystems and may favor cyanobacteria dominance. It becomes thus critical to increase our understanding of salinity effects on cyanobacterial production of saxitoxins and to determine the survival capacity of toxic freshwater cyanobacteria in salted waters. We discuss the results of ecophysiological experiments carried out on several genera of freshwater cyanobacteria known as saxitoxin producers. Direct effects of salinity stress on their growth, photosynthetic activity, oxidative stress, compatible solute synthesis and toxin production will be presented.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-015

OPTIMIZATION OF LABORATORY CULTURES OF TWO DINOPHYSIS SPECIES

J. Hernández-Urcera [1], P. Rial [1], M. García-Portela [1,*], P. Loures [1], F. Rodríguez [1], B. Reguera [1]
[1]Harmful Algae Group, Spanish Institute of Oceanography, Vigo, Spain

Abstract: The main objective of the MARBioFEED project is the mass cultivation of microalgae to obtain bioactive compounds with enhanced biorefining methods. Diarrhetic shellfish poisoning (DSP) toxins are produced by some benthic species of *Prorocentrum*, but mainly by mixotrophic species of *Dinophysis* which also produce pectenotoxins (PTX). Although PTXs have a wide spectrum of applications, their supply was hindered due to our inability to grow *Dinophysis* in laboratory cultures. The first successful culture of *D. acuminata* was established using the ciliate *Mesodinium rubrum* as prey, which in turn feeds on cryptophytes (Park et al., 2006). However, conditions for mass culturing of *D. acuminata* and *D. acuta* are not optimized yet. Small scale experiments performed to compare survival of prey-limited *D. acuminata* and *D. acuta* grown with different (*L*, *f2* and *K-Si*) culture media, showed best results were obtained with *K-Si* medium (Keller at al., 1987), especially with *D. acuminata*, where a 6 fold yield was obtained compared with the other media. Then, *K-Si* medium was selected to produce larger volumes (4L) of the two species. Maximal growth rates reached were 0.33 d^{-1} by *D. acuminata* and 0.26 d^{-1} by *D. acuta*. Next, four cryptophytes species (*Teleaulax amphioxeia*, *T. minuta*, *T. gracilis* and *Plagioselmis prolonga*) were given to *Mesodinium* grown with *K-Si* medium, to identify the optimal prey for the ciliate. Maximal ciliate yield (32.34×10^3 cells mL^{-1}) was obtained using *T. amphioxeia* while the highest growth rate was attained with *T. minuta* (0.22 d^{-1}). Establishment of optimal conditions for small-volume cultures of *D. acuminata* and *D. acuta* is a key step before scaling up to large volumes in photobioreactors.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-016

Characterization and comparison of toxin-producing strains of *Dinophysis acuta*, *Dinophysis caudata* and *Dinophysis sacculus*

V. Séchet [1,*], M. Sibat [1], N. Chomérat [2], L. Carpentier [1], S. Gaillard [1], F. Malo [1], G.-A. Rovillon [1], G. Billien [2], A. Duval [2], M. Perrière-Rumebe [3], C. Meteignier [3], M. Retho [4], A. Schmitt [4], C. Hubert [5], Z. Amzil [1], P. Hess [1]

[1]Phycotoxins, IFREMER, NANTES 44300, [2]Laboratoire Environnement Ressources de Bretagne Occidentale, IFREMER, Concarneau, [3]Laboratoire Environnement Ressources, Arcachon, IFREMER, Arcachon, [4]Laboratoire Environnement Ressources, Morbihan-Pays de Loire, IFREMER, La Trinité sur Mer/ Nantes, [5]Laboratoire Environnement Ressources, Languedoc Roussillon, IFREMER, Sète, France

Abstract: Several dinoflagellate species of the genus *Dinophysis* produce potent lipophilic toxins and have been implicated as the causative organism of Diarrhetic Shellfish Poisoning in French coastal waters since 1983. If *Dinophysis acuminata* is without doubt the main DSP-causing agent on the Atlantic coast, the role of the other species of the *Dinophysis* community is not as clear, as more information is needed on their specific toxin profiles. We isolated cells of *D. sacculus* from various Atlantic and Mediterranean sites, and strains of *D. acuta* and *D. caudata* from the Atlantic coast, that we fed on the ciliate *Mesodinium rubrum* to establish mixotrophic cultures. Morphological analyses by light and scanning electron microscopy, and molecular sequencing of ribosomal operon (ITS1, 5.8S, ITS2, partial LSU) and mitochrondrial cytochrome oxydase (*cox1*) gene were performed to identify the strains unambiguously. Intra and extracellular toxin profiles were analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). *Dinophysis sacculus* cells contained low amounts of OA and moderate levels of PTX2. The clear predominance of PTX2 over okadaic acid is in accordance with previous results obtained from laboratory culture of *D. sacculus* from the Galician Rias altas. *Dinophysis acuta* showed a more complex profile and in particular, the additional presence of DTX2 to OA and PTX2. On the contrary, the strains of the third species, *Dinophysis caudata,* contained only PTX2, while no OA was detected.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-017

Cyanobacterial seasonal fluctuation and the presence of the microcystin producing genes in Billings Reservoir (Diadema/SP – Brazil)

C. S. F. Nordi [1,*], M. Ribeiro [2], M. Matarazzo [1], B. Milz [1], C. Niero [2]
[1]Environmental Science, Federal University of São Paulo, Diadema, [2]Biology, Federal University of São Paulo, São Paulo, Brazil

Abstract: Cyanobacteria have a great potential on the occupancy of eutrophic environments, favored mainly by the increase on the concentrations of phosphorus and nitrogen. This phenomenon has also been observed in Billings Reservoir, the study area of this work. In addition, cyanobacteria are potential producers of various toxins that poses harmful effects on both public health and on the environment. Thus, the monitoring of the presence of cyanobacteria and their potential capacity to produce cyanotoxins is of paramount importance for monitoring purposes, since the reservoir is used for fishing, irrigation and public supply purposes.

The sampling was carried out in two periods of 2017: one in January (31) in the rainy season and another in August (02) during the dry season. Four points were chosen with different environmental contributions. Phytoplankton quantification and composition analysis were performed with surface samples. The countings were made in an Uthermol chamber and the density was expressed in biovolume. For molecular biology, two PCR reactions were carried out with specific primers for phycocyanin (positive control for cyanobacteria genetic material) and the microcystin *mcy*E gene.

Cyanobacteria dominated throughout the drought period, with percentages of 97, 94, 95 and 97% at points 1, 2, 3 and 4, respectively, in relation to the total biovolume. During the rainy season, eukaryotes were the dominant components of phytoplankton with 77, 63 and 74% percentages, at points 1, 3 and 4, except for point 2, where cyanobacteria dominated, with 60% of the total biovolume. The specie *Woronichinia naegeliana* was the dominant one during drought season. The cyanobacteria dominance during the dry season can be explained by some factors, such as higher concentration of nutrients and mainly by an increased stability of the water column. These results are in line with previous researches done on the Billings Reservoir. The results of molecular biology show that the *mcy*E gene, considered one of the main ones involved in the production of the toxin, is present in the genetic pool of the cyanobacteria present in the reservoir, and can be related to several genera found during the study period: *Synechocystis*, *Dolichospermum*, *Microcystis* and the species *Woronichinia naegeliana*.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-018

Is Cell Division Time a Constant for *Karlodinium veneficum*? Strain and Temperature Effects with Implications for In Situ Growth

E. L. J. E. Broemsen[1], A. R. Place[2,*], M. W. Parrow[1]

[1]Department of Biological Sciences, University of North Carolina, Charlotte, [2]Center for Environmental Science, Institute of Marine and Environmental Technology, University of Maryland, Baltimore, USA

Abstract: Estimation of the actual growth rate of microalgae in natural blooms is complicated by difficult to measure “net” population gains or losses caused by convection and cell mortality. Several methods have been proposed to address this issue by calculating *in situ* or “gross” growth rates independent of such factors, based solely on the proportion of cells in the population that are dividing. The most reliable of these methods requires determination of the duration of cell division (t_d) for the taxon of interest - a value which must, in the case of the HAB dinoflagellate *Karlodinium veneficum*, be measured in laboratory experiments. Our previous work demonstrated a rapid t_d of ~1.1 h for *K. veneficum* under standard conditions, but appreciation for temperature and strain variability in natural blooms necessitated evaluation of the relationship between these factors and t_d, a topic rarely addressed in the literature. In this study, three strains of *K. veneficum* (two from the Chesapeake Bay (CCMP 1975 and 2010 IH) and one from Norway (CCMP 416)), were used to calculate t_d at three environmentally-relevant temperatures (15 °C, 20 °C, and 25 °C) by measuring the hourly dividing cell frequencies over a 24 h period during mid-log growth. The calculated t_d values ranged from 1.1 h to 4.9 h, with the shortest and longest durations corresponding to the warmest and coldest temperatures, respectively. Interestingly, an obvious strain effect was observed wherein t_d values for strain CCMP 1975 varied negligibly between temperatures (t_d = 1.5 h at 20 °C, μ = 0.35 day^{-1}; t_d = 1.4 h at 15 °C, μ = 0.25 day^{-1}), while strain 2010IH varied significantly (t_d = 1.1 h at 25 °C, μ = 0.41 day^{-1}; t_d = 4.9 h at 15 °C, μ = 0.17 day^{-1}). The observed relationship between temperature and t_d can be useful in the field by establishing upper and lower bound limits as well as a mean t_d for measuring *in situ* growth rates in *K. veneficum* blooms. Observed differences in the duration of cell division might be explained by strain specific temperature optima among these geographically distinct isolates, suggesting the occurrence of ecotype species within *K. veneficum*.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-019

Nitzschia bizertensis: Distribution, toxicity and eco-toxicology

A. Sakka Hlaili [1,*], D. Bouchouicha-Smida [2], S. Melliti Ben Garali [3], O. Kahla [3], I. Sahraoui [2], N. Lundholm [4], P. de la Iglesia [5], F. Karray [6], S. Sayadi [7], B. Barhoumi [2], M. Tedetti [8], O. Pringault [9]

[1]prof, [2]Doctor, [3]Phd doctor, University of Carthage, Faculty of Sciences Bizerte, Bizerte, Tunisia, [4]prof, University of Copenhagen, Copenhagen, Denmark, [5]Doctor, Institut de Recherche et Technologie Agroalimentaire , Tarragona, Spain, [6]Doctor, [7]prof, Centre de biotechnologie de Sfax, Sfax, Tunisia, [8]prof, Université Toulon, Marseille, [9]prof, Université de Montpellier, Montpellier , France

Abstract: Among the HAB algae, toxic diatoms that mainly belong to the genus *Pseudo-nitzschia* have received considerable scientific attention, as several species can produce the potent neurotoxin, domoic acid (DA). In addition to *Pseudo-nitzschia* spp., species of the genus *Nitzschia*, as *N. bizertensis*, are also able to synthesize DA. *N. bizertensis* was discovered in the Lagoon of Bizerte during summer 2009, and then it was recorded during 2012 and 2013. Pronounced blooms of *N. bizertensis* (10^5 -10^6 cells l^{-1}) occurred in spring, summer, autumn and were driven by temperature, salinity, inorganic N (NO_3^-/NH_4^+) and urea. Some strains were reported as toxic, producing 0.10 - 7.29 ng DA l^{-1} in batch cultures. In spring 2018, *N. bizertensis* was found in the sediment of the Bizerte Lagoon, which is known as highly contaminated by PAHs, and hence the tolerance of the species to these pollutants was suspected. Effectively, the contamination of axenic and non-axenic cultures of *N. bizertensis* by the B(a)P (at final concentration of 3 µg l^{-1}) for 7 days was not followed by any significant effect either on the biomass or on the growth. The diatom and the associated bacteria have shown PAH degradation capabilities. This biodegradation was better when the diatom and the bacteria were in co-culture (for non-axenic cultures). This proves the synergistic power of *N. bizertensis* and bacteria in the removal of PAHs

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-020

Study of the neurotoxic dinoflagellate *Gymnodinium catenatum*, responsible of PSP outbreaks in the Mediterranean waters of Morocco

H. Aboualaalaa [1 2,*], B. Rijal leblad [2], M. laabir [3], H. riadi [1], M. daoudi [2], N. maamour [2], M. marhraoui [2], M. K. Ouelad Abdellah [2], H. taleb [4], R. amnhir [4]

[1]Applied Botanical Laboratory, Abdelmalek Essaadi University, Faculty of Sciences, Tetouan, [2]Toxic Microalgae Laboratory, Institut National de Recherche Halieutique, Tanger, Morocco, [3]Center for Marine Biodiversity, Exploitation and Conservation (MARBEC), Montpellier University, CNRS, IRD, Ifremer, montpellier, France, [4]Phycotoxins Laboratory, Institut National de Recherche Halieutique, casablanca, Morocco

Abstract: The chain-forming dinoflagellate *Gymnodinium catenatum* is responsible for outbreaks of paralytic shellfish poisoning (PSP) worldwide. In Morocco, Shellfish poisoning were recorded early in 1969. Since then numerous intoxications and some deaths were reported in Moroccan Atlantic waters. Moreover, the Mediterranean coastline of Morocco is recurrently and highly impacted by PSPs affecting the exploited mollusks and in particular the cockle *Acanthocardia tuberculatum* which showed persistent high levels of PSTs exceeding the threshold level (Taleb et al. 2003). The causative species have been previously identified as *G. catenatum* (Tahri 1998). In our study, we performed a long term survey (2008-2009) in M'diq bay and Oued Laou estuary (Western Moroccan Mediterranean) which showed that *G. catenatum* developed in these ecosystems along the year. *A. tuberculatum* showed an important intoxication during sampling period with PSTs ranging from 1000 to 7000 µg Equivalent Saxitoxin/kg flesh with higher concentrations being registered in Oued Laou Estuary. To go further, several strains of *G. catenatum* were isolated from M'diq Bay and Oued Laou and monoclonal cultures were established. The isolated strains were identified as *G. catenatum*. To determine the optimal conditions for *G. catenatum* growth, this species was cultivated at 23±2 °C in L1 medium (Guillard et Hargraves, 1993), using different Selenium concentrations (0 to 10^{-6} M) and light intensities. Results showed that the growth of *G. catenatum* varied significantly with these experimental conditions. The maximum growth was recorded with 10^{-8} M of Selenium (0.22 day^{-1}). In the absence of this oligo-element growth rate decreased (0.14 day^{-1}) with a cell yield of 1000 $cells.ml^{-1}$). Increasing light intensity leaded to high growth rate. Results from these ecophysiological experiments suggest that Se plays a key role in the growth of the Moroccan strain of *G. catenatum*

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-021

Karenia mikmotoi's inability adapting to fluctuating light may control their migration to mid-deep layer

Y. Nakajima [1], S. Hirae [2], T. Yamatogi [2], K. Koike [1,*]
[1]Graduate School of Biosphere Science, Hiroshima University, Higashihiroshima, [2]Nagasaki Prefectural Institute of Fisheries, Nagasaki, Japan

Abstract: *Karenia mikimotoi,* a notorious fish killer in Japanese waters, often grows and forms blooms in the middle layer because of its preference to low light regimens. In contrast, several culture experiments showed that it could adapt to higher light levels, even over 600 µmol photon m^{-2} sec^{-1}. To know which photoadaptation strategies may control its mid-deep migration, by using a pulse-amplitude modulation (PAM) fluorometry, we compared photosynthetic responses of *K. mikimotoi* and another dinoflagellate species, *Prorocentrum dentatum*, which often co-occurs with *K. mikimotoi* but stays at the shallower depth.

Strains of *K. mikimotoi a*nd *P. dentatum* were co-cultured in a custom-made cylindrical acrylic vessel (76 x 2000 mm) illuminated by a 400W metal halide lamp from the top, and their vertical distributions and the photosynthetic parameters were monitored every 4 h for more than 24 h. The experiment reproduced the phenomena observed in the field waters; in the daytime, *P. dentatum* stayed at the shallower depth while *K. mikimotoi* at beneath the *P. dentatum* layer. Under strong light at the upper layer, *P. dentatum* showed decreases of the photosynthetic electron transportation rate (ETR) and the photochemical quenching (qP) and an increase of the heat dissipation of excess light energy (NPQ). In contrast, *K. mikimotoi* kept higher ETR and lower NPQ values, indicating effectively photosynthesized at the mid-layer than *P. dentatum* at the upper.

In the another experiment in which both species were exposed to 800 µmol m^{-2} s^{-1} for 3 h then followed by a 1 h relaxation period under 40µmol m^{-2} s^{-1}, *P. dentatum* showed excessive NPQ increase (~1.97) under the high light exposure, and even during the relaxation period, the effective quantum yield of the Photosystem II (ΦII) was not fully recovered. In *K. mikimotoi*, the NPQ value did not increase significantly (~0.44) and the ΦII maintained original level after under the low light relaxation. It seemed inconsistent with the fact that *P. dentatum* prefers shallower layer and *K. mikimotoi* does deeper. However, when each species was exposed to rapid cycles (every 15 min) of a light shift (40 ↔ 800 µmol photon m^{-2} sec^{-1}), *P. dentatum* coordinately responded each high and low light shifts by rapidly controlling NPQ and thus maintained relatively higher ΦII; in contrast, NPQ responses in *K. mikimotoi* could not catch up with such rapid cycles and overall ΦII values were gradually slowed down. We therefore conclude that *K. mikimotoi* may adapt to longer high light exposure but its photosystem may not cope with rapid light fluctuation, and hence *K. mikimotoi* prefers mid-deep layer at where light fluctuations are relatively narrower than the shallower layers.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-022

Characterization of *Chattonella* spp. of Todos Santos Bay, and the effects on early-stages of the Pacific oyster Crassostrea gigas.

N. V. Ahumada [1,*], E. Garcia [1], M. C. Ruiz [2], R. Cruz [1]
[1]CICESE, [2]uabc, ensenada, Mexico

Abstract: *A Chattonella spp* bloom caused mass mortality of farmed Bluefin tuna in summer 2016 in Todos Santos Bay, Baja California, Mexico. Also, coincident with mass mortalities on early stages of the Pacific Oyster Crassostrea gigas. Therefore, two strains of *Chattonella* were established, isolated from the region, which presented different morphology and growth characteristics. Strains were identified as Chattonella marina var. ovata and C. minima, by morphological observations and sequencing of D1/D2 domains of the large-subunit rDNA. The highest growth rates of 1.44 and 0.68 day^{-1} were detected at 16 ° C and 190 µmol quanta m^{-2} s^{-1} for C. marina var. ovata and C. minima, respectively. Also, it was identified that the D and umbonada larvae of *Crassostrea gigas* reduce their movement following direct exposure to *Chattonella* strains, and depends on the abundance (1x10^3, 1x10^4, 2x10^4 cell mL^{-1}). The negative effect was recorded after the first 12 hours. Likewise, the crude extract (2x10^4 cell mL^{-1}) has a paralyzing effect on the early larval stages of the oyster. Therefore, Chattonella marina var. ovata and C. minima of the BTS presents an important harmful potential for early stages of the oyster *Crassostrea gigas*.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-023

EFFECT OF BACTERIA IN THE GERMINATION BIOCHEMICAL COMPOSITION AND GROWTH OF *Gymnodinium catenatum*

Dulce V. Ramírez Rodríguez[1], Christine J. Band-Schmidt[1], Bárbara González Acosta[1], David J. López-Cortés[2], José J. Bustillos-Guzmán[2], Francisco E. Hernández-Sandoval[2], Claudia Hernández Guerrero[1], Andrea Lourdes Arriaga Pizano[3]
[1]Centro Interdisciplinario de Ciencias Marinas (CICIMAR-IPN), Ave. Instituto Politécnico Nacional s/n, La Paz, B.C.S. 23096, Mexico. dulval01@gmail.com, [2]Centro de Investigaciones Biológicas del Noroeste, A. C. (CIBNOR), Ave. Instituto Politécnico Nacional s/n, La Paz, B.C.S. 23096, Mexico, [3]Centro Médico Nacional Siglo XXI, Ave. Cuauhtémoc 330, Ciudad de Mexico. 06720, Mexico.

Abstract: Dinoflagellates have a close relationship with bacterial communities that influence their growth, physiology and population dynamics. Compared to the relationship between dinoflagellates-abiotic factors, the bacteria-dinoflagellate relationship is less clear. In this work, changes in protein content, carbohydrates, lipids, pigments and paralyzing toxins were quantified along the growth curve of three strains of *Gymnodinium catenatum*. The effect of *Alteromonas* spp. and *Roseovarius* spp. was tested in the cyst germination, changes in the content of primary metabolites, profile of photosynthetic pigments and paralyzing toxins. Both bacteria were isolated from the strain G7 of the dinoflagellate by cellular sorting and cell staining; when they were inoculated with an initial concentration of 10^5 cells/mL in strain GCCV7 there was a significant increase ($p<0.001$) in the cell biomass. In addition, a higher growth rate and generation time were observed, as well as an increase in the lipid and carbohydrates concentration. In the remaining strains, significant differences were recorded ($p<0.001$) between the acclimation-exponential and exponential-decline phases in proteins, carbohydrates and lipids, respectively. Both bacteria promoted the germination of *G. catenatum* cysts in 96%, observing smaller vegetative cells with reduced pigmentation. These results corroborate the synergy between both organisms and indicate that the interaction between bacteria and *G. catenatum* is key to the success of the sexual process of the dinoflagellate, as well as favoring its growth by doubling the growth rate (0.176 - 0.353 div / day) and doubling its biomass (3153 - 6402 cells / mL). Although the toxicological profile did not change, the presence of *Alteromonas* spp. and *Roseovarius* spp. increased the production of the sulfocarmaboil and decarbamoil analogs.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-023b

The control of microcystin content in low water intake by using the intracellular dynamics of blue green-algae

I. Makino [1,*], Y. Yahagi [2], T. Nakayama [3], A. Kobayashi [4]

[1]Assistant Professor, Tohoku University, Sendai, [2]Professor, Shibaura Institute of Technology, Tokyo, [3]Dr, Tohoku Chemical Co., Ltd., Iwate, [4]Professor, Nihon University , Fukushima, Japan

Abstract: Continuing expansion in the scale of blue-green algae proliferation over the past several decades has become a common phenomenon in freshwater ecosystems worldwide. Microcystin, which is produced by a blue-green algae Microcystis, is the most significant threat to human health, because we often need to intake water from reservoirs regardless of the presence or absence of blue-green algae. To maintain the quality of drinking water under such a situation, quantification of microcystin in intake water source must be required; however, quantification of microcystin has not been established due to simultaneous production of a lot of derivatives by Microcystis. Microcystin, a non-ribosomal peptides, that is synthesized by a series of microcystin synthases. Therefore, analysis of spatio-temporal change of gene expression level and metabolite must be effective for estimation of microsystin production level. In this paper, we discuss whether gene expression level and other metabolite correlate with microsystin production level. We collected blue green-algae during periods of active growth in a man-made lake in August 2015, in which blue-green algae actively reproduces, on a sunny day at 10:00 a.m., 2:00 p.m., 4:00 p.m. and 2:00 a.m. from mid-depth water layers (0.3-0.4 m depth). The blue-green algae samples were subjected to gene analysis and metabolome analysis. The four samples of 10:00 a.m. to 2:00 a.m. were analyzed by RNA-seq representing an independent biological sample for *Microcystis*. The mcy*C* gene transcript peaked at 4:00 p.m. Then, the remaining eight mcy genes (mcy*ABDEFGH*) excluding mcy*C* gene and mcy*I* gene had markedly decreased at midnight (2:00 a.m.). The expression level of the mcyC gene was the lowest at 10:00 a.m. and was increasing in the same way in other time periods. The mcy*I* gene was hardly detected in any samples. Thus, Wild-type *Microcystis* gradually increases the expression of microcystin biosynthesis genes during the day. Then, it dramatically decreased during the night. Metabolomics was performed on four samples sampled at the same timing in parallel with gene analysis to confirm the dynamic metabolic fluctuation of *Microcystis.* Every sample had at least minimum 95 metabolites detected, 82 metabolites of these were decreasing at midnight (2:00 a.m.). The decreased amino acid species contained aspartic acid, glutamic acid, etc. directly related to the microcystin biosynthesis, and were linked with the variation in the amount of the microcystin biosynthesis genes expression. For this reason, it was strongly suspected that the decrease in the synthesis of amino acids influences the decrease in the amount of the microcystin biosynthesis genes expression.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

P-024

Improving the predictability of HABs around the Shetland Islands, Scotland

P. Dees [1,*], K. Davidson [2], A. Dale [3], B. Mouat [4], A. Turner [5], C. Whyte [6]

[1]PhD Student, [2]Microbial Ecology and Modelling, [3]Physics, SAMS, Oban, [4]Joint Head of Marine Science and Technology, NAFC Marine Centre, Scalloway, [5]Food Safety Group, Cefas, Weymouth, [6]Microbial and Molecular Biology, SAMS, Oban, United Kingdom

Abstract: Aquaculture is an important industry in the Shetland Islands which provides employment for around 12-13% of islanders. Employment opportunities will likely increase with ambitious growth targets set by the Scottish government. This increase in aquaculture may be limited by sporadic and difficult to predict harmful algal blooms around Shetland. Large numbers of farmed salmon have suffered gill damage due to high biomass HABs, and shellfish have incorporated algal toxins. This may have a detrimental effect on the industry directly through loss of sales, and indirectly through decreased consumer confidence. Many of the HABs occurring in Shetland have been advected, rather than caused by *in situ* growth alone. Observation of wind patterns and satellite temperature records surrounding HAB events led to the hypothesis that a mixing front west of Shetland is providing a location favourable for HAB initiation. Data from CTD casts have been used to model the structure of the water column. Water column structure within 10 km of shore shows distinct thermal stratification from late Spring until Autumn, making it unlikely that a mixing front is forming in this area. The use of drifters has tested the likelihood of advection taking place before and after thermal stratification, showing the timing of thermal stratification may be more important than the location of HAB initiation. Model output was also supplemented by measurements of HAB species cell abundance, nutrient concentration in the water column, and shellfish toxin concentration.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

P-025

Algal blooms: how are they harming models used for climate management?

I. R. Jenkinson [1 2,*], F. Elias [3 4], H. Ding [5], T. Wyatt [6], L. Seuront [7] and International Working Group on Rheology micro/nanoFluidics and bioFouling in the Oceans and other natural waters (RheFFO WG)

[1]CAS Key Laboratory of Marine Ecology and Environmental Science, Chinese Academy of Sciences Institute of Oceanology, Qingdao, China, [2]Agence de Conseil et de Recherche Océanographiques, La Roche Canillac, France, [3]Laboratoire Matière et Systèmes Complexes (MSC), Université Paris Diderot, CNRS, [4]UFR 925, Sorbonne Universités, UPMC Université 6, Paris, France, [5]Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education,, Ocean University of China, Qingdao, China, [6]Barrio A Tomada, Borreiros, Gondomar, Pontevedra, Spain, [7]UMR 8187, Laboratoire d'Océanologie et de Géoscience, CNRS, Wimereux, France

Abstract: Algae bloom in ocean and inland waters. They produce and consume CO_2 and other greenhouse gases as well as O_2, and these processes are incorporated into models of climate. These algae also produce most of the organic matter (OM) in offshore, coastal and inland waters. Climate models, however, rarely take this OM into account. We will explain why they should.

Much of this OM, as exopolymeric polysaccharides (EPS), is hydrophilic and forms a layer of gel in the surface microlayer (SML) just below the water-air surface. A smaller proportion of the OM forms hydrophobic or amphiphilic molecules mainly of lipids and surfactants (*sensu stricto*), which form an overlying thinner layer at the water-air surface. Hitherto unmodeled variation in the quality and quantity of OM in the SML may be harming climate prediction and management by partly invalidating the climate models used. Such surface-associated OM reduces exchange of O_2, CO_2 and other greenhouse gases, and alters exchange of salts, humidity, aerosols and both thermal and mechanical energy. Such OM in the surface film also damps ripples, gravity waves and even low-frequency ocean swell. Algae-produced OM, through its rheological (mechanical) properties, interacts with turbulence produced by wind, waves and other processes to produce whitecaps, and more long-lived foam. For example, high levels of dissolved organic matter in the Trade-Wind areas of the oceans are associated with whitecaps of abundance comparable with that in the Southern Ocean, where winds are much stronger, but dissolved organic matter, including EPS, is less. Such foam uplifted by wind adds its own dynamics to water-atmosphere exchanges, as well as increasing albedo. So far, measurement of fluxes have been made only under calm ($U_{10} = 0 – 3.6$ m s^{-1}) and moderate (3.6 – 13 m s^{-1}) winds and sea states, where U_{10} is wind speed at a height of 10 m. Currently, studies of such processes take the presence of EPS into account without considering its polymeric structure or rheological properties. Measurements of algal-produced OM have shown huge inter- and intra-taxon variations in its rheological properties. This suggests that sudden shifts in the taxon composition of phytoplankton, particularly in large areas of the oceans could produce abrupt changes to ocean modulation of climate.

We suggest that there is a need to characterize the tertiary chemical structure of OM in the SML in relation to both its rheological and surface properties, as well as the taxonomic composition of blooming phytoplankton throughout the oceans at all seasons and in all weathers. Such characterization should be combined with measurements of gas exchange reduction.

Algal blooms that, by producing varying OM, damage models and predictions of weather and climate represent a hitherto unaddressed type of HAB. The harm they do can be mitigated by conceiving and validating models of this harm.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

P-026

Spatial-temporal distribution of the *Pseudo-nitzschia seriata* and delicatissima complexes in the Sub-Antarctic Ecoregion, South Pacific

M. Pinto-Torres[1 2 3,*], G. Pizarro[4 5], M. Frangópulos[3 6], B. York[7], C. Alves de Souza[7], C. Tomas[7], C. Alarcón[4 5], H. Pacheco[4 5], C. Toro[4 5], C. Mora[8], J. Iriarte[1 2 3 9 10]

[1]Escuela de Graduados, Instituto de Acuicultura, [2]Programa de Doctorado en Ciencias de la Acuicultura, Universidad Austral de Chile, Los Pinos S/N. Puerto Montt, [3]Centro FONDAP IDEAL, Av. El Bosque 01789, Punta Arenas, [4]División de Acuicultura, Departamento de Medio Ambiente, [5]Centro de Estudios de Algas Nocivas (CREAN), Grupo Magallanes, Departamento de Medio Ambiente, Instituto de Fomento Pesquero (IFOP), Enrique Abello 0552, Punta Arenas, [6]Centro de Investigación GAIA Antártica, Universidad de Magallanes, Punta Arenas, Chile, [7]Algal Resources Collection, University of North Carolina Wilmington, Marine Biotechnology Facility, 5600 Marvin K. Moss Ln., Wilmington, NC 28403, USA, [8]División de Acuicultura, Servicio Nacional de Pesca y Acuicultura, 21 de Mayo 1490, Punta Arenas , [9]Centro COPAS-Sur Austral, Universidad de Concepción, Víctor Lamas 1290, Concepción, [10]Centro de Investigación de Ecosistemas de la Patagonia (CIEP), José de moraleda 16, Coyhaique, Chile

Abstract: *Pseudo-nitzschia* blooms and the presence of domoic acid (DA) in shellfish have been poorly characterized in the Sub-Antarctic Ecoregion of the South Pacific. Here, we present the space-time distribution of *Pseudo-nitzschia* spp. in the northern area of the Magellan region (49-52° S), distributed over a period of 10 years, using a monthly sampling frequency, from May 2006 to December 2017. The identification of the complexes was carried out with light and scanning electron microscopy, identifying five species of the genus *Pseudo-nitzschia* belonging to the *seriata* and *delicatissima* complex. There was an increase in abundance (100 and 2.660.700 cells L^{-1} at the beginning and end of the study period, respectively) and the recurrence of these two complexes over the years, with their distribution and associated relative contribution to two water mass present in the study area: Subantarctic Surface Water (Subantarctic Mode Water or SAMW) and Brackish Estuarine Water (BEW). A predominance of the *P. seriata* complex was observed, mainly during the winter months characterized by the input of SAMW into the system of fjords and channels with low temperatures (6-8 °C) and high salinity (30 to 33). Conversely, *P. delicatissima* complex was associated with the BEW, which increases its presence between the summer months, when a high input of freshwater cause's stratification in the water column associated to relatively higher temperatures (10-12 °C) and lower salinities (26-27).

Financial support: CONICYT-PCHA Scholarship / National PhD -2016-folio number 21160047 / Operational Expenses 7332/2017 / FONDECyT code 1170474 / PhD Program in Aquaculture Sciences.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

P-027

The origin of *Dinophysis acuta* blooms which impact Irish aquaculture

R. Raine [1,*], C. Gregory [1], S. Cosgrove [2], H. Farrell [3], R. Cave [1], C. Cusack [4]

[1]Earth and Ocean Sciences, NUI Galway, Galway, Ireland, [2]Marine Science Institute, University of Texas , Port Aransas, Texas, USA, [3]Biosecurity and Food Safety, Department of Primary Industries, Silverwater NSW, Australia, [4]Ocean Science, The Marine Institute, Galway, Ireland

Abstract: The main unresolved issue with *Dinophysis* blooms and their contamination of shellfish with DSP toxins has been the identification of their source. From results of surveys carried out in the summers of 2013 to 2017, we show that *Dinophysis acuta* is advected from the Northeast Atlantic onto the northwest European shelf, including the Celtic Sea to the south of Ireland, in low numbers. Here, substantial populations ($<5*10^3$ cells L^{-1}) develop locally and rapidly in productive regions near the Celtic Sea Front, a tidal front between the Celtic Sea and the Irish Sea. Subsequently, developed blooms advect westwards along the south coast of Ireland in a coastal current towards the economically important shellfish culture region of southwest Ireland, where they cause substantial economic damage through contamination of shellfish with DSP toxins. The source of these *Dinophysis* blooms is thus in excess of 300 km from their point of impact. We also show that in this region *D. acuta* has an origin and an ecological niche separate from *D. acuminata*, another toxin producing *Dinophysis* species which is common here in summer.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

P-028

Imaging phytoplankton – analyzing the Baltic Sea phytoplankton communities using Imaging FlowCytobot

K. Kraft [1,*], J. Seppälä [1], H. Hällfors [1], S. Suikkanen [1], P. Ylöstalo [1], S. Anglès [1], S. Kielosto [1], H. Kuosa [1], L. Laakso [2], S. Lehtinen [1], J. Oja [1], T. Tamminen [1]

[1]Marine Research Centre, Finnish Environment Institute SYKE, [2]Finnish Meteorological Institute, Helsinki, Finland

Abstract: An Imaging FlowCytobot (IFCB) has been deployed at the Utö Atmospheric and Marine Research Station (Finland, northern Baltic Sea) since spring 2017. It is connected to a flow-through system which provides continuous observations of the physical, chemical and biological state of the outer Archipelago Sea, with a sampling inlet at 5 m depth. Phytoplankton images are acquired at 20 min intervals and analyzed using an image recognition and classification algorithm. The algorithm is continually being manually trained using images of cells identified and named by the users. We provide the first results on the functionality and applicability of the IFCB in the Baltic Sea. Especially, we demonstrate the strengths and weaknesses of the technology in identifying key phytoplankton species and groups in the northern Baltic Sea. Our first scientific target in using IFCB data is to analyze climate change related shifts in the northern Baltic Sea phytoplankton communities. In addition, IFCB data is used in the weekly harmful algal situation reports covering the summertime cyanophyte bloom development in the Baltic Sea.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

P-029

Distribution and abundance of toxic HABs and oceanographic conditions during summer 2017 in sub antarctic waters of Southern Chile.

M. Frangopulos [1 2,*], J. Iriarte [3 4 5], H. González [3 6], H. Pacheco [7], E. Menschel [6], M. Pinto [2 4], C. Alarcón [7], C. Toro [7]

[1]Centro de Investigación GAIA Antártica (CIGA), Universidad de Magallanes, [2]Centro FONDAP de Investigación Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL), Punta Arenas, [3]Centro FONDAP de Investigación Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL), Valdivia, [4]Escuela de graduados, Universidad Austral de Chile, Puerto Montt, [5]Centro COPAS Sur Austral, Universidad de Concepción, Concepción , [6]Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, [7]Centro de Estudios de Algas Nocivas (CREAN) grupo Magallanes, Instituto de Fomento Pesquero (IFOP), Punta Arenas, Chile

Abstract: The fjords and channel of southern Chile (between Los Lagos, Aysén and Magallanes regions), has historically presented Harmful Algal Blooms (HABs) associated with marine toxins, affecting public health, economic and social activities, particularly those linked to fishing, aquaculture and tourism. Two oceanographic campaigns were carried out in the summer of 2017, aboard the R/V *Mirai* (January) and the Chilean Navy ship *Marinero Fuentealba* (February), both in the outer and inland waters of the South Pacific (45°-53° S) respectively. Several sampled stations were selected to determine phytoplankton abundance and composition and the presence of HAB species and associated oceanographic parameters. Water samples were taken at different depths and the phytoplankton was counted in laboratory under inverted microscope. The phytoplankton in both cruises was diverse, dominated by diatoms and dinoflagellates; in the first group, high abundances of species of the *Pseudo-nitzschia* complex were observed. In dinoflagellates, an important presence was registered, probably multispecific, of the toxic genus *Karenia*, with a wide geographic distribution in the monitored area, but with higher values of abundance at the stations located in the oceanic and inner zone of the Gulf of Penas. Also, we highlight the detection for the first time within the fjords and channels system of South Pacific Patagonia, of high abundances of the lipophilic toxins producer dinoflagellate *Azadinium* spp. The oceanic and inland waters detection of ichthyotoxic microalgae *Karenia* spp. match with the massive mortality caused by a bloom of this genus observed at the end of January 2017 in the wellboats that moves between Magallanes and Los Lagos region, suggesting the occurrence large spatial and geographical scale blooms in the area and with a probably oceanic origin. Finally, the presence of *Azadinium* spp. confirms the previous detections of azaspiracid toxins in mussels from the area. The implications of the detection of new phytoplankton toxic species for this area of southern Latin America are discussed.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

P-030

Combination of “machine learning” methodologies and imaging-in-flow system to detect Harmful Algae semi-automatically.

G. Wacquet [1,*], A. Lefebvre [2], C. Blondel [2], A. Louchart [1], P. Grosjean [3], L. F. Artigas [1]
[1]Laboratory of Oceanology and Geosciences, CNRS, Wimereux, [2]Laboratory of Environment and Resources, IFREMER, Boulogne-sur-mer, France, [3]Laboratory of Numerical Ecology of Aquatic Systems, UMONS, Mons, Belgium

Abstract: In recent years, improvements in data acquisition techniques have been carried out in order to sample, characterize and quantify phytoplankton communities with a special focus on potential harmful algae during oceanographic campaigns or in the frame of monitoring networks. However, these acquisition and digitization techniques, including those concerning «imaging-in-flow» systems, still generate an important quantity of data in which the presence of target events might not be detected. Indeed, as for traditional samples analysis with inverted microscope, a full manual quantification of the particles based on a simple visual inspection can be time-consuming, tedious and consequently lead to erroneous or missing identifications. For this purpose, a specific R-package, named “zooimage”, was and is still being developed to allow greater automation in data analysis and classification while permitting a limited user-interaction during the process. The proposed methodology consists in combining few expert knowledge and some “machine learning” algorithms at different levels: (i) to classify particles into different groups based on the definition of a specific training set; (ii) to detect and partially validate the “most suspect” predictions which can represent until 90% of the global error; (iii) to automatically estimate the number of cells for each colonial form. Moreover, in order to orientate the automated classification and consequently to reduce the global error rate, an active learning process consisting in adapting the training set to the phytoplankton communities generally encountered in the studied area, was developed. For this, some samples were chosen at regular time intervals, manually classified and used as “contextual data”. Thanks to this technique, the initial recognition rate can be significantly improved, and even reach 95% when 20-25% of the particles are manually validated. These different semi-automated tools were applied on the *in vivo* image dataset acquired with the FlowCam system during the September-October CAMANOC 2014 (IFREMER) cruise in the English Channel, in order to evaluate their operational ability to automatically monitor the diversity of samples for the microphytoplankton, and especially to detect, track and count the most frequent potentially harmful algae found in this area at that period, like species belonging to the genera *Pseudo-nitzschia*, *Dinophysis, Prorocentrum* and *Phaeocystis*. A spatial distribution of these target groups was computed and could allow to highlight different sub-regions in the English Channel during the late summer-fall transition.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

P-031

Automated techniques to follow the spatial distribution of *Phaeocystis globosa* and diatoms spring blooms in the Channel and North Sea

A. Louchart [1,*], R. de Blok [2], E. Debusschere [3], F. Gomez [1], A. Lefebvre [4], F. Lizon [1], J. Mortelmans [3], M. Rijkeboer [5], K. Deneudt [3], A. Veen [5], L. F. Artigas [1]

[1]Laboratory of Oceanology and Geosciences, CNRS, Wimereux, France, [2]Laboratory of Protistology and Aquatic Ecology, University of Ghent, Ghent, [3]Flanders Marine Institute, Ostend, Belgium, [4]Laboratory of Environment and Resources, IFREMER, Boulogne sur mer, France, [5]Laboratory of Hydrobiological Analyses, Rijkswaterstaat, Lelystad, Netherlands

Abstract: The eastern Channel and southern North Sea are continuously influenced by the Atlantic waters and freshwaters inputs, as well as by tidal fronts. At the French coast, flows from local estuaries are driven from the Channel to the North Sea by the residual tidal current creating a “coastal flow” separated from Channel central waters by a tidal front. It leads to a series of different brackish water systems from the Bay of Seine to the Scheldt and Rhine estuaries, supplementing and maintaining high nutrient concentrations along the French, Belgian and Dutch coast. During spring, phytoplankton blooms are mainly dominated by a haptophyte harmful algal species, *Phaeocystis globosa,* preceded and succeeded by diatom blooms including potentially harmful genera (i.e. *Pseudo-nitzschia* spp.). Moreover, in the English coastal area, the Thames river plume provides a local area of important continental influence. Semi-automated techniques are being applied in this area recently, at high resolution to highlight spatio-temporal patterns in phytoplankton successions and outbursts. They provide rapid estimates of abundance and/or chlorophyll *a* content for the whole community and/or at the single-cell level for small picoeukaryotes up to large colonies of microphytoplankton.

The study was performed during a series of three consecutive spring cruises 2017 (PHYCO, CNRS, “Côtes de la Manche” R/V; Lifewatch, VLIZ, “Simon Stevin” R/V; RWS, “Zirfaea” R/V) in the frame of monitoring or research projects, as well as of the European JERICO-Next H2020 European research infrastructure. The cruises started after the onset of spring blooms in the Channel and followed their development along the eastern Channel towards the southern North Sea. Multi-spectral fluorometers and automated flow cytometers were deployed in continuous and profiling mode. They highlighted patchiness in abundance and fluorescence per group as well as some inshore-offshore gradients and contrasts when considering the distance to main estuaries in the area. The deployment of these automated techniques made it possible to discriminate the optical signatures of *P. globosa and Pseudo-nitzschia* spp., mainly found in the brackish waters and to point out their substantial contribution to total phytoplankton abundance and biomass during the progression of the spring bloom. Multivariate analysis were applied to show relations between phytoplankton communities and hydrological and biogeochemical features in the system considered. Automated flow cytometry analysis suggested that *Pseudo-nitzschia* spp. were mostly found in the Scheldt and Rhine estuaries whereas *P. globosa* was widely spread in the system. In brackish waters, the application of a haptophyte signature by multispectral fluorescence showed that this species would have represented more than 75% of the total pigmentary biomass.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

P-032

Micro-phytoplankton and *Alexandrium catenella* abundances, in relation to climatic-oceanographic indices in the Magellan region, Chile

G. Pizarro [1,*], L. Guzmán [2], N. Butorovic [3], J. Blanco [4]

[1]IFOP, Punta Arenas, [2]IFOP, Puerto Montt, [3]Universidad de Magallanes, UMAG, Punta Arenas, [4]Bluewater-consultant, Castro, Chile

Abstract: Since 1972 *Alexandrium catenella* and Paralytic Shellfish Toxin (PST) has gradually extended its distribution from high towards lower latitudes (55° to 41° S) through the Chilean fjords. Currently, its expansion covers up to 36° S along the coastal eastern Pacific Ocean. We hypothesized that micro-phytoplankton abundance and relative abundance of *A. catenella* (RA) in the Magellan region are related by teleconnections, with climatic-oceanographic conditions associated to El Niño Southern Oscillation (ENSO). For this region the major outbreaks of *A. catenella* and PSP have been observed, at the end of the La Niña events but prior to an intense or medium intensity El Niño. From time series available since 1994 for the Magellan region, we contrasted standardised anomalies of micro-phytoplankton abundance and *A. catenella* RA against pluviometry, air and water temperatures, salinity and atmospheric pressure (AP), through non-parametric correlations. Multivariate ENSO Index (MEI), Southern Oscillation Index (SOI) and Antarctic Oscillation (AAO) were also included within the analyses. Results showed a higher Spearman correlation between AP - *A. catenella* RA (-0.41 to -0.65, $p < 0.05$) and MEI - AP (0.44, $p < 0.05$), but other relationships are explored and discussed. Results suggest that large-scale processes related with the atmospheric pressure variability, such as the South Pacific Anticyclone and its disturbances over the westerly winds, may be regulating the micro-phytoplankton and *A. catenella* distributions at high latitude over the southeastern Pacific.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

P-033

FORECASTING ‘EVERY BEACH, EVERY’ DAY TO IMPROVE PUBLIC HEALTH INFORMATION DURING KARENIA BREVIS BLOOMS

R. P. Stumpf [1], B. Kirkpatrick [2], R. W. Litaker [3], M. C. Tomlinson [1,*], R. D. Currier [2], R. Hardison [3], W. C. Holland [3], A. Reich [4], T. Fanara [5], D. Burris [5], A. Busse [5], F. Otis [5]

[1]NOAA's National Centers for Coastal Ocean Science, Silver Spring, MD, [2]Department of Oceanography, Gulf of Mexico Coastal Ocean Observing System, Texas A&M University , College Station, TX, [3]NOAA's National Centers for Coastal Ocean Science, Beaufort, NC, [4]Florida Department of Health, Tampa, FL, [5]Mote Marine Laboratory, Sarasota, FL, USA

Abstract: Improving the forecasts of respiratory risk from *Karenia brevis* aerosols involves the integration of several types of data sets. Any one data set is insufficient to locate blooms at the required spatial and temporal resolution to achieve “every beach, every day”. Our project uses three data sets to improve the respiratory forecasts from the county level to the individual beach level.

First, we use ocean color satellite data to detect blooms that are likely to be *Karenia brevis*. The analysis includes a standard ensemble of algorithms to process data from multiple sensors (MODIS, VIIRS, OLCI). Using multiple satellites reduces the impact of clouds—improving temporal resolution. This information allows us to identify areas that warrant beach monitoring.

Second, we use observations. Field monitoring programs typically sample once a week to determine *Karenia* concentration through taxonomic microscopy. We have developed HABscope, a rapid detection system that combines a microscope with smartphone acquisition, then subsequent real-time automated image recognition software. HABscope is intended for deployment by volunteers. The backbone of the Gulf Coastal Ocean Observing System, combines these concentrations with state sampling and respiratory impacts reported from the Beach Conditions Reporting System.

Third, we use models. Respiratory irritation is forecast from the cell concentration and the onshore wind component. Replacing the daily marine forecasts with the National Digital Forecast Database (NDFD), will provide hourly, 2.5 km resolution forecasts. NDFD allows forecasting at a resolution not previously achieved, leading to respiratory forecasts every day at every beach with samples.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

P-034

Cyanobacteria and Brazil's greatest environmental impact: a worrying past.

F. C. O. Martins [1,*]

[1]IFES - Instituto Federal de Educação, Ciência e Tecnologia do Espírito Santo, Guarapari, Brazil

Abstract: The objective of this work is to present the spatiotemporal dynamics of the cyanobacterial community of the Juparanã lagoon (Linhares, Espírito Santo, Brazil) of the largest and latest research conducted in this ecosystem (2010) before the environmental impact caused by the rupture of ore tailings dam of iron that affected the river Doce in 2015, with which the lagoon communicates, through the Rio Pequeno. The Doce river basin is divided into 24 sub-basins, the largest being in Espírito Santo, the São José river (2,310.13 km² area), where the Juparanã lagoon (62.06 km^2) is located. Because it is a tropical, shallow, vulnerable to the action of the winds and affected by the anthropic activities, mainly agriculture and pasture, the cyanobacteria are favored to the development of blooms. 11 samples were taken at 4 seasons and 3 depths (surface, 1% light and background) of the lagoon, 6 in summer (rainy season) and 5 in winter (dry period). In addition to the qualitative and quantitative evaluation of the phytoplankton community, in particular cyanobacteria, water temperature, turbidity, electrical conductivity, transparency (and euphotic zone determination), oxygen concentration and nutrients (nitrogen and phosphate) were determined. Trophic state index was also calculated. Spearman's correlation coefficient was used to verify the correlations between the variables. The Cyanophyceae Class predominated (33% of the taxa) in the 4 sampling stations and 3 depths, but the occurrence of the taxa was different between summer and winter. Comparing the population density of cyanobacteria with the limits established by Ministry of Health Ordinance No. 2,914 / 2011, they were above the established standard for weekly monitoring (>10,000 cells/mL) in 85.2% of the samples during the study period. In relation to the percentage contribution of Classes in the total population density, different distribution patterns were observed between summer and winter, with Cyanophyceae predominating in the summer (over 90% of the total density). In winter, contributions from other Classes reduced the density of cyanobacteria, but without decreasing their predominance in total density at any sampling station and depth. The total density was negatively correlated with rainfall, wind velocity, which is associated with the lagoon circulation; and, positively, the electrical conductivity and concentration of chlorophyll *a*. The trophic status index presented positive correlations with the density of the Class Cyanophyceae and chlorophyll *a* concentration. The most frequent species of cyanobacteria, responsible for the high densities, were *Anabaena planktonica*, *Cylindrospermopsis raciborskii*, *Oscillatoria simplicissima*, *Microcystis aeruginosa* and *Plankthotrix agardii*, potentially toxic. The results showed worrisome conditions and demand new assessments after the environmental impact of the rupture of the dam.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

P-035

Pulse-Amplitude Modulated (PAM) Fluorometry to Assess N, P, and Fe-Limitation in Freshwater Harmful Bloom-Forming Cyanobacteria

K. Perri [1,*], S. Manning [2], G. Boyer [1]

[1]Chemistry, State University of New York College of Environmental Science and Forestry, Syracuse, [2]Molecular Biosciences, University of Texas at Austin, Austin, USA

Abstract: Harmful algal blooms have been widely linked to excessive phosphorus (P) loading to inland waters, but increasing evidence shows that growth can be limited by other nutrients, notably nitrogen (N) and iron (Fe). Deficiency in each of these nutrients differentially affects the structure of the cell's photosystem and can be manifested as changes in photosynthetic yield, calculated as the ratio of variable to maximal fluorescence (F_v/F_m) and light-modulated electron transport rate. Several instruments have been used to measure F_v/F_m as a rapid *in situ* gauge of nutrient deficiency, but there have been few comparisons of their sensitivity and their ability to resolve among specific nutrient stresses. In this study, we used controlled assays with the bloom-forming cyanobacterium, *Microcystis aeruginosa* UTEX 3037, grown under nutrient-replete (R), nitrogen-limited (LN), phosphorus-limited (LP), and iron-limited (LFe) conditions. F_v/F_m was measured using three pulse-amplitude modulated (PAM) fluorometers and the more traditional method using the photosystem inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). F_v/F_m is usually measured on dark-adapted samples to fully oxidize the photosystem prior to a saturating pulse of light, but several PAM fluorometers (two of which are tested here) are often deployed in the field to measure photosynthetic yield without a dark-adaptation period (F_v'/F_m'). Our results showed no significant differences in F_v/F_m measurements among PAM fluorometers, but they were all significantly higher than the DCMU-based measurements. There was no significant difference in F_v/F_m between R and LFe treatments (0.37 ± 0.06, 0.38 ± 0.15, respectively), but these were significantly higher than the average yield in LP treatments (0.26 ± 0.05). LN yield was significantly lower on average (0.30 ± 0.17) than nutrient-replete measurements (0.37 ± 0.06). F_v/F_m was not statistically different from F_v'/F_m' across all treatments, suggesting that dark-adaptation does not significantly bias measurements made from prokaryotic-dominated assemblages. Field-based nutrient enrichment experiments showed that F_v/F_m did not significantly change (i.e., $p > 0.05$) in cyanobacterial populations, but were either significantly reduced or increased in the eukaryotic populations. The AlgaeOnlineAnalyser PAM fluorometer used in some field experiments produced varied and sometimes incomplete data, making analyzing the effects of nutrient amendments on microalgal field populations extremely difficult. These data suggest that this metric alone cannot resolve limitation by different nutrients in *M. aeruginosa* or cyanobacterial populations and should be used in conjunction with other assays.

Disclosure of Interest: None Declared

Biological oceanography and limnology of HABs

P-036

Dinoflagellate dense bloom and the enhancement of sea surface temperature stratification

M. C. Ruiz-de la Torre [1,*], B. Martin-Atienza [1]
[1]Marine Science Faculty, UABC, Ensenada, Mexico

Abstract: Algal blooms formed by potential yessotoxins producers dinoflagellates are a recurrent phenomenon of potentially socio-economic impact in many coastal waters including the upwelling region off northern Baja California, Mexico. A crucial question is why the blooms persist so long, considering the low growth rates in a highly dispersive environment. Near Surface Temperature Stratification (NSTS) reduce the frictional coupling of the surface layer from deeper waters and allow for its more efficient wind transport, an increased heat retention in the surface layer was expected as a result of visible day light attenuation in surface blooms. The presence of a consistent thermal structure in the first 2.8 meters of the water column during a dense algal bloom led to the question if high concentration of pigments near the sea surface help in the temperature stratification, which might result in differences in thermal stratification inside and outside the bloom patches. The objective was to demonstrate significant differences between NSTS during a dense algal bloom. To determine the presence of stratification within the water column, temperature profiles inside and outside the bloom were analyzed. We used the first and second derivative methods. First derivative produced a highlight of the areas where there is a noticeable change in the slope of the temperature profile, second derivative must be zero in zones where the temperature value was constant. We found that the second derivative showed a significant change of sign in the position of the interface between two adjacent layers, called a "zero-crossing". The steeper the slope change, the easier it was to detect the boundary between two layers. When the second derivative had a narrow peak, the interface between the layers was sharp, if the interface was not sharp or it was wide, the peak in the second derivative was wide too, so that the interface could be determined by locating the point where the second derivative becomes zero or changes its sign. Thus, by analyzing the profile of the second derivative of the temperature we could detect the presence of stratification in the water layer and evaluate its differences inside and outside the patches. The enhancement of sea surface temperature stratification mechanism would indirectly promote the transport of bloom patches towards the coast by thermal wind supporting our hypothesis about the mechanism that may help to maintain dense algal blooms of phytoplankton that exhibits specifically behavioral and life history traits type in regions where inter-annual variabilities might change the frequency and durability of harmful algal blooms.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-037

Recruitment of bloom-forming cyanobacteria, particularly Nodularia spumigena, in the Baltic Sea

N. Wasmund [1,*]

[1]Biological Oceanography, Leibniz Institute for Baltic Sea Research, Rostock-Warnemünde, Germany

Abstract: Despite intense public and scientific interest in cyanobacterial blooms in the Baltic Sea, their origin and generation was not well investigated. Especially the overwintering strategies of the potentially toxic *Aphanizomenon, Dolichospermum* and *Nodularia* are still not well understood. Overwintering populations are undetectably low in routine monitoring samples. The presence of start populations of these three genera should be identified by their growth in mesocosm experiments in March 2009. The results suggested that *Aphanizomenon* derives primarily from the surface water, whereas *Dolichospermum* is known to derive from benthic akinetes. *Nodularia* was of particular interest as its recruitment from akinetes was still under debate. The results verified that *Nodularia* does not develop in water from below the halocline, and therefore not from benthic akinetes, but most likely overwinters in the upper water layers. The next question was if the extremely small winter/spring population has the growth potential to form the large summer blooms. A mesocosm experiment from May 2006 confirmed that the small planktonic start population alone may give rise to the summer blooms.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-038

Thermal tolerance of non-toxic and toxic phytoplankton: Implications to their biogeography in the future ocean

B. Edullantes [1 2,*], T. Cameron [2], E. Low-Decarie [2]

[1]Department of Biology and Environmental Science, College of Science, University of the Philippines Cebu, Cebu City, Philippines, [2]School of Biological Sciences, University of Essex, Colchester, Essex, United Kingdom

Abstract: Toxic algal blooms appear to expand globally and may be intensified in the future, where their duration, frequency, and intensity may increase in response to climate change. It is important to assess the sensitivity of toxic phytoplankton growth to temperature, which is expected to change with climate. Temperature sensitivity of growth can be described by a bell-shaped curve in which biologically meaningful parameters can be obtained to infer the thermal tolerance of a species. Understanding the thermal growth curves is crucial in predicting the biogeography of non-toxic and toxic phytoplankton species in the context of ocean warming. In this present work, we aimed to (1) evaluate existing models to identify the best equation for fitting the temperature-dependence of growth in non-toxic and toxic phytoplankton, (2) examine the variations in thermal growth tolerance between non-toxic and toxic phytoplankton, and (3) evaluate the implication of these differences to their biogeography in future climate scenario. Using non-toxic strains (*Alexandrium tamatum* RCC 3034, *Prorocentrum micans* CCAP 1136/15 and unknown strain of *P. minimum*) and toxic strains *(A. minutum* RCC 2649*, P. lima* CCAP 1136/11, and *P. minimum* RCC 291) strains of dinoflagellates as model organisms, we measured their growth rates along a wide temperature gradient (7 – 35°C) using high-throughput microtiter based culturing and phenotyping approach. The growth rates were then fitted against temperature using 12 non-linear models to estimate the thermal growth parameters (i.e. maximum growth rates, cardinal thermal limits, thermal curve shape and thermal niche), and the correlative trend between these parameters was then evaluated to explain the variation in thermal growth response in non-toxic and toxic phytoplankton. The data obtained from this study was supplemented with the datasets compiled from laboratory culture experiments to allow comparison with an adequate number of observations. Among the models used, temperature sensitivity of phytoplankton growth could be well described by mechanistic and empirical Gaussian models, which had better predictability of growth with temperature compared to other models. Findings in the analysis of pooled experimental and published datasets revealed differences in the thermal growth parameters, which may suggest the potential influence of toxigenicity to the thermal response. Furthermore, results showed that the distribution and dominance of toxic phytoplankton may shift in the warming ocean which may due to alteration in their thermal tolerance.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-039

Mixotrophy in the bloom-forming euglenophyte *Eutreptiella eupharyngea*

Y. D. Yoo [1,*], K. A. Seong [1], W. Yih [1], H. J. Jeong [2]

[1]Department of Marine Biotechnology, Kunsan National University, Kunsan, [2]School of Earth and Environmental Sciences, Seoul National University, Seoul, Republic of Korea

Abstract: The phototrophic euglenophyte *Eutreptiella eupharyngea* often causes red tides in the coastal waters of many countries. To understand the mechanisms of the outbreak, persistence, and decline of its red tides, its trophic mode should be identified. This species had been thought to be an exclusively autotrophic organism. To explore whether *E. eupharyngea* has an ability of mixotrophy, the protoplasms of *E. eupharyngea* cells were examined using an epifluorescence microscope and a transmission electron microscope after heterotrophic bacteria, the cyanobacterium *Synechococcus* sp., and the diverse algal prey species were provided. Furthermore, the ingestion rates of *E. eupharyngea* on heterotrophic bacteria or *Synechococcus* sp. as a function of prey concentration were measured. In addition, grazing by natural populations of euglenophytes on natural populations of heterotrophic bacteria in Masan Bay were investigated. This study reported for the first time that *E. eupharyngea* is a mixotrophic species. Among the prey organisms offered, *E. eupharyngea* fed on only heterotrophic bacteria and *Synechococcus* sp. The maximum ingestion rates of *E. eupharyngea* on heterotrophic bacteria and *Synechococcus* sp. measured in the laboratory were 5.7 and 0.7 cells predator^{-1} h^{-1}, respectively. During the field experiments, the maximum ingestion rates and grazing impacts of euglenophytes including *E. eupharyngea* on natural populations of heterotrophic bacteria were 11.8 cells predator^{-1} h^{-1} and 1.228 d^{-1}, respectively. Therefore, the euglenophytes may sometimes have a considerable potential grazing impact on populations of marine bacteria.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-040

Harmful Algal Blooms along the Red Sea

A. Ismael [1,*]

[1]Oceanography, Faculty of Science, Alexandria University, Alexandria, Egypt

Abstract: In recent years, harmful algal blooms (HABs) have been increased worldwide causing a serious problem to marine and public health. Despite of its importance, limited studies were carried out along the Red Sea with lack of information along its western side except for Gulf of Suez.

Although the Red Sea is an oligotrophic basin, harmful algal blooms occurred at different areas along the Gulf of Suez and its Eastern side causing fish and invertebrate mortalities. Forty seven potentially harmful species both planktonic and benthic species were recorded belonging to Five groups; dinoflagellates (33 sp.), diatoms (8 sp.), cyanophytes (4 sp.), chrysophytes and raphidophytes (one species each).

Among the Red Sea areas, the Red Sea Proper along Saudi Arabia and Yemeni Coasts are the most affected areas with 44 species, followed by Gulf of Suez (17 species) and Gulf of Aqaba (4 species). The increase of HAB species in the Red Sea is associated with increasing nutrients along the coastal areas especially along the Gulf of Suez coast due to increase of wastewater discharge. By continuous increasing of HAB events, HAB monitoring programs along the Red Sea should be established to avoid the health threats and the economic loss.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-041

Genetic diversity of the golden tide forming alga Sargassum horneri in the Yellow Sea inferred from multiple organelle DNA markers

F. Liu [1 2 3,*]

[1] CAS Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, [2]Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, [3]Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao, China

Abstract: Recent years have seen *Sargassum* golden tides become commonplace along the Chinese coastline. Large scale drifting *Sargassum horneri* biomass became stranded on *Pyropia* (nori) cultivation rafts and destroyed the floating nori cultivation infrastructure. A dangerous combination of wave and tide conditions led to an economic loss of at least ¥0.5 billion (Chinese yuan) in the nori industry of Dafeng in Jiangsu Province, China. To understand the genetic diversity of the golden tide forming alga *S. horneri* in the Yellow Sea, and as part of an effort to track the source of the floating biomass, this study performed large-scale spatio-temporal sampling during nine cruises in the Yellow Sea as well as five coastal surveys, and analyzed the genetic diversity of *S. horneri* inferred from multiple organelle DNA markers. A total of 196 *S. horneri* samples had identical sequences of partial *cox3* and *rbc*L-S spacer region, revealing very low genetic diversity in the floating biomass. A total of 19 haplotypes for partial *cox3* previously found in the Yellow Sea were not detected in our large-scale sampling. Considering *Sargassum* mtDNAs had an approximately three-fold greater mutation rate than cpDNAs, four novel mtDNA markers (including regions of *rnl-atp9*, *rps12-rps7*, *rpl5-rps3*, and *cob-cox2*) were designed to perform molecular typing of *S. horneri* samples. The 196 samples could be further distinguished into two forms, which varied in proportions at various locations, but coexisted in each of the spatio-temporal sampling. These results indicated that the floating *Sargassum* biomass in the Yellow Sea came from only two dominating haplotypes. The novel findings uncovered by this work will provide further insight into the underlying mechanisms of reoccurring golden tides in the Yellow Sea.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-042

Assessing realised niche of toxic phytoplankton species along the French Atlantic coast

C. Guallar [1 2 3,*], A. Chapelle [1], C. Bacher [4]

[1]DYNECO/PELAGOS, IFREMER, Plouzané, France, [2]Oceanografia Física i Tecnològica, Institut de Ciències del Mar (ICM-CSIC), [3]Ecologia, Universitat de Barcelona, Barcelona, Spain, [4]DYNECO, IFREMER, Plouzané, France

Abstract: Harmful algae species are responsible of toxic proliferations affecting human health, aquaculture and ecosystems. Among them, the dinoflagellate *Alexandrium minutum* (associated to Paralytic Shellfish Poisoning - PSP - events), and the diatoms *Pseudo-nitzschia fraudulenta* and *P. australis* (both associated to Amnesic Shellfish Poisoning - ASP - events) are recurrent toxic bloom-forming phytoplankton species frequently identified in the French Atlantic coast. Since late 80's, the French programme for phytoplankton and phycotoxin monitoring (REPHY), together with research projects, are collecting environmental, species abundance and toxicity data. We used this *in-situ* long-term data (n = 9 280), together with satellite and model output data, to assess and compare the realised niche of these three phytoplankton toxic species. To obtain them we applied a kernel density estimation framed in a multivariate environmental space and then tested them for local and global niche differences.

In the French-Atlantic coasts, water column temperature, irradiance, salinity, river flow and turbidity segregate the realized niches of the three species studied, highlighting differences on seasonal and spatial distributions. *A. minutum* niche is mainly distributed in summer months, while Pseudo-nitzschia species niches show a bimodal distribution peaking in spring and autumn. The ecosystems studied may present optimum conditions for three, two, one or none of the species niches analysed. In addition, some regions present optimum conditions for the species development although the corresponding species abundances are very low or 0cells L^{-1}, targeting a potential ecosystem for future colonisations or showing a lack of some species key variable in the analysis. According to statistical tests, *P. australis* presents the most different niche among the three species studied, especially from *A. minutum*. The local differences test reveals that in the species inter-comparisons, *A. minutum* and *P. australis* always present favourable conditions in the same region of the defined environmental space. Contrary, *P. fraudulenta* switches the favourable condition region depending on the compared species niche, that may indicate some competitive exclusion.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-043

Submarine groundwater nutrients inputs drive exceptional blooms of *P.micans* in Kinvara Bay, Ireland.

C. Gregory [1 2,*], R. Raine [1 2], R. Cave [1 2], G. McDermott [3]

[1]Earth and Ocean Sciences, School of Natural Sciences, [2]Ryan Institute, NUI Galway, Galway, [3]Environmental Protection Agency, Office of Environmental Assessment, Castlebar, Ireland

Abstract: There is a history of exceptional blooms of the dinoflagellate *Prorocentrum micans* in Kinvara Bay, a semi-enclosed bay on the southern shore of Galway Bay, Ireland. Fresh water inputs to Kinvara Bay are dominated by karst aquifer fed submarine groundwaters (SGD). Nutrient levels in SGD are generally high, of the order 10^1-10^2 mmoles L^{-1} of inorganic dissolved oxidised nitrogen, even in summer. This nutrient input drives phytoplankton blooms, the most striking of which are the *P. micans* blooms, which attain cell densities of $>5x10^5$ cells L^{-1} and often result in discoloured water. We provide data showing the relationship between these exceptional blooms and rainfall, which drives the nutrient inputs from SGD. We also show the link between bloom cell density and tidal state. Tracked drifter studies show that Kinvara bay flushes well on spring tides, but not so on neap tides during which the blooms can develop before being flushed from the system. The relationship between rainfall, nutrient input and tidal state allows a level of prediction of exceptional *P. micans* blooms.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-044

An exceptional summer bloom of *Dinophysis acuta* in a Chilean fjord

P. A. Díaz [1,*], I. Pérez-Santos [1], A. Baldrich [1], P. Montero [2], G. Igor [2], G. Daneri [2], M. Seguel [3], G. Álvarez [4], L. Guzmán [5], G. Pizarro [5], L. Norambuena [5], J. I. Mardones [5], P. Carbonell [5], F. Rodríguez [6], B. Reguera [6]

[1]Centro i-mar, Universidad de Los Lagos, Puerto Montt, [2]Centro de Investigaciones en Ecosistemas de la Patagonia (CIEP), Coyhaique, [3]Centro Regional de Análisis de Recursos y Medio Ambiente (CERAM), Universidad Austral de Chile, Puerto Montt, [4]Facultad de Ciencias del Mar, Departamento de Acuicultura, Universidad Católica del Norte, Coquimbo, [5]Centro de Estudios de Algas Nocivas (CREAN), Instituto de Fomento Pesquero, Puerto Montt, Chile, [6]Centro Oceanográfico de Vigo, Instituto Español de Oceanografía, Vigo, Spain

Abstract: Diarrhetic shellfish poisoning (DSP) toxins and pectenotoxins (PTX) produced by endemic species of the genus *Dinophysis*, mainly *D. acuta* and *D. acuminata*, pose a big threat to public health, artisanal fisheries and the mussel industry in Southern Chile. Nevertheless, little is known about the environmental factors controlling these outbreaks in the Chilean fjords. During summer-autumn (January-April) 2018 several cruises were carried out in Puyuhuapi fjord (44.65ºS-72.79ºW) to parameterize fjord conditions associated with bloom development and fine-scale distribution of *Dinophysis* populations. Here we present results from the survey carried out on February 16, 2018 along nine sampling stations in a 25-km long transect, which coincided with the onset of an exceptional bloom of *D. acuta* in the Patagonian fjords. Vertical profiles of density (kg m^{-3}), temperature (°C), in vivo fluorescence (mg equiv. Chl a L^{-1}) and dissolved oxygen (mL L^{-1}) (CTD casts) as well as water samples at 2m intervals from surface to 20 m depth (Niskin bottles) for microphytoplankton analyses were collected at each station. Vertical hauls (0-20m) with a 20-mm mesh net were collected for lipophilic toxin analyses. Our results evidenced a strong thermohaline stratification along the fjord transect. *Dinophysis acuta* was present as a sub surface thin layer with a cell maximum of 118,000 cells L^{-1}. Due to the overwhelming dominance of *D. acuta*, the bloom gave a strong fluorescence signal coinciding with the fluorescence maximum (rather unusual with the typical low biomass blooms of *Dinophysis*) at 6 m depth, within the layer of maximal water column stability. Further, *D. acuta* seemed to avoid the low salinity values from Puyuhuapi fjord surface waters, because no cells were detected above the 16psu isohaline at 2m depth. Toxins analysis of the haul samples showed the presence of OA, dinophysistoxin-1 (DTX1) and PTX2 in addition to yessotoxins (YTX) associated with the co-occurrence of *Protoceratium reticulatum*. This exceptional summer bloom of *D. acuta* observed in Puyuhuapi fjord, represents one of the highest density blooms ever reported worldwide for this species and a record for Chile. The identification and parameterization of the key factors that triggered this intense bloom are discussed here.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-045

Water Hyacinths as Cyanobacterial Bloom Control

H. Qin [1 2,*]

[1]Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, Netherlands, [2]Jiangsu Academy of Agricultural Sciences, China, Nanjng, China

Abstract: The occurrence of harmful cyanobacterial blooms is harmful to aquatic ecosystems and poses risks to public health. Phytoremediation is a method in which plants are used for the removal of nutrients and cyanobacteria. It has advantages because plants are solar-driven, economical, and help us to achieve a sustainable environment. Water hyacinths are free-floating perennial aquatic macrophytes with long and dense hairy root systems which are conducive to absorbing nutrients and a medium for the filtering out and attachment of particulate matter including cyanobacteria. Water hyacinths generally grow well even in heavily polluted waters, and have a wide tolerance to environmental conditions, so they can be very well used for harmful cyanobacterial bloom control and nutrient removal in algae dominated waters. An eco-engineering project with water hyacinths planted in large-scale enclosures was conducted, and the results confirmed the great potential to use water hyacinths for cyanobacterial bloom control and nutrient removal in algal lakes such as Lake Dianchi. The pilot test *in situ* suggested that water hyacinths achieved high efficiencies on algal (92%) and chlorophyll *a* (92%) removal from a domestic sewage contaminated pond by using self-designed experimental devices. Over 87% cyanobacterial N could be assimilated by water hyacinths by using ^{15}N labeling technique for nitrogen transfer from *Microcystis aeruginosa* to water hyacinths. The results show a new strategy for cyanobacterial bloom control, nitrogen removal and nitrogen cycling in eutrophic waters.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-046

Environmental factors leading to the occurrence of a harmful alga, *Vicicitus globosus*, in the center of the Seto-Inland Sea, Japan

S. Ohara [1,*], R. Yano [2], E. Hagiwara [3], H. Yoneyama [3], S. Sakamoto [4], K. Koike [1]

[1]Graduate School of Biosphere Science, [2]Faculty of Applied Biological Science, Hiroshima University, Higashihiroshima, [3]Hiroshima Prefectural Agriculture, Forestry and Fisheries Bureau, Hiroshima, [4]National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research and Education Agency, Hatsukaichi, Japan

Abstract: The largest inland sea in Japan, the Seto-Inland Sea, was once eutrophicated and thus experienced severe red tides. Strict waste water management laws have been enforced in the surrounding area, and the sea is now rather oligotrophicated. However, the occurrences of red tides have not been obviously decreased, and there are approximately 100 incidences per year. Beginning in 2014, we have conducted monthly phytoplankton monitoring in the center of the Seto-Inland Sea and found notable occurrences of a silicoflagellate, *Vicicitus globosus* (previously *Chattonella globosa*), from late spring to early summer. Blooms of this species have also been reported in various coastal areas around the world. In our sampling area, this species co-occurred with *Pseudochattonella verruculosa* and produced red tide in 2006, causing the mass mortality of caged and wild fishes (Kawaguchi et al. 2007 in Japanese). In this study, data on environmental factors leading to the occurrence of *V. globosus* were extracted from the datasets obtained during our surveys. In the center of the Seto-Inland Sea, *V. globosus* cells were observed from April to September. The maximum cell density was 117 cells mL^{-1} (May 2014). Except 2016, May is the month of notable *V. globosus* occurrence, and the species accounted for 66 to 95% of the total phytoplankton cells. The environmental datasets obtained in May from 2014 until 2017 were subjected to PCA (principal component analysis) to identify the factors causing *V. globosus* occurrence. The blooms were linked with increases in surface light and salinity and with decreases in the inorganic phosphate concentration and maximum quantum yields of PSII (*Fv/Fm*) in the phytoplankton community. These results suggest that *V. globosus* could be dominant under sunny and less rainy weather, which might lead to a low nutrient supply from rivers and lower photosynthetic activity in the whole phytoplankton community. In contrast, the diatom density was negatively linked with *V. globosus* density, and many diatoms were thus observed in nutrient-rich environments. However, such low-nutrient conditions may not be suitable for diatoms or *V. globosus*; the addition of nitrate and phosphate to the *V. globosus*-dominated seawater (May 2017) initially showed a low *Fv/Fm* value (0.53) and caused the *Fv/Fm* to increase to 0.68 in the nitrate-added seawater; this drastic activation of photosynthesis was mainly due to an increase of the activity of *V. globosus* (0.47 to 0.61), not in diatoms (0.59 to 0.59), which were individually assessed using microscopy-PAM. This result suggests that *V. globosus* cells experienced nitrogen depletion even in the seawater in which they were dominant; however, this oligotrophic situation hindered the growth of other phytoplankton, which may have positioned *V. globosus* as the dominant species.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-047

New quantitative real-time PCR assay to detect and quantify the azaspiracid-producing dinoflagellate *Amphidoma languida*

S. Wietkamp [1,*], U. Tillmann [1], K. Toebe [1]
[1]Alfred-Wegener-Institut, Bremerhaven, Germany

Abstract: Azaspiracids (AZA) are a group of lipophilic toxins, which are produced by a few species of the marine nanoplanktonic dinoflagellate genera *Azadinium* and *Amphidoma* (Amphidomataceae). Amphidomataceae were found to be globally distributed in coastal waters and new areas of occurrence are regularly discovered. The AZA toxins accumulate mainly in shellfish and - when consumed by humans - can lead to the so-called azaspiracid shellfish poisoning syndrome (AZP). Given this serious threat to seafood production and to deepen knowledge about the distribution and risk potential of AZA-producing algae, an appropriate detection method enabling a fast identification and quantification for these toxigenic species is needed.

Traditional light microscopy is time-consuming, requires expertise and is getting rather difficult when it comes to the detection, identification and quantification of small-sized plankton. To overcome this challenges, quantitative real-time PCR (qPCR) assays are increasingly used as a molecular additive. Basically, when amplifying the extracted DNA and using DNA standards, the amplification threshold (C_T) gives information about the number of target species in the sample. For two AZA-producing species, *Azadinium spinosum* and *Azadinium poporum*, quantitative PCR assays have already been developed and successfully applied in the field.

Another AZA-producing species, *Amphidoma languida*, was discovered in 2012 in Irish coastal waters and discovered as a new species within the group of Amphidomataceae - in close relationship with *Azadinium spp*. All available strains from Ireland, Iceland, Norway, Denmark and Spain produce azaspiracids. Moreover, *Am. languida* from the Atlantic coast of southern Spain was responsible for AZA amounts in shellfish above the EU regulatory limit, emphasizing the need for further investigations.

We thus developed a quantitative TaqMan PCR assay, amplifying 60bp of the D2 region (located on the LSU/28S) of the ribosomal DNA (rDNA) to detect toxic *Am. languida*. To confirm assay specificity *in vitro*, cross-reactivity tests with DNA of a variety of related organisms were performed. This included 12 different *Am. languida* strains as positive controls, *Amphidoma parvula*, 10 *Azadinium* species (each including different strains), as well as 10 further related dinoflagellate species (*Alexandrium spp.*, *Gymnodinium spp.*, *Heterocapsa spp.*, *Karlodinium sp.*, *Prorocentrum spp.* & *Scripsiella sp.*). The developed probe and primer set successfully detected only *A. languida* strains. Currently, we perform tests of the newly-designed assay on spiked field samples to test and optimize the quantification ability of the assay.

With this assay, we provide a tool for the rapid and distinctive quantification of the toxic dinoflagellate *Amphidoma languida* to be used in monitoring programs and bio-geographic studies.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-048

Revealing the distribution and relative abundance of harmful microalgae species in the South China Sea by metagenomics analysis

K. S. Hii [1], I. K. Law [1], W. L. S. Lau [1], H. Gu [2], Z. Luo [2], C. P. Leaw [1], P. T. Lim [1,*]

[1]Bachok Marine Research Station, Institute of Ocean and Earth Sciences, University of Malaya, Bachok, Malaysia, [2]Thrid Institute of Oceanography, Xiamen, China

Abstract: Harmful algal blooms (HABs) occur due to the proliferation of some harmful microalgae under optimum environmental conditions in the coastal ecosystem. HABs has been increasingly reported over wider geographical regions, with no exception in the South China Sea. To clarify the distribution of HABs species along the east coast of Peninsular Malaysia, the metagenomic approach was adopted in this study. A total of thirty subsurface water samples were collected by a submersible pump and filtered through a 15-micrometer net during a 10-day research cruise (*RV Discovery*) in August 2016. Samples were preserved in saline-ethanol and kept in -20^{o}C until further analysis. Environmental genomic DNA was extracted and analyzed using an Illumina MiSeq platform. Phytoplankton community composition was assessed in 30 sites by metagenomics analysis of the amplicons of two variable domains of 18S ribosomal RNA gene, V4 and V9 regions. At least 70 taxa of phytoplankton species were identified from the V9 metagenome data, with the sequence length ranged from 110-500 bp. The known and potential HABs species were identified from these locations. The Paralytic Shellfish toxin producer, *Alexandrium tamiyavanichii* was found to be widely distributed in the water. The data revealed the distribution of additional nine *Alexandrium* species (*A. affine, A. andersonii, A. fraterculus, A. leei, A. margalefii, A. monilatum, A. ostenfeldii, A. insuetum and A. tamerense*). Several ichthyotoxic species, *Cochlodinium polykrikoides*, *Karlodinium veneficum* and *Chattonella minima* were identified genetically. *Azadinium dexteroporum* and *Protoceratium* sp. were found but with low relative abundances. Factors regulating the distribution and dispersion of HABs species in the water remained to be investigated. The metagenomic analysis provides a fast and reliable tool for risk assessment of HABs that could be served as a useful baseline data on distribution and occurrence of HABs species along the east coast of Peninsular Malaysia.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-049

Wide distribution of the domoic-acid-producing benthic diatom *Nitzschia navis-varingica* at mangrove forests along the Strait of Malacca

S. N. Tan [1], P. T. Lim [1], K. S. Hii [1], S. T. Teng [2], H. C. Lim [3], Y. Kotaki [4], S. S. Bates [5], C. P. Leaw [1,*]

[1]Bachok Marine Research Station, Institute of Ocean and Earth Sciences, University of Malaya, Bachok, [2]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Kota Samarahan, [3]Tunku Abdul Rahman University College, Segamat, Malaysia, [4]Food and Nutrition Section, Fukushima College, Fukushima, Japan, [5]Fisheries and Oceans Canada, Gulf Fisheries Centre, New Brunswick, Canada

Abstract: Amnesic Shellfish Poisoning (ASP) is caused by a group of neurotoxins, domoic acid (DA), that binds irreversibly to the glutamate receptor in the vertebrate central nervous system. Contamination of wild shellfish mollusks and crustacean crabs by DA has resulted in concerns for public health and seafood safety. The benthic pennate diatom *Nitzschia navis-varingica* is one of the diatom species known to produce DA. This species is widely distributed in the Southeast Asian region but information on the occurrence and distribution of this toxigenic *Nitzschia* species along the Strait of Malacca is scarce. In this study, the presence of *Nitzschia* species from mangrove habitats along the Strait of Malacca was documented. A total of 148 *Nitzschia* strains was isolated and cultures established. Other than *N. navis-varingica*, three morphotypes were identified based on the frustule morphology. The phylogenetic relationships of *Nitzschia* species were supported by the nuclear-encoded ribosomal DNA (rDNA) in the large subunit (LSU) and the internal transcript spacer (ITS) regions. Production of DA and its isomers, isodomoic A (IA) and isodomoic B (IB), was detected in some strains by liquid chromatography (FMOC LC-FLD) and further confirmed by mass spectrometry (LC-MS/MS). DA contents in the strains were recorded in the range of 0.27 to 11.06 pg/cell. This study documented the occurrence of toxigenic *Nitzschia* species in the water, with extensive morphological and genetic information. The finding suggests a potential risk of ASP in the coastal waters of Malaysia.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-050

Application of Solid Phase Adsorption Toxin Tracking (SPATT) for the field detection of toxins in Sfax Region (Gulf of Gabès, Tunisia).

W. Medhioub [1,*], A. Hamza [2], M. Mahfoudhi [2], L. Dammak [2], F. Hervé [3], E. Abadie [4], M. Laabir [5], P. Hess [3]

[1]Laboratoire Milieu Marin, National Institute of Marine Sciences and Technologies, Monastir, [2]Laboratoire Milieu Marin, National Institute of Marine Sciences and Technologies, Sfax, Tunisia, [3]Laboratoire Phycotoxines, Institut Français de Recherche pour l'Exploitation de la Mer, Centre Atlantique, Nantes, [4]MARine Biodiversity, Exploitation and Conservation, Institut Français de Recherche pour l'Exploitation de la Mer, [5]MARine Biodiversity, Exploitation and Conservation, Université de Montpellier, Montpellier, France

Abstract: Passive sampling of toxins in seawater has been successfully implemented using Solid Phase Adsorption Toxin Tracking (SPATT) devices which present a simple and sensitive technique for retrieving lipophilic toxins from sea water.

Passive samplingusing SPATT was carried out during one yearat the site Ras Younga in the region of Sfax. SPATTs containing 20 g of HP-20 resin were suspended at 1m sea water depth and retrieved monthly. In parallel, one liter for sea waterwas collected at each sampling pointfor identification of toxic phytoplankton by conventional light microscopy.The resin from the mesh bags was extracted with methanol and analysed for lipophilic toxins by liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS).

Results showed the i) detection of trace concentrations of Gymnodimine (GYM-A), Spirolide(SPX-1) and Pinnatoxin(PnTx-G)ii) increase of concentrations of Okadaic Acid (OA) and Dinophysistoxins (DTX-1) from March to June associated with the presence*of Prorocentrum lima* cells at the surface of sediment. This study exemplified the usefulness of the SPATT technique for sites in the Gulf of Gabès as many other coastal areas. It is anticipated that passive sampling will eventually help to better understand and monitor toxic events.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-051

Annual blooms of a resident *Dinophysis acuminata* population in Port Underwood, Marlborough Sounds, New Zealand

L. Mackenzie [1,*]
[1]Cawthron Institute, Nelson, New Zealand

Abstract: Port Underwood is a 24 km^2 inlet on the north-east coast of the South Island, New Zealand that is regarded as one of the most productive Greenshell mussel (*Perna canaliculus*) growing regions of the Marlborough Sounds. The high productivity of the inlet is attributed to the fertilising effects of local wind induced upwelling and its proximity to the Wairau River out-welling plume. Since weekly toxic phytoplankton and marine biotoxin monitoring began in the early 1990s, it has become apparent that the inlet has a resident population of *Dinophysis acuminata* that blooms for short periods every year between spring (September-October) and autumn (March-April). Annual maximum cell numbers ranged from 1,500- 75,000 cells/litre. and, over 25 years of weekly monitoring, the *D. acuminata* bloom has never failed to appear at some time over this interval. Bloom occurrence may be coupled to the abundance of *D. acuminata* prey (*Mesodinium rubrum*) but the mechanism by which it maintains its residence in this hydrologically dynamic environment is unknown. The toxin profile of *D. acuminata* is dominated by pectenotoxn-2 (PTX2) and dinophysis toxin-1 (DTX1) but the cellular toxin content is low and it was very rare (twice in the last 9 years) that the levels of mussel contamination exceeded the regulated level of 0.16 mg/kg of total DTX1. The *D. acuminata* trigger level of 1000 cells/litre was often exceeded and a good case can be made that this level is too conservative.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-052

Generalist life cycle despite strong seasonality – peculiarities of the dinoflagellate *Alexandrium ostenfeldii* in the Baltic Sea

J. Jerney [1 2,*], S. Suikkanen [1], P. Hakanen [1], A. Kremp [3]

[1]Marine Research Center, Finnish Environment Institute, [2]Department of Environmental Sciences, University of Helsinki, Helsinki, Finland, [3]Leibnitz Institute for Baltic Sea Research Warnemuende, Roshtock, Germany

Abstract: Strong seasonality characterizes the ecosystems inhabited by the toxin producing dinoflagellate *Alexandrium ostenfeldii* in the Baltic Sea. The shallow coastal embayments are exposed to strong gradients of temperature, light and nutrients, which are known to impact the life cycle of other temperate phytoplankton. We hypothesize that life cycle transitions of *A. ostenfeldii* are regulated by environmental factors (temperature and nutrients) and that germination is temporally limited in these habitats due to endogenous regulation. We collected field data (temperature, salinity) as well as sediment surface samples of a bloom hotspot and carried out germination and encystment experiments under controlled laboratory conditions, to unravel the secrets of *A. ostenfeldii's* life cycle in the Baltic Sea. As expected, we found that germination is dependent on temperature: cysts did not germinate below 8 °C and rapid germination only occurred above 16 °C. The formation of resting stages was found to be independent of nutrient limitation, but increased when a drop of temperature from 16 to 10 °C was imposed in laboratory experiments. At 16 °C high germination success occurred in cysts collected from the field throughout the year, despite a reduction to 40-50% just after the bloom peak in September. In addition, no pronounced dormancy period was observed in incubation experiments with cysts produced in the laboratory and a large proportion of thick walled cysts were degraded after 8 months storage period. We did not observe a seasonal germination window set by an endogenous rhythm, as found in other seasonal dinoflagellates from the area. However, cyst germination is dependent on temperature and synchronizes conditions of germination with seasonal warming and cooling, thus setting the timing for the growth period. We conclude that *A. ostenfeldii* follows a generalist germination and cyst formation strategy despite the strong seasonality of its environment, which is unusual and calls for more investigations on drivers of life cycle strategies.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-053

To be or not to be: identifying genetic variation in toxic and non-toxic strains of the cyanobacteria *Microcystis botrys*

E. Johansson [1,*], C. Björnerås [1], H. Mazur-Marzec [2], C. Legrand [3], A. Godhe [4], K. Rengefors [1]

[1]Department of Biology, Lund University, Lund, Sweden, [2]Institute of Oceanography, University of Gdansk, Gdańsk, Poland, [3]Department of Biology and Environmental Science, Linnaeus University, Kalmar, [4]Department of Marine Sciences, University of Gothenburg, Gothenburg, Sweden

Abstract: Cyanobacterial blooms in freshwater systems are often dominated by species within the genus *Microcystis*. These blooms are often correlated with high levels of the cyanotoxin microcystin, which is a potent hepatotoxin. However, both temporal and spatial variance of cyanobacterial biomass and microcystin concentrations occur within a system. In Southern Sweden, *Microcystis botrys* is one of the most commonly occurring species within the genus. We hypothesize that *M. botrys* consists of both toxic (i.e. microcystin producing) and non-toxic (not producing microcystins) strains, which form genetically differentiated subpopulations that are favored under different environmental conditions. The objectives of the present study was to determine the composition of toxic and non-toxic *M. botrys* during an entire growth season, perform in depth toxin profiling, and sequence the entire genomes.

One hundred *M. botrys* strains were isolated during 5 sampling occasions (June-September) in Lake Vomb, Sweden. Screening of strains for microcystin profiles using Q-TOF MS/MS showed that toxic and non-toxic strains of *M. botrys* coexisted during all sampling occasions. Preliminary results indicate that toxic strains increased in proportion during the growth season. In accordance to previous studies, we found that microcystin production was strain-specific, i.e. that all toxic strains displayed unique toxin profiles. The number of microcystin isomers produced in each toxic strain varied, from one up to 12 different isomers. The most common microcystin was MC-RR, which was produced by the vast majority of toxic strains. For the population genomic analyses, our approach was to establish reference genomes of the toxic and non-toxic *M. botrys*, and re-sequence the remainder of the strains. Two strains (one toxic and one non-toxic) were chosen for reference genomes (PacBio de novo deep sequencing). Re-sequencing (Illumina) of all strains will be performed during autumn 2018, and sequences will be mapped against 1) the two reference genomes and 2) published genomes of *Microcystis aeruginosa*. Based on re-sequenced strains, we will investigate the microcystin gene complex for e.g. SNPs, deletions, insertions. We will also perform population genomic analyses to determine whether differentiated toxic and non-toxic subpopulations are present.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-054

Pseudo-nitzschia specific diversity in the Gulf of Trieste

V. Natali[1], A. Beran[1], F. Cerino[1], D. Fornasaro[1], V. Torboli[2], A. Calfapietra[3], M. Cabrini[1,*]
[1]INOGS BIO, INOGS, [2]Dep. Life Science, Università Trieste, Trieste, [3]Centro Ricerche Marine, Centro Ricerche Marine, Cesenatico, Italy

Abstract: The genus *Pseudo-nitzschia*, including some potentially toxic species producing domoic acid, a neurotoxin responsible in humans of the Amnesic Syndrome (ASP) following the ingestion of bivalves, is often present in the phytoplankton community of the Gulf of Trieste. A taxonomic identification up to the species level is needed for the development of adequate measures for the containment and the mitigation of possible negative impacts.

Physico-chemical data and *Pseudo-nitzschia*abundance from 2010 to 2016, monthly sampled at the coastal long-term Ecological Research station C1-LTER in the Gulf of Trieste (northern Adriatic Sea), were analysed to describe the mean seasonal cycle and analyse the interannual variability of the genus. Fixed samples, selected according to periods of maximum abundance of the genus in the time series, were analysed by TEM. Additionally, live samples were collected in October 2016 to isolate different *Pseudo-nitzschia*strains. They were analysed by light and transmission electron microscopy (TEM) for their morphology and by DNA-barcoding, using the ITS2 marker, for genetic characterization. Furthermore, to investigate the occurrence of domoic acid, toxicological analysis was carried out by liquid chromatography associated with mass spectrometry on these strains.

The analysis of the time series 2010-2016 showed a high interannual variability of the *Pseudo-nitzschia* genus with a mean seasonal cycle characterized by the presence of three peaks in spring, summer and autumn. From the observation of the fixed samples with TEM, five species were identified: *P.* cf. *calliantha*, *P.*cf *delicatissima*, *P.* cf. *pseudodelicatissima*, *P. pungens*, and *P. fraudulenta*, all potentially toxic species. Three species were identified from the live samples with TEM and by molecular analysis: *P. calliantha*, *P. mannii*, and *P. subfraudulenta*. Although *P. calliantha* and *P. subfraudulenta* have been reported to be toxic in other studies, none of the strains isolated from the Gulf of Trieste was found to produce toxins. *P. subfraudulenta* has been reported in previous studies only once in the Mediterranean Sea, along the Greek coasts.

The observed seasonal variability indicates the presence of different species with different blooming periods, as suggested by the identification of multiple species from fixed samples. However, to get a complete picture of the *Pseudo-nitzschia*species present in this area and describe their seasonal cycles, it would be necessary to isolate a greater number of strains in different periods of the year. Furthermore, it would be worthwhile to verify the production of toxins under different cultivation conditions to evaluate the toxic potential of these strains under different environmental conditions.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-055

A hypervariable mitochondrial gene associated with geographical origin in a cosmopolitan bloom-forming alga, *Heterosigma akashiwo*

S. Ueki [1,*]

[1]IPSR, Okayama University, Kurashiki, Japan

Abstract: Geographic distributions of phytoplankton species can be defined by events on both evolutionary time and shorter scales, e.g., recent climate changes. Additionally, modern industrial activity, including the transport of live fish and spat for aquaculture and aquatic microorganisms in ship ballast water, may aid the spread of phytoplankton. Obtaining a reliable marker is key to gaining insight into the phylogeographic history of a species. Here, we report a hypervariable mitochondrial gene in the cosmopolitan bloom-forming alga, *Heterosigma akashiwo*. We compared the entire mitochondrial genome sequences of seven *H. akashiwo*strains from Japanese and North American coastal waters and identified a hypervariable segment. The region codes for a hypothetical protein with no defined function, and its variations between Japanese and North American isolates, were prominent, while the sequences were more conserved among Japanese strains and North American isolates. Comparison of the sequence in isolates obtained from different geographical points in theNorthern Hemisphere revealed that the sequence variations largely correlated with latitude and longitude (i.e. Pacific/Atlantic oceans). Our results demonstrate the usefulness of the sequence in determining the phylogeographic history of *H. akashiwo*.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-056

Association of *Alexandrium ostenfeldii* with spirolide accumulation in cultured mussels (*Mytilus galloprovincialis*) from northwest Mexico

P. Paredes-Banda [1,*], E. García-Mendoza [2], E. Ponce-Rivas [3], J. Blanco [4], A. Cembella [5], A. Almazán-Becerril [6], C. Galindo-Sánchez [3]

[1]PhD student, [2]Biological Oceanography , [3]Marine Biotechnology , Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico, [4]Toxic Episodes, Centro de Investigacións Mariñas, Vilanova de Arousa , Spain, [5]Ecological Chemistry , Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, [6]Water Sciences Unit, Centro de Investigación Científica de Yucatán , Cancún, Mexico

Abstract: Spirolides are polyether cyclic imines considered as “fast acting toxins”. Long-term human health consequences of spirolides ingestion via shellfish consumption are uncertain. The lack of recorded acute toxicity effects has led to not being included in sanitary regulations. Nevertheless, their interference with the interpretation of mouse bioassays for regulated lipophilic shellfish toxins requires continued attention within regulatory regimes. Todos Santos Bay (TSB) on the northwest Baja California peninsula is an important shellfish cultivation and fish-farming location in Mexico. The spirolide analogue 13-desmethyl-spirolide-C (13 desm SPXC) has been reported in cultured mussels at TSB, but the causative species associated with accumulation of this toxin has not been previously identified. In this work, we assessed the presence of the dinoflagellate *Alexandrium ostenfeldii*, a known producer of spirolides, by light microscopy (Utermöhl method) and by polymerase chain reaction (PCR) with species-specific oligonucleotides designed for ITS and 18S rDNA gene. We evaluated the presence of this species at the surface and 10 m depth from water samples and from vertical net tows collected from TSB every two weeks from July, 2013 to June, 2014 and from August, 2016 to August, 2017. The accumulation of spirolides was also evaluated by LC-MS/MS in cultured mussels (*Mytilus galloprovincialis*). During the 2013-2014 sampling period, *A. ostenfeldii* was detected in 44% of the plankton samples analyzed by light microscopy. The highest cell abundance (≈3.6×10^3 cells L^{-1}) occurred in October 2013. PCR results confirmed the presence of the species in 52.6% of the discrete water column samples. During 2016 to 2017, *A. ostenfeldii* was detected only in August and at abundance of only 200 cells L^{-1}. PCR analysis confirmed the absence of the species in samples collected from this period. 13 desm SPXC was the only spirolide found by LC-MS/MS in mussels. This analogue was detected mainly in the 2013-2014 sampling period, with the highest concentration of 1.05 µg kg^{-1} detected in June 2014. During winter, toxin concentration in mussels remained at or below the detection limit. Spirolides were not detected during the 2016-2017 sampling period corresponding to the very low cell abundance (<500 cells L^{-1}) or absence of the putative causative species. Although *A. ostenfeldii* cell abundance and spirolide concentration in mussels could not be tightly correlated throughout the entire sampling regime, cells of this species were mostly detected when sea surface temperatures were from 17 to 20 °C. This study represents the first record of *A. ostenfeldii* in TSB and provides the confirmation that this species is the primary if not exclusive origin of spirolide accumulation in mussels from the region.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-057

Detailed insights into *Alexandrium catenella* (Dinophyceae) (Group 1 genotype) population structure and evolution

U. John [1 2,*], T. Alpermann [3], S. Nagai [4], W. Litaker [5], S. Murray [6], D. M. Anderson [7], C. Bolch [8]

[1]Scientist, Helmholtz Institute for Functional Marine Biodiversity, 26129 Oldenburg, [2]Chemical Ecology, Alfred-Wegener-Institut Helmhotz center for Polar and Marine Research, 27570 Bremerhaven, [3]Scientist, Senckenberg Research Institute and Natural History Museum Frankfurt, Frankfurt, Germany, [4]National Research Institute of Fisheries Science, Yokohama, Japan, [5]National Oceanic and Atmospheric Administration, National Centers for Coastal Oceans Science, Beaufort, USA, [6]5Plant Functional Biology and Climate Change Cluster, UTS, Sydney, Australia, [7]Woods Hole Oceanographic Institution, Woods Hole (Massachusetts), USA, [8]Institute for Marine and Antarctic Studies, University of Tasmania, Launceston, Australia

Abstract: The *Alexandrium tamarense* species complex is one of the most studied marine dinoflagellate groups because of its ecological, toxicological and socio-economic importance. Members of this complex produce saxitoxins, potent neurotoxins that cause paralytic shellfish poisoning. Isolates of *A. catenella* (Group 1 genotype) from geographically separated populations have been examined. Thirteen microsatellite markers from divergent *A. catenella* populations such as Bay of Fundy (East Coast of North America), Greenland, North Sea, Alaska (Gulf of Alaska and Bering Strait), Japan and Australia have been analysed in order to provide insights into population genetic sub-structure and patterns of differentiation. The results illustrate the biogeographic dispersal pattern and evolutionary history among and between the populations of *A. catenella*. Interestingly, genetic relatedness including gene flow are not primarily correlated with geographic distance among populations. The course of evolution of the species complex is reconsidered in a paleogeographic context.

*Persons who contributed cultures to the analyses: Michael Broshanan[6], Mandy Richlen[6], Urban Tillmann[1], Ichiro Imai[8]

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-058

DINOFLAGELLATE CYST ASSEMBLAGE OF WEST JOHOR STRAIT, SINGAPORE

S. Yim [1,*], L. Miranda [2]

[1]Research Assistant, [2]Research Fellow, Tropical Marine Science Institute, Singapore, Singapore

Abstract: This is the first study of dinoflagellate cyst assemblage in Singapore. Surface sediment was collected from 32 stations in West Johor Strait to study the spatial distribution and diversity of dinoflagellate cysts. Fifteen genera of dinoflagellate cysts from two orders (Gonyaulacales and Peridiniales) and three families (Gonyaulacaceae, Peridiniaceae and Protoperidiniaceae) were identified. The study site was dominated by cysts of Protoperidininiaceae, notably *Brigantedinium* spp., which constituted 84.1% of the samples. It is also the most diverse genus with 10 morphotypes. *Islandinium* spp. is the second most abundant dinoflagellate cyst, which comprised 3.9% of the samples. *Protoperidinium* spp. is the third most common genus comprising 2.7% of the samples, followed by *Echinidinium* spp. at 1.4%, *Lejeunecysta* spp. at 2.4%, *Quinquecuspis* spp. at 1.5% and *Trinovantedinium* spp. at 0.9%. Even though *Spiniferites* spp., *Alexandrium* spp., *Scrippsiella* spp. and *Cryodinium* spp. were encountered, they were rarely observed in the samples. In addition, 11 morphotypes were also present but have yet to be identified. The total dinoflagellate cyst concentration has a range of 5.36–167.87 cyst g^{-1} dry weight. Dinoflagellate cyst concentration ranged 2.66–134.04 cyst g^{-1} dry weight in the 0–2 cm layer and ranged 0–125.82 cyst g^{-1} dry weight in the 2–5 cm layer. Dinoflagellate cysts in the 0–2 cm layer has shown to be more densely distributed towards the coastal end of the Strait, while cyst abundance in the 2–5 cm layer is generally higher in the inner Strait, where water exchange is minimal.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-059

Polycyclic Aromatic Hydrocarbons effect on the growth rate and photosynthetic activity of the toxic *Alexandrium pseudogonyaulax*

H. Zmerli Triki [1], M. Laabir [2,*], C. Mrabet [1], H. Ben Gharbia [1], D. Couet [3], M. Tedetti [4], O. Pringault [5], O. Kéfi - Daly Yahia [1]
[1]Tunisian National Institute of Agronomy , Tunis, Tunisia, [2]Montpellier University, Montpellier, France, [3]IRD, Tunis, Tunisia, [4]Aix Marseille University, Marseille, [5]IRD, Montpellier, France

Abstract: Polycyclic aromatic hydrocarbons (PAHs) are relatively persistent organic pollutants in marine coastal ecosystems. It's interesting to know the toxicity and lethality effect of PAHs on phytoplankton. Until now the effects of PAHs on Harmful Algal Blooms (HABs) species have been poorly investigated. The goal of this study was to evaluate the impact of a cocktail of four PAHs (Benzo(a) Anhracene, Pyrene, Chrysene and Fluoranthene) on the growth and the photosynthetic activity of the toxic dinoflagellate *Alexandrium pseudogonyaulax*. These PAHs are extensively distributed in the anthropized lagoon of Bizerte (Tunisia, Mediterranean Sea) where *A. pseudogonyaulax* can develop and produce Goniodomin A, a highly toxic compound. For this purpose, controlled laboratory experiments were conducted on *A. pseudogonyaulax* cultures exposed to six different PAH concentrations, C0 (DMSO, control), C1 (8 µg L^{-1}), C2 (30 µg L^{-1}), C3 (120 µg L^{-1}), C4 (360 µg L^{-1}) and C5 (720 µg L^{-1}).The temporal evolution of abundance and morphology of the phytoplankton species was determined by microscopy and the efficiency of the photosynthetic activity was assessed with a portable pulse amplitude modulated fluorometer. Results showed that the growth rate of *A. pseudogonyaulax* decreased since C3 concentration ($\mu = 0.169\ d^{-1}$) and was highly inhibited at C4 ($\mu=0.05\ d^{-1}$) in comparison to control ($\mu=0.235\ d^{-1}$). A lethal effect with lyses of the algal cells was observed for the C5 cocktail concentration corresponding PAHs level measured in the sediment of the Bizerte lagoon. The photosynthetic activity was strongly inhibited at C5 with no significant effects for the lower concentrations. Altogether, results showed that *A. pseudogonyaulax* was sensitive to PAHs which imply that these pollutants could impact its dynamic in anthropized marine coastal ecosystems.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-060

Harmful epibenthic dinoflagellates in two Southern Mediterranean ecosystems: The Bizerte Bay and Lagoon (North of Tunisia)

H. Ben Gharbia [1], M. Laabir [2,*], S. Gueroun [3], O. Kéfi - Daly Yahia [1]

[1]Tunisian National Institute of Agronomy , Tunis, Tunisia, [2]Montpellier university, Montpellier, France, [3]Faculty Science of Bizerte, Bizerte, Tunisia

Abstract: Macrophytes can be overgrown by epiphytic microalgae. Among the epibenthic species, many dinoflagellates are known to be toxin-producers. Harmful events associated with benthic algal blooms (BHABs), have been reported more frequently over the last decades. Nevertheless, reports on BHABs are particularly scarce in the Southern Mediterranean Sea. Potentially toxic benthic dinoflagellates, associated with *Cymodocea nodosa* leaves, were surveyed monthly over one year in the bay and the lagoon of Bizerte (North of Tunisia, Southern Mediterranean Sea). This shellfish production area is dominated by dense *C. nodosa* meadows. Several potentially toxic dinoflagellate species, belonging to the genera *Prorocentrum*, *Ostreopsis, Coolia* and *Amphidinium,* were identified. Densities of the epibenthic dinoflagellates varied according to the collection site, and a great disparity was observed between the bay and the lagoon. The highest epibenthic dinoflagellate abundances were recorded on *C. nodosa* leaves from the Bizerte bay, while lower densities were associated with this magnoliophyte in the highly anthropized lagoon. Consequently, *C. nodosa* seems to offer a suitable biotic substrate for the settlement of epibenthic dinoflagellates. This corroborated our previous co-incubation laboratory experiments showing that *C. nodosa* has a relatively low negative allelopathic effect. The highest mean abundances were recorded for *Ostreopsis* cf. *ovata* in the Bizerte bay, and results revealed significant positive correlations between this toxic dinoflagellate and temperature. Our study supports the idea that global warming and high sea water temperatures may be considered among the major factors affecting *Ostreopsis* blooms and explaining their expansion in the Mediterranean basin.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-061

Species dynamics and associated toxin composition of harmful algae at a shellfish aquaculture site on the Bohus coast of western Sweden

A. D. Cembella[1,*], B. Karlson[2], E. Krieger[1], L. Arneborg[3], A. Godhe[4], W. Eikrem[5], M. Brosnahan[6], B. Krock[1], U. John[1 7]
[1]Ecological Chemistry, Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar-und Meeresforschung, Am Handelshafen 12, 27570 Bremerhaven, Germany, [2]Swedish Meteorological and Hydrological Institute, 426 71 Västra Frölunda, [3]Swedish Meteorological and Hydrological Institute, Folkborgsvägen 17 60176 Norrköping, [4]Department of Marine Sciences, University of Gothenburg, Box 461, 40530 Göteborg, Sweden, [5]Norwegian Institute for Water Research, University of Oslo, Postboks 1172 Blindern0318, Oslo, Norway, [6]Woods Hole Oceanographic Institution, 266 Woods Hole Rd. MS# 32. Woods Hole, MA 02543-1050, USA, [7]Helmholtz Institute for Functional Marine Biodiversity, Ammerländer Heersstraße 231, 26129 Oldenburg, Germany

Abstract: The Skagerrak-Kattegat region exhibits a complex hydrology influenced by variable water currents and advection supplemented by flux from adjacent fjord systems. Frequently occurring HAB events and intense aquaculture in the region, particularly within the fjords, pose challenges for understanding bloom dynamics and implementing strategies for HAB monitoring and prediction. This study was therefore conducted to examine the harmful algal community and related toxins in various ecological compartments, size-fractions and at different water depths at a mussel aquaculture site within a fjord on the west coast of Sweden. The objective of the programme was to identify and define plausible linkages between the driving environmental parameters and shifts in the distribution of HAB taxa and associated toxins over a summer-fall transition. Plankton sampling was conducted at weekly intervals at the Tangesund aquaculture site for identification and quantification of key HAB species and toxins. Toxin concentration and composition profiles were determined weekly from plankton cell concentrates from the water column, from solid phase adsorption toxin tracking (SPATT) resin bags deployed near the mussel raft and from suspended whole mussels (*M. edulis*). The diversity and abundance of HAB taxa was assessed by morphological identification and next generation sequencing (Illumina) of the 18s rDNA gene. Toxin profiles and concentrations of polyether toxins and domoic acid were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Seven HAB taxa were confirmed at the species or generic level and ten related toxins, including one novel spirolide, were associated with these respective key taxa in the region. Non-metric multidimensional scaling (NMDS) analysis revealed no significant differences between the HAB species communities or toxin profiles over time or geographical distance. Nevertheless, clear associations between particular HAB taxa and their known toxins could be defined throughout the season. Trends indicated predominance of dinoflagellate species/genera and related toxins when the water column was stratified. Toxin-producing diatom species (*Pseudo-nitzschia*) were dominant when stratification was weak or absent. Redundancy analysis (RDA) showed that the water temperature, salinity, depth, nitrite levels and distance to the coast were associated with the changes in species cell abundances and/or toxin concentrations. This study provides the first comprehensive evidence of the rich diversity of polyether lipophilic toxins - azaspiracids, spirolides, yessotoxins, pectenotoxins and goniodomins, in plankton and aquaculture shellfish from the Swedish west coast, and helps to refine monitoring and mitigation strategies for aquaculture.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-062

Marine dinoflagellate assemblage in the Galápagos Marine Reserve

O.Carnicer[1]*, P.De La Fuente[2], A.Canepa[3], I.Keith[4], J.Yépez[1], E.Rebolledo[1], J.Diogène[5], M.Fernández-Tejedor[5]
[1]Pontificia Universidad Católica del Ecuador, Sede Esmeraldas, PUCESE, Calle Espejo y subida a Santa Cruz, Casilla 08-01-0065, Esmeraldas, Ecuador; [2]Institut de Ciències del Mar, CSIC, Passeig Maritim de la Barceloneta 37-49, 08003 Barcelona, Spain; [3]Escuela Politécnica Superior, Universidad de Burgos, Avda. de Cantabria s/n, 09006 Burgos, Spain [4]Charles Darwin Research Station, Charles Darwin Foundation, Galapagos Islands, Ecuador; [5]Institut de Recerca i Tecnologia Agroalimentària, IRTA, Carretera de Poble Nou, km 5.5, 43540 Sant Carles de la Ràpita, Spain.

Abstract: It is likely that harmful algae blooms (HABs) have increased in frequency, intensity and geographic distribution in the last decades in response to anthropogenic activities but, there are still some areas in the globe where data is scarce increasing the lack of understanding of distributional patterns of HABs in these areas. The Galapagos Islands are renowned for their unique biological diversity, high levels of endemism, and the unique currents and oceanographic features that allow a variety of habitats to exist. However, marine dinoflagellates community has a lack of representation in biodiversity assessment. In that sense, this study aims to provide key information about dinoflagellates diversity and abundances, related to physical parameters, in the Galapagos Marine Reserve. Samples were collected in April 2016 at 2, 5 and 10 miles north, south east and west on four Islands (Santa Cruz, Santa Fé, Seymour and Pinzón) belonging to southern Galapagos Marine Reserve, 48 sampling sites. A total of 155 species were observed, 26 of them potentially toxic. Among these genera, *Tripos, Protoperidinium* and *Dinophysis* were the most diverse. At species level, *T. furca, Oxytoxum tesselatum* and *Podolampas bipes* were observed in more than 60% of the samples. It is important to mention the presence of benthic species in the water column such as *Ostreopsis* sp., *Gambierdiscus* sp. and *Prorcentrum lima*. In addition, water samples were taken at 0, 15 and 30 meters in 21 sampling sites where no blooms of toxic species were found, just *Karlodinium* sp. was registered in low abundances (3940 cells. L^{-1}) at 2 miles, 30 meters depth northern Seymour Island. According to Sorensen Index, the community structure was homogenous, with slight differences in Santa Fé Island, associated to oceanographic variability. These results represent the first register of several species in the area and highlights the high diversity values of dinoflagellates. The presence of toxic species should be considered by policy makers regarding the implementation of phytoplankton monitoring programs in order to prevent ecological, sanitary and economic impacts in the islands considering the high species diversity and tourism influx.

Disclosure of Interest: None Declared

__Ecology – from the ecological niche to population dynamics and biogeography__

P-063

Protist functional diversity across size-fractionated coastal planktonic communities

P. Ramond [1], M. Sourisseau [1], C. de Vargas [2], R. Siano [1,*]
[1]Dyneco-Pelagos, Ifremer, Brest, [2]Station Biologique de Roscoff, CNRS - UMR7144 , Roscoff, France

Abstract: Studying the coupling between functions and taxonomy is a major challenge of microbiology. The objectives are to understand the structure, dynamics and influence of micro-organisms communities on their environment. We analysed the coupling between functional and taxonomic diversity in communities of marine protists across coastal ecosystems. We used the metabarcoding of the V4-18srDNA genetic region to describe the taxonomic diversity of protists across three different plankton size classes. In parallel, starting from previous theoretical functional diversity framework elaborated for phytoplankton, we developed a trait-based conceptual frame to describe the functional diversity of both autotrophic and heterotrophic marine protists. Thirty traits were used to describe morphological, trophic and physiological aspects of protistan ecological-strategies. The trait 'toxin production' has been included in our framework because it could represent a selective ecological advantage (*eg* allelopathy, predation avoidance).

The sequences obtained from 277 samples were clustered into 111 089 Operational Taxonomic Units (OTUs) corresponding to 2007 distinct taxonomic references, annotated to different taxonomic levels (from species to families). After a review of 717 bibliographical sources, 13 out of 30 traits, mostly corresponding to morpho-trophic characteristics, could describe more than half of our taxonomical references. The trait 'toxin production' was annotated for 37 distinct taxonomic references (2136 OTUs) of harmful protists. Trade-offs between the well-annotated traits were depicted and six functional groups were identified, corresponding to ecological strategies and functional roles of marine protists. Unlikely, the trait 'toxin production' was considered not useful to describe functional groups because annotated for a too small number of taxonomic references. However, harmful protists were well annotated for other traits and thus included in our functional groups. The read abundances of each OTU assigned to a taxonomic reference was cumulated into the corresponding functional group. Finally, the relative abundance of each functional group was analysed across samples and size classes.

The results of this study suggest a tight coupling between the taxonomy and functions in protistan marine coastal communities and demonstrate that study of their diversity is informative on their functional roles. However, functional diversity patterns were distinct between large and small protists. Indeed, micro-plankton seemed more prone to the domination of few species and one or few functional groups while the pico-nanoplankton maintained the coexistence of a more various assemblage (such as phototrophic and heterotrophic groups) with higher species richness. The application of our functional diversity approach to other marine ecosystems will help in corroborating this pattern.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-064

Ecological niches of the five epiphytic dinoflagellates genera in the T-S-host space quantified at coastal waters of Jeju Island Korea

B. Jang [1], H. S. Kim [1], Y. D. Yoo [1], S. Lee [1], J. R. Rho [1], J. W. Park [2], W. Yih [1,*]
[1]Dept. Marine Biotechnology, Kunsan National University, Kunsan, [2]Dept. Marine Biotechnology, National Institute of Fisheries Science, Busan, Republic of Korea

Abstract: We estimated the abundances of the epiphytic dinoflagellate (EPDs) species belonging to the five gernera, *Amphidinium, Coolia, Gambierdiscus, Ostreopsis, and Prorocentrum,* from macroalgal samples collected every two months in 2011 from 6 stations in Jeju coastal waters of Korea. Total averaged mean of the total EPDs counts (cells g^{-1} wet weight) on 81 macro-algal species showed maximum at Station 1 (690) spatially and in June (756) temporally. *Ostreopsis* spp. did not exhibited any limited pattern of distribution on the scatter-diagram planes of temperature-abundance axes or salinity-abundance axes whilst the other 4 genera did. In the spaces of temperature-precipitation-abundance axes the 5 genera showed different distribution patterns each other, which was relatively more evident in those of temperature-salinity-abundance. Meaningful and characteristic patterns were also resulted when the 5 genera were plotted in the spaces of HMAS(host macroalgal species)-T-abundance axes. Such kind of differential distribution patterns among five EPD genera shown here might invoke us some ecological insights to better understand their responses to the changes in environmental factors such as T, S, and host macroalgal species.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-065

Harmful species associated to massive Atlantic salmon mortalities while transported through the Gulf of Penas, southern Chile.

C. A. Toro [1,*], C. Alarcón [1], H. Pacheco [1], P. Salgado [1], M. Frangópulos [2 3], F. Rodríguez [4], G. Fuenzalida [5], R. Raimapo [1], G. Pizarro [1], L. Guzmán [5]

[1]Centro de Estudios de Algas Nocivas (CREAN) (Punta Arenas working group) , Instituto de Fomento Pesquero (IFOP), Enrique Abello 0552, Punta Arenas, [2]Centro de Investigación GAIA-Antártica (CIGA), Universidad de Magallanes, Av. Bulnes 01855, Punta Arenas, [3]Centro FONDAP IDEAL , Universidad Austral de Chile, Av. El Bosque 01789, Punta Arenas, Chile, [4]Instituto Español de Oceanografía (IEO), Subida a Radio Faro 50, 36390 Vigo, Spain, [5]Centro de Estudios de Algas Nocivas (CREAN) (Puerto Montt working group), Instituto de Fomento Pesquero (IFOP), Padre Harter 574, Puerto Montt, Chile

Abstract: During January (Austral Summer) 2017, a mortality of around 130 ton of farmed smolts of Atlantic salmon occurred in the Gulf of Penas (47° S) while were transported by three wellboats from the Aysén region (45° S) to the western area of the Strait of Magellan (54° S). The ichthyotoxic *Kareni*a cf. *mikimotoi* was identified as the likely mortality cause. To verify the abundance and distribution of *Karenia*, the gulf and surrounding areas were visited in four occasions on February and March of 2017 and 2018. Also, data from the Magellan region between March 2017 and February 2018 provided by the Fjord System Red Tide-Monitoring Programme, were considered.

The results showed different species of *Karenia* (*K.* cf*. mikimotoi*, *K.* cf. *brevis*, *K*. cf. *bicuneiformis*). The highest abundances occurred in the Gulf of Penas (700 and 4,500 cells L^{-1}, February 2017 and 2018, respectively). The distribution of these species ranged from Paso Tamar (52°S), Magellan region (100 cells L^{-1}) to Inchemo island (45°S), Aysén region (200 cells L^{-1}), both in March 2017. Interestingly, *Azadinium* spp. for the first time were also identified at the Magellan region during 2017, between Puerto Edén (49° S) and Mariotti Islets (55°S), reaching up to 2,800 cells L^{-1} at Estero Wickham (53° S) in February 2018.

The abundances and distributions of *Karenia* spp*.* and *Azadinium* spp*.,* suggest its densities increased initially in the Pacific Ocean, followed by a passive hydrodinamical transport, carrying cells to the Gulf of Penas and later to the fjords of the Magellan region.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-066

Relationships between environmental conditions and phytoplankton in the Mellah lagoon (Algeria), with an emphasis on HABs species

D. Mohamed Anis [1,*], F. Hocine [2], B. Chahinez [3], G. Anne [4], L. Mohamed [5]

[1]PhD Student, Laboratoire des Ecosystèmes Aquatiques et Terrestres. Faculté des Sciences de la Nature et de la Vie. Université M.C. Messaadia - Souk Ahras., Souk Ahras, [2]Sciences de la Mer, Laboratoire Bioressources Marines. Faculté des Sciences. UniversitéBadjiMokhtar , Annaba, [3]Biologie, Laboratoire des Ecosystèmes Aquatiques et Terrestres. Université M.C. Messaadia , Souk Ahras, Algeria, [4]Oceanology, FOCUS Research Unit,MARE Centre, University of Liège, Liège, Belgium, [5]Center for Marine Biodiversity, Exploitation and Conservation (MARBEC), IRD, Ifremer, CNRS, Université de Montpellier, Montpellier, France

Abstract: For the first time, a bi-monthly monitoring of environmental parameters (temperature, salinity, nutrients) and phytoplankton assemblages was conducted in the well-preserved Mellah lagoon ecosystem (South Western Mediterranean, Algeria). Sampling was performed at 3 stations in 2016. The objective was to study the evolution of the microphytoplankton community with a focus on HABs species in relation with the environmental characteristics.

In total, 227 microphytoplankton species belonging mainly to Diatomophyceae (160 species) and Dinophyceae (53 species), were inventoried in the Mellah lagoon. There was a clear dominance of diatoms (63%), compared to dinoflagellates (37%). Proliferations of some potentially toxic species such as *Prorocentrum micans* (7 200 cells.l^{-1}) and *Gymnodinium sanguineum* (6 000 cells.l^{-1}), were observed in summer and autumn respectively. Other HABs species as *Alexandrium tamarense/catenella* (1 350 cells.l^{-1}) and *Alexandrium minutum* (1 420 cells.l^{-1}), were present in winter and spring respectively.

Temporal differences in the qualitative composition and quantitative phytoplankton communities in the three stations are discussed in relation with environmental variables. Microphytoplankton populations in the Mellah show diversity and equitability indices of 3.04 bits.cell^{-1} and 0.94 respectively, showing a structured community during winter and spring. The rest of the year is characterized by unstable phytoplankton populations, where only opportunistic species develop.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-067

Co-occurrence of *Pyrodinium bahamense* var. *bahamense* and *Pyrodinium bahamense* var. *compressum* (Gonyaulacales) in the Pacific, Guatemala

L. Carrillo-Ovalle [1], J. Garcia-Perez [1,*], E. Blanda [1], M. Vargas-Montero [2]

[1]Centro de Estudios del Mar y Acuicultura, Universidad de San Carlos de Guatemala, Guatemala, Guatemala, [2]Centro de Investigación en Estructuras Microscopicas , Universidad de Costa Rica, San Jose, Costa Rica

Abstract: Harmful algal blooms (HABs) are natural events that occur when one or more species of microalgae proliferate at concentrations that can cause damage to aquatic organisms and to those who consume them. In Guatemala very little information exists on HABs events, although in recent decades it is reported worldwide that there has been an increase in alarming mortality events of aquatic organisms and intoxications in humans, caused by toxins produced by microalgae. Therefore, a monthly monitoring program was established to investigate the presence of harmful algal blooms in the Pacific coasts of Guatemala and to prevent intoxication events. From June to October 2011 the identified samples showed the presence of the species *Pyrodinium bahamense*. Even though the most frequent variety reported is *Pyrodinium bahamense* var. *compressum*, during the analysis for the October sample, another variety was observed. To confirm the varieties, a morphological analysis was carried out taking photos with a scanning electron microscope. This study confirmed the co-occurrence of *Pyrodinium bahamense* var. *bahamense* and *Pyrodinium bahamense* var. *compressum* (Gonyaulacales) for the first time in Guatemala for October 2011. Although the presence of potentially toxic species was described, no human poisoning or aquatic organism mortalities were reported.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-068

Diversity of the potentially toxic diatom genus *Pseud- nitzschia* in the northern Adriatic Sea

D. Maric Pfannkuchen [1,*], M. Smodlaka Tankovic [1], A. Baricevic [1], N. Kuzat [1], M. Pfannkuchen [1]
[1]Center for marine research, Rudjer Boskovic Institute, Rovinj, Croatia

Abstract: Phytoplankton samples were collected at two stations in the northern Adriatic Sea from 1972 to 2017. The analysis of this 45 years long data set provided significant insights in the phytoplankton dynamics, species succession and changes in the phytoplankton community. Species from the genus *Pseudo-nitzschia* were among the most abundant and most frequent diatoms during the entire investigated period. The genus *Pseudo-nitzschia* is associated with the production of domoic acid (DA), a strong neurotoxin. Changes in the species composition within the genus have been observed. In the 1980ies the phytoplankton community was dominated by the *P. seriata* group. While the group *P. delicatissima* first appeared in the '80s and since then their frequency continuously increased. *P. delicatissima* grup has a frequency of appearance from 76-97% in the last decade. *Pseudo-nitzschia* are important costituents of HAB blooms worldwide as well as a DA producers which in turn calls for in depth analysis of the species diversity of the genus for the area. Monoclonal cultures were established for taxonomical and molecular identification. The existence of 7 different *Pseudo-nitzschia* species is confirmed in the northern Adriatic on the basis of detailed morphological characteristics and/or molecular feautures. *P. fraudulenta*, *P. pungens* and *P. subfraudulenta* as a rapresentatives from *P. seriata group*, and five species in *P. delicatissima* group: *P. mannii*, *P. pseudodelicatissima*, *P. delicatissima*, *P. calliantha*.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-069

Diversity, population dynamics and ecology of cyanobacteria in the lagoon Aghien (Ivory Coast)

M. Ahoutou [1,*], R. Djeha [1], K. E. Yao [1], M. Koné [1], J. E. Ebrottié [2], C. Quiblier [3], J. Humbert [4], F. Konan [5], K. J. Coulibaly [1], M. Dosso [1]

[1]Institut Pasteur, [2]Université Nangui Abrogoua, Abidjan, Côte d'Ivoire, [3]MNHN, [4]iEES Paris, Paris, France, [5]Université de Daloa, Daloa, Côte d'Ivoire

Abstract: Due to the population growth in Ivory Coast and the subsequent increase of anthropogenic activities, many freshwater ecosystems have become eutrophicated in the ten past years. These ecosystems are used for many purposes, including washing and cooking. Some of them are also used for the production of drinking water or should be in the next years. This is the case of the lagoon Aghien, which is located close to Abidjan. In all these ecosystems, the issue of the cyanobacterial blooms and of the associated health risks has not been addressed until yet despite the fact that blooms have been already observed in several of them.

In the framework of an international program dealing with the monitoring and the sustainable management of surface water sources in three African countries (http://humbert19.wixsite.com/wasaf), a two-year monitoring program has been implemented since January 2017 on the freshwater lagoon Aghien. The main goal of this monitoring was to assess the composition and the dynamics of the cyanobacterial community and to estimate the potential toxicity for human populations.

Six sampling stations are investigated, four of them being located along a longitudinal transect in the lagoon while the two others are located close to two villages. In addition to several physico-chemical parameters, the phytoplankton community was followed and the potential toxicity of cyanobacteria will be estimated. Moreover, knowing that fishes of the lagoon are an important source of proteins for local populations, the consumption of cyanobacteria by fishes is currently studied and the potential accumulation of microcystins in fishes will be later evaluated.

Our first findings have shown that the lagoon is highly eutrophicated as illustrated by the high phosphorus and nitrogen concentrations and phytoplankton biomasses. Concerning the composition of the phytoplankton community, 48 genera belonging to five phyla have been identified until now, knowing that Chlorophyceae and Cyanobacteria contain respectively 46% and 25 % of these genera. The most abundant genera belong to Cyanobacteria (Microcystis, Limnothrix, Cylindrospermopsis and Oscillatoria), with densities reaching up to 388,000 cells/mL. Both temporal and spatial variations were found in the composition and the abundances of phytoplankton. In particular, it has been evidenced that two phytoplankton communities located at each side of the lagoon sometimes coexist, depending on the hydrological regimes of the three rivers draining into the lagoon. Finally, the first findings on the fish diet have shown that three cyanobacterial genera (Oscillatoria, Limnothrix and Microcystis) are commonly encountered in the stomach contents of herbivorous fishes while some other ones, including Cylindropermopsis, seems to be marginally consumed.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-070

Emerging Chemical Ecology Paradigm or New Plankton Paradox: Phycotoxins as Allelochemical Drivers of Harmful Algal Dynamics

A. D. Cembella [1,*]

[1]Ecological Chemistry, Alfred-Wegener-Institut, Helmholtz Zentrum für Polar-und Meeresforschung, Am Handelshafen 12, 27570 Bremerhaven, Germany

Abstract: Marine phytoplankton produce a wide array of potent phycotoxins, with toxicity defined primarily with respect to mammalian cell targets. Among eukaryotic microalgal species a chemically diverse array of potent bioactive secondary metabolites are produced, including linear and macrocyclic polyethers, tetrahydropurine alkaloids and neurotoxic secondary amino acids. These phycotoxins are typically defined in terms of mammalian cell targets, where the bioactive mechanisms as ion-channel effectors or enzyme inhibitors are generally well known. Early hypotheses of their functional importance to the producing species generally stressed their critical role in chemical defence – as agents in the watery arms race for survival and dominance in the plankton. Laboratory studies have shown that simple bilateral species competition experiments with toxigenic versus non-toxigenic strains often contradict or fail to support the acute toxicity hypothesis as a general mechanism. Many allelochemicals are poorly chemically defined, but are structurally and functionally unrelated to classic phycotoxins, and also exhibit potent biological activity (cell lysis, immobilization, membrane disruption) against co-occurring species. Recent evidence indicates that chemical ecological interactions can affect and even regulate processes such as competition, predator-prey relationships and chemical communication. The bewildering variation in toxin composition and biosynthetic capacity within and among populations of certain species implies a possible alternative resolution to the Hutchinson plankton paradox, as driven by resource limitation and competition. In this scenario, chemical diversity and toxin interactions could contribute to the maintenance of contemporaneous disequilibrium (hence high diversity) but not necessarily by eliciting an acute lethal toxic response. Benthic dinoflagellate species are heavily overrepresented among toxigenic eukaryotic microalgae, particular as producers of polyketide-derived ion-channel effectors. The chemical ecological role of these polyether toxins remains elusive and poorly defined, but competition for substrate space and defensive function against predators or bacterial biofouling are plausible scenarios. The biosynthetic pathways for polyether toxins, synthesised via modular polyketide synthase genes, have been elucidated in a few cases. Further molecular analysis of the respective gene clusters will assist in defining phylogenetic affinities and eventually the functional role of these toxins in pelagic and benthic ecosystems.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-071

CIANOBACTERIA IN THE GUARAPARI LAGOONS (ES, BRAZIL): UNKNOWN BEINGS IN ECOSYSTEMS ALSO UNKNOWN

F. C. O. Martins [1,*], S. Lourencini [1], A. Lourencini [1]
[1]IFES - Instituto Federal de Educação, Ciência e Tecnologia do Espírito Santo, Guarapari, Brazil

Abstract: This study aimed to survey the microalgae community, with emphasis on cyanobacteria, in lacustrine ecosystems of Guarapari (ES, Brazil) so that the identified taxa contribute to the elaboration of a biological collection of microalgae, attend scientific and technological purposes and subsidize public and private institutions in the development of basic research related to biology, taxonomy and biogeography of the registered species, and applied research aimed at biotechnological applications. The selection of coastal aquatic ecosystems for sampling was based on their economic, social and ecological relevance for the Guarapari region. It was also considered the existence of past data on the structure and dynamics of algae communities already studied in ecosystems of the region. In this way 3 coastal lagoons were selected: Lagoa Mãe-Bá, already studied ecologically and Lagoon 1 and 2, so called in this research because they do not have names, record in environmental agency of the region and no ecological data. Samples for qualitative analysis of the cyanobacteria community occurred from May/2014 to January/2015, encompassing the dry and rainy periods of the region. In the Mãe-Bá lagoon the occurrence of cyanobacteria in phytoplankton ranged from 45.2% to 50% of the taxa collected, being recorded taxa of the genera *Aphanocapsa, Aphanothece, Chroococcus, Cylindrospermopsis, Gloecapsa, Leptolyngbya, Lyngbya, Merismopedia, Microcystis, Oscillatoria, Phormidium, Pseudoanabaena* and *Synechococcus*. The Mãe-Bá lagoon receives domestic and industrial effluents and its waters are used for fishing and irrigation. In Lagoon 1, the occurrence of cyanobacteria ranged from 0% to 22.7%. The registered genera were: *Aphanocapsa, Chroococcus, Cylindrospermopsis* and *Merismopedia*. This ecosystem is surrounded by a pasture and its waters are used for animal watering and artisanal fishing. In Lagoon 2, the occurrence of cyanobacteria ranged from 0% to 36.8%. The registered genera were: *Microcystis* (75%) and *Spirulina* (25%). This lagoon is used for landscaping purposes, leisure and tourism. In general, 14 genera of cyanobacteria were recorded in the three lagoons, with taxa of biotechnological and public health relevance, depending on the toxicity potential associated with the various uses discussed. Represent the first records of cyanobacteria in two unidentified lagoons (Lagoons 1 and 2), contributing to the monitoring by environmental agencies.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-072

Variability patterns of toxic epibenthic microalgae *Prorocentum lima* in the Gulf of Gabès (south-eastern Mediterranean Sea)

L. W. Dammak [1 2,*], F. Abdmouleh [1 2], M. Abdennadher [1], A. Hamza [1], C. Sammari [1]

[1]Laboratoire Milieu Marin, National Institute of Science and Technology of the Sea, [2]Faculté des Sciences de Sfax, Université de Sfax, Sfax, Tunisia

Abstract: In the last decades, the frequency of the toxic epibenthic dinoflagellates has increased in the Mediterranean Sea. These blooms are associated with harmful effects on human health and the environment. In the Gulf of Gabès (south-eastern Mediterranean Sea), the toxic epibenthic dinoflagellate *Prorocentrum lima* have been recorded as a common component of epiphytic assemblages and it has been observed in the water column and attached on various substrata. The present work provides the spatio-temporal distribution of *P. lima* on different substrates (macroalgae *Ulva rigida*, rock, sediment and biofilm) for one year (from March 2015 to Febrary 2016) in five stations (El Aouabed, Tabia, Skhira, Port Gabès and Zarrat) within the monitoring sites implanted along the Gulf of Gabès coastline. The relation between the physico-chemical parameters and the *P.lima* abundance was also carried out. A post-hoc test (SNK) revealed a significant difference between stations and substrates. *P. lima* was more abundant in Skhira site in the biofilm in March, September and January, in rock during February and August and in the sediment in January and March. This station was characterised by high ammonium concentration. Furthermore, it was frequently observed on *Ulva rigida* in Zarrat station during December. The seasonal variation of *P. lima* were influenced by temperature as well as nutrients, particularly nitrogenous. High mean abundance relative to total dinoflagellates of *P. lima* were preferentially hosted by rock in all sampling sites.

Disclosure of Interest: None Declared

Ecology – from the ecological niche to population dynamics and biogeography

P-073

THE GROWTH FEATURE OF ULVA PROLIFERA FROM ROOTED TO FLOATED GROWING PROCESS IN SUBEI SHOAL

X.-T. Han [1 2,*], T. YAN [1 2], W.-H. ZHAO [1 2]

[1]Institute of Oceanology, Chinese Academy of Sciences, [2]Qingdao National Laboratory of Marine Science and Technology, Qingdao, China

Abstract: In this paper, the community structure of the rooted green algae was studied at the end of March and the beginning of May. The growth rate of different algae was conducted by the experiments of ship-based enclosure and shoal enclosure. The results showed that the main dominant species of green algae community at the end of March were Ulva flexuosa and Blidingia minima, but there was no U. prolifera. The dominant species at the beginning of May were Blidingia minima and U. compressa. U. prolifera accounted for 15% in the total biomass of rooted green algae at the end of May, but the proportion increased to 75% after they floated on the sea surface. The results of ship-based enclosure experiment showed that the relative growth rate of floating green algae with the highest proportion of U. prolifera was 13.4d-1, while the relative growth rate of the rooted green algae was 10.%d-1. In this research it showed that the strong floating ability and high growth ability of U. prolifera were the main reasons for becoming the dominant species in the green tide.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-074

Analysis of trace levels of domoic acid in seawater and plankton net samples from the Golden Horn Estuary (Sea of Marmara, Turkey)

F. Dursun [1,*], S. Ünlü [1], T. Yurdun [2]

[1]Marine Biology, Istanbul University- Institute of Marine Sciences and Management, [2]Pharmaceutical Toxicology, Marmara University-Faculty of Pharmacy, Istanbul, Turkey

Abstract: The major objective of this research is to characterize spatio-temporal distributions of domoic acid (DA), an algal biotoxin produced by the genus *Pseudo-nitzschia*, in seawater and plankton net samples from the Golden Horn Estuary (GHE), between August 2011 and July 2012. DA concentrations were measured with high-performance liquid chromatography (HPLC), using a fluorenylmethoxycarbonyl (FMOC) derivatization technique. Results of laboratory analyses showed that DA concentrations in seawater ranged from 0.24 to 21.03 µg mL^{-1} while varied between 0.36 and 94.34 µg mL^{-1} in net samples. Also the biotoxin levels showed remarkable seasonal variations and the highest values were detected in May 2012. Moreover, DA values reached the maximum value when *Pseudo-nitzschia* abundance was counted at the highest level (196×10^3 cells L^{-1}). Monthly and biweekly data were evaluated with environmental variables, and their influence on DA production is discussed. The relationship between DA and environmental parameters was investigated by Spearman correlation analysis and the influencing factors of the DA distribution were evaluated with Principal Component Analysis. The biotoxin production was mostly controlled by temperature, with nitrate and silicate limitations being secondary factors that influenced DA concentrations. This study presents the first evaluation of DA levels along the GHE and especially highlights the spring season as a potential risk period for Amnesic Shellfish Poisoning events linked to *Pseudo-nitzschia* growth in the GHE. Therefore, further studies on DA and other marine biotoxins should be performed in this area.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-075

SEASONAL PATTERNS IN THE OCCURRENCE OF RED TIDES IN SANTA MARTA AND SURROUNDING BAYS, COLOMBIAN CARIBBEAN

N. M. Arbeláez [1,*], J. Franco [1], L. Espinosa [1]
[1]Scientific researcher, Marine and Coastal Research Institute-INVEMAR, Santa Marta, Colombia

Abstract: Red tides are a phenomenon that could cause changes in the color of water related to the increase of some photosynthetic microorganisms densities. These events are becoming more frequent, particularly in coastal areas with high nutrients concentration, being in some cases harmful for living organisms including humans. Since 2010, the frequency of red tides has been increasing in the coastal areas of Santa Marta Colombia, for this reason and with the aim of analyzing the influence of environmental and climatic variability on the occurrence of red tides events between 2010 and 2017, surface water temperature, salinity, dissolved oxygen and pH were measured and water samples were collected to determine microalgal densities and the concentration of dissolved inorganic nutrients (nitrogen and phosphorus). Additionally, precipitation data and the ONI index were consulted. The results showed that the dinoflagellate *Cochlodinium* sp. was responsible for three events occurred in October 2010, October 2011 and November 2015, when the precipitation increased (>90 mm), the temperature was greater than 29.7 °C and salinity decreased (<35.5). During these events, *Cochlodinium* sp. was the dominant species (90 % of relative abundance), reaching a maximum density of 5×10^{6} cells L^{-1}. On the other hand, the ciliate *Mesodinium* cf. *rubrum* was responsible for three events occurred in January 2015, January 2017 and March 2017, with concentrations between 5.5×10^{4} and 9.2×10^{6} cells L^{-1}. During these events there was absence of rainfall, high concentrations of phosphates (up to 224 µg L^{-1}), increase in salinity (>36.6) and temperature lower than 26 °C. Analyzing red tides events recorded and the ONI index, it was evident that *Cochlodinium* sp. blooms occurred in years influenced by moderate-strong climate anomalies (ONI ≥ ± 1.0), whereas those caused by *M.* cf. *rubrum*, occurred during neutral years (ONI ≤ 0.5).These blooms have been recurrent in Santa Marta, without representing a risk to human health and to other organisms, however, considering the history of mortality of organisms in several countries because of *Cochlodinium* spp. proliferations, these dinoflagellates are considered potentially harmful, representing a risk in the area. The episodes of red tides have had a cyclic pattern of occurrence in Santa Marta and surrounding bays, influenced by the environmental conditions and the climatic variability of each season. This information together along with the permanent monitoring of potentially harmful algae in the area, which has been implemented with the support of the International Atomic Energy Agency, could be considered as a relevant input for the construction of a predictive system against red tides both harmless and noxious in the Colombian Caribbean region.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-076

Inhibition of massive blooms of *Cochlodinium polykrikoides* due to abnormal high temperature from Changjiang River discharge in Korea

S. H. Baek [1,*], M. Lee [1], J. H. Kim [1], Y. O. Kim [2], Y. K. Lim [1], C.-Y. Ahn [3], B. S. Park [4]

[1]Korea Institute of Ocean Science and Technology, Geoje, [2]Korea Institute of Ocean Science and Technology, Busan, [3]Korea Research Institute of Bioscience & Biotechnology, Daejeon, Republic of Korea, [4]University of Texas, Austin, USA

Abstract: For the past 20 years, blooms of *Cochlodinium polykrikoides* have been consistently present along the Tongyeong coast, Korea, but they abruptly disappeared in 2016. Despite extensive studies on this dinoflagellate, the cause of this abrupt decline remains largely unknown. To understand these phenomena, physico-chemical and biological data were collected along the Tongyeong coast through a biweekly field survey from June to September. The environmental differences between 2016 and previous years (2012-2015) were investigated. In August, the water temperatures (ca. 30°C) and the salinity (ca. 30) were outside the optimum ranges for *C. polykrikoides* growth in the study area. Moreover, the averages of these factors were significantly different from previous years ($p<0.001$). In 2016, the amount of Changjiang River discharge, which can affect coastal environments via ocean currents, was relatively larger than in the past four years, reducing the salinity in August. Increased stratification and diatom dominance, both negatively associated with *C. polykrikoides* growth, were consistently observed during the study period. In the Goheung-Oenarodo area, which is the western part of the southern Korean coastal waters (KCW), *C. polykrikoides* blooms were observed, but they did not expand to the entire southern KCW, unlike in previous years. At that time, the strong winds from the *Malakas* typhoon may have terminated the bloom. In addition, westward winds may have inhibited the spread of the bloom eastward along the Tongyeong coast. The combination of these environmental factors may have contributed to the decline of massive *C. polykrikoides* blooms in southern KCW in 2016.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-077

Co-development for HAB climate services

B. A. Siemering [1,*], E. O'Rourke [1], C. Cusack [1], T. Dabrowski [1], M. Garcia-Jove [1], J. Silke [1]
[1]Marine Institute, Rinville, Ireland

Abstract: Climate change, in combination with other anthropogenic pressures, may have dramatic effects on coastal marine ecosystems and influence the dynamics and occurrence of HABs. Changes in water temperature, stratification, current systems and seasonality are known to affect harmful phytoplankton growth, toxin production, and bloom dynamics. Unfortunately, describing the full impact of climate change remains a challenge as the response of HABs to environmental factors can be highly site and species specific. Despite these challenges, coastal stakeholders are in need of high quality information regarding the specific impact of climate change on HABs that is understandable, relevant and can support informed decision making. The EU JPI Climate ERA4CS funded project, CoCliME, aims at co-developing such, much needed, climate services with a strong focus on stakeholder specific needs and expectations. Among other transdisciplinary methods, statistical regression models are used to analyse over 20 years of HAB and environmental data around the Irish Atlantic coast. Preliminary modelling results suggest that occurrence and toxin production of some harmful species can be partly explained by a combination of oceanographic, meteorological and hydrodynamic model data including water temperature, wind, currents and turbulence. While water temperature and stratification were the most significant factors in the model description of *Dinophysis* and *Pseudo-nitzschia* spp., amnesic shellfish poisoning and diarrhetic shellfish poisoning in blue mussels were also significantly related to turbulence and water current dynamics respectively. Models also indicated a quick response of phytoplankton to environmental drivers, as data from the same week gave better modelling results than using past weeks' data. In some cases, anomalies in temperature and stratification were as relevant to the model as the original data. In conclusion, the availability of 20+ years of data can help to describe toxic events as regional specific combinations of environmental drivers. This is the first step in the development of regional climate services and future work will focus on using these results to determine how HABs might respond to our changing environment.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-078

Web-tool services for investigation of sea anomalies by abundance of harmful algal blooms retrieved from satellite monitoring data

V. Savorskiy [1,*], O. Lavrova [2], I. Uvarov [2], A. Kashnitskiy [2]
[1]FIRE RAS, Fryazino, [2]IKI RAS, Moscow, Russian Federation

Abstract: Environmental researches, which are directed at estimations of anomalies in marine environment caused by both anthropogenic and climatic factors, composed important scientific task to be solved at present. One of the most prominent pointer at the negative consequences of such anomalous changes are the abundance of harmful algal blooms. Presented work applies this pointer for investigations of marine environment of intracontinental seas. The choice of such pointer is due to the urgency of the problem of water reservoir, lake, and sea eutrophication, which growth is significant in recent decades. This problem is especially acute for the Azov Sea, where the cyanobacteria, which are most dangerous to humans, marine fauna, and, possibly, flora, are "blooming". The main goal of the work is to work out software tools enabling identification of patterns in the intensification of flowering of harmful algae, the impact on them of various anthropogenic and climatic factors, and their possible negative impact on the coastal zone of the Russian Federation. To achieve the goal a working model of the remote coastal zone monitoring system, which uses long-term (above 20 years) data sets received from satellite monitoring, is developed. Web-tools, which implement information system services, are presented. In order to ensure compatibility of experimental data estimations, which were retrieved from different satellite instruments during multi-year monitoring, abundance of harmful algal blooms is estimated via application of special spectral indices, such as FAI (Floating Algae Index), adopted to use in Azov Sea environment. Developed web-tools provide not only presentation services but allow fine tuning spectral indices in remote user access mode as well. These tools present via common web interface the spatial dynamics of intensity of harmful algal blooms, river flow discharges, and agricultural activity characteristics. As a result developed tools suit for establishment a connection of the studied dynamics of harmful bloom with river discharges, with agricultural activities in the catchment areas, for revealing processes that may pose environmental threat to the coastal zones.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-079

Habitat effects on *Ostreopsis* cf. *ovata* bloom dynamics

L. Meroni [1,*], M. Chiantore [1], V. Asnaghi [1]
[1]DiSTAV, University of Genoa, Genoa, Italy

Abstract: *Ostreopsis* cf. *ovata* is a toxic benthic dinolagellate of tropical origin that raised a large interest in the last decades also in the Mediterranean Sea, where several bloom events have been observed. *O.* cf. *ovata* ecology and proliferation dynamics are driven by complex interactions among biotic and abiotic drivers, and understanding mechanisms triggering bloom events is still far from being complete. The aim of the present study is to highlight the role of the habitat, disentangling the effects of i) exposure to hydrodynamic conditions, ii) macroalgal community and iii) urbanization level, in driving *O.* cf. *ovata* bloom dynamics. A significant effect of hydrodynamics was observed only for cells in seawater, with higher abundances in sheltered zones, irrespective of urbanization level. Similarly, a significant effect of the dominant macroalgal community, with higher abundances in Corallinales and turf dominated communities, and lower in *Cystoseira amentacea* canopies, has been recorded, consistently in the differently urbanized sites. Lastly, we observed that stretches of coast suffering from a more intense anthropic exploitation are in general more prone to the proliferation of potentially toxic benthic microalgae. All these results imply a larger risk exposure to toxic effects for humans, being anthropized and sheltered areas usually more frequented by swimmers and bathers, and underline the need to preserve and eventually restore canopy dominated assemblages, which presently are under regression because of human threats, providing a straightforward example that restoration implies amelioration of human welfare.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-080

Fundamental study on the pratical application of eco-friendly materials for the removal of *Cochlodinium polykrikoides*

J.-W. Park [1,*]

[1]Researcher, National Institute of Fisheries Science, Busan, Republic of Korea

Abstract: HABs (Harmful Algal Blooms) have repeatedly occurred in the southern part of Korea since 1995. For the damage mitigation by HABs, application of natural yellow loess has been adapted in the field. However, the controversy over removal efficiency on HABs and ecological risk using natural yellow loess have been lasted in academic circles and the media. This study evaluated the approval of use for the several removal materials with removal efficiency and field adaptation. As the results, the four eco-friendly removal materials finally selected were applied to the government legislation of the Ministry of Oceans and Fisheries (Korea). Also, the field application manual of these four materials was prepared and disseminated to local governments and related organizations. The field application manual contained not only the field sprinkle methods and the usage of each materials but also the method to mix with natural yellow loess and selected material for considering the economical efficiency. In addition, marine ecosystem monitoring was carried out in the application area of removal materials on HABs in this study, and the quality standardization and management plan of new removal materials and yellow loess will be conducted hereafter.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-081

Mediterranean long-term study shows correlation between *Ostreopsis* cf. *ovata* bloom timing and spring temperature

K. Drouet [1,*], S. Gasparini [1], R. Siano [2], C. Jauzein [2], V. Davenet-Sbirrazuoli [3], E. Berdalet [4], R. Lemée [1]

[1]Sorbonne Université, Laboratoire d'Océanographie de Villefranche (UMR 7093), CNRS, Villefranche-sur-mer, [2]Ifremer, DYNECO Pelagos, Plouzané, France, [3]Direction de l'Environnement, Monaco, Monaco, [4]Institut de Ciències del Mar, Barcelona, Spain

Abstract: Benthic toxic dinoflagellate *Ostreopsis* cf. *ovata* events are responsible for ecological, sanitary and economic issues in different temperate areas and need better understanding. Every summer, from 2007 to 2017, *Ostreopsis* cf. *ovata* blooms have been monitored in Larvotto beach (Monaco, NW Mediterranean Sea), allowing for the achievement of one of the longest time series of *Ostreopsis* blooms worldwide. Five sites were sampled each year during the bloom period, from mid-June to end August, with collection of benthic cells.

Blooms phenology (timing, length and maximum cell abundance) was highly variable across the study period. Variations of net growth rate estimated during the phases of bloom development were analyzed as a function of Sea Surface Temperature (SST). Surprisingly, the highest growth rates were not associated with the maximal temperature records (27.5°C) but were estimated when temperature ranged between 21°C and 25°C. Many authors suggested that global warming might have influenced *Ostreopsis* expansion from tropical areas to temperate waters, such as the Mediterranean Sea. Current results do not directly support this hypothesis, but suggest a more complex role of temperature in bloom dynamics than a simple facilitation factor for algal growth, at least in temperate areas.

In order to characterize in more details the role of SST on *O.* cf. *ovata* bloom phenology, SST anomalies were calculated as differences between temperature values and the mean SST value over the 11 years of survey. A positive correlation (Spearman test, r_s= 0.766, $p < 0.01$) was observed between positive anomalies of SST and the bloom precocity. This result indicates that blooms seem to occur earlier in the season when spring is warmer. The relation between SST and net growth rate as well as between SST anomalies and precocity of the blooms should be of great interest for modelers. Predicting the distribution and the phenology of *Ostreopsis* in European coastal waters is a crucial challenge, especially in a context of global warming.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-082

Urea-induced toxic *Pseudo-nitzschia seriata* bloom in Danish waters

A. J. Olesen [1,*], S. Hardardottir [1 2], M. Moltke Lyngsgaard [3], P. Andersen [4], N. Lundholm [1]

[1]Natural History Museum of Denmark, University of Copenhagen, [2]The Geological Survey of Denmark and Greenland, Copenhagen, [3]Orbicon, [4]Niras, Aarhus, Denmark

Abstract: A bloom of *Pseudo-nitzschia seriata* heavily affected the mussel industry in otherwise productive areas in spring 2016 in Denmark. The bloom commenced approximately one month subsequent to a huge spill of urea to the environment. The magnitude of the outlet was >4000 tons liquid nitrogen fertilizer, of which the majority was urea. To give an idea of the relative significance of the outlet, this represents additional 93-132% of the average yearly land-based runoff of nitrogen in this area. The fertilizer spill was the result of a breakdown of an overloaded fertilizer storage silo located on the harbor of Fredericia. This massive amount of fertilizer was released to the environment in just a couple of hours. One month after the outlet, the monitoring data showed inorganic nitrogen at normal levels. Nevertheless, *P. seriata* produced domoic acid (DA) at levels measured in blue mussels (*Mytilus edulis*) up to 47 mg DA kg$_{-1}$. Densities of *P. seriata* peaked at 110.800 cells L$_{-1}$ (two weeks prior to the toxicity peak) which is a relatively low concentration in comparison to the magnitude of the toxicity. It has previously been shown that urea has the potential to heavily induce production of DA in some *Pseudo-nitzschia* species, but the effect of urea has not been examined in any North Atlantic *Pseudo-nitzschia* strains. The monitoring measurements did not include measures of organic nitrogen, thus urea might have been overseen as the potential trigger for an increase in DA production in *P. seriata*. This study wishes to present the potentials of urea as a trigger for DA induction in Danish *Pseudo-nitzschia* strains.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-083

Long term study of the environmental factors associated with the dynamics of the toxic *dinoflagellate Gymnodinium catenatum* in Uruguay

S. M. Mendez [1,*], O. Galli [2], W. Norbis [2]
[1]Phytoplankton Laboratory, [2]Fishery Biology, National Direction of Aquatic Resources, Montevideo, Uruguay

Abstract: The chain-forming dinoflagellate *Gymnodinium catenatum* is the main agent of Paralytic Shellfish Poisoning events in Uruguay. Existing records of 22 years (1991-2013) show that blooms of this species occur mainly in summer (January-March) at a regional scale (Northern Argentina to Southern Brazil) with its gravity centre (highest cell densities and shellfish toxicities) along the Uruguayan margins of the Rio de la Plata estuary. A Principal Component Analysis (PCA) of the phytoplankton counts from three selected locations of the Uruguayan harmful algae monitoring programme (period 1991-2013) and meterorological data (wind speed and direction from a local meteorological station and river outflowfrom the Instituto Nacional del Agua, Argentina) was carried out to study the effect of environmental conditions on the dynamics of *G. catenatum* blooms in Uruguay. *G. catenatum* thrived during years with positive sea surface temperature anomalies in summer; bloom densities above 3 x 10^4 cells · L^{-1} were recorded within a narrow temperature window (22 - 24ºC), but a wide range of salinity (16.8 to 32). Wind velocities above 35km h^{-1} and increased river outflow had a negative effect for *G. catenatum* growth mainly in the estuarine locations (Piriápolis and Punta del Este), whereas SW winds blowing to the coast caused accumulation of cells in the estuary and intensificacion of the PSP events.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-084

Distribution of marine phycotoxins along the Greenland west coast during summer 2017

B. Krock [1,*], J. Nieva [1], U. Tillmann [1]
[1]AWI, Bremerhaven, Germany

Abstract: From 25th July to 19th August 2017 an oceanographic expedition was performed along the west coast of Greenland up to 75 °N with special emphasis on Nuup Kangerlua (Gothaab Fjord, 64 °N) and Qeqertarsuup Tunua (Disko Bay, 69 °N) regions. During the expedition planktonic net tow (NT) samples were taken at 50 stations and analyzed for marine biotoxins. Among the detected toxins were paralytic shellfish poisoning (PSP) toxins, spirolides (SPX), pectenotoxin-2 (PTX-2), yessotoxin (YTX) and domoic acid (DA). Toxin levels were generally low, never exceeding 1 µg NT^{-1} except for total spirolides at three stations in Disko Bay. PSP toxin levels ranged from detection limit (LOD) to 340 ng NT^{-1} close to Maniitsoq (65 °N). The occurrence of PSP toxins was sporadic but highest values were detected in open coastal waters, whereas within Nuup Kangerlua and Qeqertarsuup Tunua total PSP abundance did not exceed 24 ng NT^{-1}. Besides the quantitative differences, open coastal waters profiles were dominated by gonyautoxins 1/4 (GTX1/4) and neosaxitoxin (NEO), whereas profiles of Nuup Kangerlua and Qeqertarsuup Tunua only contained GTX2/3 and saxitoxin (STX). SPX were the most abundant toxin class and were detected at almost all stations. Toxin profiles were very complex consisting of five known SPX (SPX-1, SPX-A, SPX-C, 20-methyl SPX-G, SPX-H) and six yet unreported SPX. SPX profiles were distinct in Nuup Kangerlua and Qeqertarsuup Tunua clearly demonstrating the presence of different populations. PTX-2 produced by several species of the genus *Dinophysis* was only detected in embayments and at low abundances up to 50 ng NT^{-1}, but not in open coastal waters. An exception was a high abundance of *Dinophysis* and PTX-2 in central Labrador Sea. YTX was sporadically detected at very low abundances and not restrained to any of the geographic regions. DA was found at almost all stations and showed highest abundances exceeding 300 ng NT^{-1}at the northernmost stations (75 °N).

Of the potentially YTX-producing species two strains of *Gonyaulax* sp. and seven strains of *Protoceratium reticulatum* were isolated from Nuup Kangerlua and Qeqertarsuup Tunua. Whereas *Gonyaulax sp.* did not contain any detectable YTX, all *P. reticulatum* strains produced YTX as major variant at a cellular level between 4.5 and 24 pg cell^{-1} with up to six yet unspecified YTX variants as trace components. Five strains of *Alexandrium catenella* were isolated from inner Nuup Kangerlua and six from inner Qeqertarsuup Tunua. Total cellular PSP toxin content was very variable among these strains and ranged from 0.7 to 55 pg cell^{-1}. Interestingly, PSP toxin profiles of strains isolated from Nuup Kangerlua were dominated by GTX2/3 and STX and strains isolated from inner Qeqertarsuup Tunua were dominated by GTX1/4 and NEO. None of the Greenland strains of *A. catenella* produced any *N*-sulfocarbamoyl toxins (B- and C-toxins).

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-085

Impact of global change (irradiance and temperature) on growth and ovatoxins production of *Ostreopsis* cf. *ovata*.

M.-P. Gémin [1,*], D. Réveillon [1], F. Hervé [1], S. Bertrand [2], V. Séchet [1], Z. Amzil [1]

[1]Ifremer - Laboratoire Phycotoxines, [2]EA 2160 Mer, Molecules, Santé, Nantes, France

Abstract: The benthic dinoflagellate *Ostreopsis* cf. *ovata* is a known producer of potent marine toxins such as palytoxin and its congeners the ovatoxins (-a to -l identified so far). For a decade, this species has spread in the Mediterranean Sea forming dense blooms on Italian, Spanish, and French coasts and more recently on Greek and Tunisian coasts. Blooms of *Ostreopsis* can induce intoxications (fever, water rhinorrhea, pharyngeal pain, dry or mildly productive cough, headache, nausea/vomiting, and bronchoconstriction) as it was observed on Genoa in 2005. The environmental factors responsible for the increasing incidence of these toxic blooms have not yet been determined even if global change has been pointed out. Some ecological scenario suggests an acidification of oceans, an increase of sea surface temperature and salinity, and a decrease of precipitation. In this context, we investigated the effect of temperature and light intensity on the growth of *Ostreopsis* cf. *ovata* and toxin production. Cultures were acclimated at three temperatures (23, 27 and 31 °C) and three different light intensities (200, 400 and 600 µmol m^{-2} s^{-1} in media) in L1 medium. Then, the growth and physiological state were monitored during 21 days by cell counting and fluorescence measurements (Fv/Fm). Nitrate and phosphate concentrations in the medium were measured at the end of exponential growth phase. Cells were harvested at days 7, 10, 14, 18, 21 and toxin content was quantified by targeted LC-MS/MS analysis. Therefore, those data should provide precise information about *Ostreopsis*-derived risk in the Mediterranean Sea in the context of global climate change.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-086

Cyanobacteria dynamics: Comparison of two different ecological modeling approaches

F. Piccioni [1,*], B. Vinçon-Leite [1], Y. Hong [1], C. Casenave [2], D. Plec [1], B. J. Lemaire [1], C. Li [1], M.-H. Le [3], N. Goutal [3]
[1]LEESU, Ecole des Ponts ParisTech, AgroParisTech, UPEC, Champs-sur-Marne, [2]UMR MISTEA, Univ Montpellier, INRA, Montpellier SupAgro, Montpellier, [3]LHSV, Ecole des Ponts ParisTech, CEREMA, EDF R et D, Chatou, France

Abstract: The accelerated changes in land use and human society during the last decades, and the consequent anthropogenic stresses applied to water resources, have led eutrophication to be one of the major freshwater environmental problems. At the same time, the unprecedented rate of climate warming contributed to an increase in frequency, intensity, and duration of cyanobacteria blooms.

For these reasons, an ever increasing interest is given by the scientific community to ecological modeling in order to give water quality managers reliable projections for decision making. Numerous ecological models based on various approaches arose in the last decades. However, modeling the dynamics of cyanobacteria in inland water bodies remains a challenging task. This is mostly due to the generalized lack of suitable data, crucial to calibrate and validate model results as well as to keep improving the formulations of models themselves.

We analyze here the performances of two different approaches in the modeling of cyanobacteria dynamics by confronting model results to field data. The first ecological model is Aquatic EcoDynamics (AED), a customizable modular model library based on the conceptualization of biogeochemical cycles and limitations in growth rates for numerous phytoplankton functional types in the classic form of ordinary differential equations [1]. The second one is the BLOOM module of the Delft3D modeling suite [2]. It also involves nutrients cycles and growth rates in its formulation, but relies on a competition principle that is able to choose, at every time step and each grid point, the best adapted phytoplankton functional type consistently with the available resources and the existing biomass level. Both ecological models are coupled to three-dimensional hydrodynamic models, Telemac3D and Delft3D-Flow respectively.

The study site, Lake Champs-sur-Marne, is a small and shallow urban lake (area 0.12 km², average depth 2.3 m), located in the metropolitan area of Paris and used for bathing and sailing. It suffers from repeated severe blooms of cyanobacteria from spring to fall and since 2015 it has been more regularly monitored with high frequency measurements and bi-weekly surveys.

Simulations of both ecological models have been carried out over three consecutive summers during critical blooming periods. The modeling results on each blooming period have been compared to the collected data from Lake Champs in order to evaluate the reliability of their different formulations in the context of future projections under climate change scenarios.

[1] M. R. Hipsey, L. C. Bruce, and D. P. Hamilton, *Aquatic Ecodynamics (AED) Model Library. Science Manual*. 2013.
[2] Deltares, *Delft3D-FLOW - Simulation of multi-dimensional hydrodynamic flows and transport phenomena, including sediments. User Manual Version: 3.15.30059*. Delft: Delt Hydraulics, 2013.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-087

Occurrence of bloom-forming *Microcystis* sp. in an oligotrophic North Patagonian lake of Chile

N. N. Fuentes [1], P. A. Díaz [2,*], C. Ríos [1]
[1]Laboratorio de Limnología, Departamento de Acuicultura y Recursos Agroalimentarios, Universidad de Los Lagos, Casilla 933, Osorno, [2]Centro i-mar, Universidad de Los Lagos, Casilla 557, Puerto Montt, Chile

Abstract: Demographic growth, together with a concept of development whose economic-productive dimension does underestimates, the associated environmental and social costs has generated drastic change and the environmental degradation of continental aquatic ecosystems over the last few decades. In response to this situation cyanobacterial harmful blooms (mainly *Microcystis* blooms) are more recurrent. In Chile, toxic blooms of cyanobacteria have been reported in lakes with high levels of nitrogen and phosphorus, coinciding with the highest temperature during the summer. Nevertheless, in recent years these blooms have also been reported in oligotrophic north Patagonian lakes. To evaluate temporary changes of the microalgae communities of Rupanco Lake (40.8°S - 72.5°W), we obtained monthly samples from March 2016 to February 2018 at different fixed depths (2-5-10-15- 30 m), from the deepest zone of the Lake (maximum depth: 274 m). This North-Patagonian lake is classified as warm monomictic (summer temperature: 16–22°C) and oligotrophic (dissolved inorganic nitrogen: <0.1–3 mmol L^{-1}; soluble reactive phosphorous: <0.1–0.4 mmol L^{-1}). Microalgae analysis showed the presence of the bloom-forming cyanobacteria *Nostoc* sp., during spring-summer associated with stratified conditions. Nevertheless, in June 2017 (austral winter), during periods of mixing of the water column (high turbulence), low temperature (11±0.1°C), high wind speed (16.9 km h^{-1}, annual maxima) and intense daily precipitations (51.1 mm day^{-1}) a bloom of *Microcystis* sp. (667 cells L^{-1}) was observed at 30 m depth. Considering that Rupanco Lake is nitrogen-limited, the effects of the global warming and changes in local hydrological conditions over this *Microcystis* bloom are widely discussed.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-088

The effect of temperature on physiology and toxicity of the benthic dinoflagellates *Coolia* spp. isolated from Hong Kong waters

M. Yan [1 2,*], P. T. Leung [1 2], J. Gu [2 3], X. Li [2 3], V. T. Lam [2], T.-C. Wai [1 2], S. K. Yiu [2], P. K. Lam [1 2 3]

[1]Research Centre for the Oceans and Human Health, City University of Hong Kong Shenzhen Research Institute, Shenzhen, [2]State Key Laboratory in Marine Pollution, [3]Department of Chemistry, City University of Hong Kong, Hong Kong, China

Abstract: The emerging threat of benthic and epiphytic toxic algae (BETA) to marine ecosystems has attracted worldwide attention. Seawater temperature is one of the major factors influencing the growth and distribution of BETA. *Coolia* spp. are one of the cosmopolitan group of BETA, and are potentially toxic. In this study, we aimed to elucidate the effect of different seawater temperatures on physiological performance, toxicity, toxin production and molecular responses of *C. malayensis* (a toxic species) and *C. canariensis* (a less toxic species). *Coolia* monocultures were exposed to 7 temperatures i.e., 16, 18, 20, 22, 24, 26 and 28°C for more than 5 generations before sampled for growth curves determination, photosynthesis efficiency, phaeo-pigments amount measurement, bioassay, toxin analysis and gene expression. Our results showed that the optimal temperature for growth of *C. malayensis* was 24°C while its photosynthesis was steady across the range of temperatures. The growth rate of *C. canariensis* was highest at 20°C. Lower temperatures, i.e., 16 and 18°C inhabited its growth and photosynthesis. The amount of phaeo-pigments (in 1×10^5 cells) reached the highest at 26°C for both *C. malayensis* (3.06 µg/ml) and *C. canariensis* (15.61 µg/ml), but the level reached the lowest at 20°C for *C. malayensis* (0.90 µg/ml) and at 16°C for *C. canariensis* (2.76 µg/ml). The bioassay with *Artemia* larvae showed that the 48 h LC_{50} values of *C. malayensis* decreased as temperature increased. The current results suggested that *Coolia* may produce more pigments and toxins as temperatures increased. Our preliminary analytic analysis showed that C. *malayensis* produces okadiac acid (OA) and dinophysistoxin 1 (DTX1). With more upcoming results on toxin analysis and transcriptome profiles, we could have gain further insight on possible mechanisms that would associate with different toxicities of *Coolia* species due to global changes of seawater temperature.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-089

Alexandrium species, harmful or environmentally friendly?

A. Caruana [1,*], M. Le Gac [2], S. Geffroy [1], F. Hervé [1], G.-A. Rovillon [1], F. Malo [1], Z. Amzil [1]
[1]Phycotoxin Laboratory, IFREMER , Nantes, [2]PELAGOS Laboratory, IFREMER, Plouzané, France

Abstract: The dinoflagellate genus *Alexandrium* is well-known for its toxic species. These toxic species are able to produce a complex and variable set of saxitoxin derivatives (STXs). Besides their toxicity, *Alexandrium* species are also able to produce DMSP, a compound that contributes to climate regulation and is the major natural source of sulfur. French coasts are frequently invaded by toxic blooms of *Alexandrium minutum* in Brittany and *A. catenella* (also named *A. pacificum*) on the Mediterranean side, both species showing different toxin profiles. This is the first study on the DMSP content of *Alexandrium* French strains in culture. Our results show that both species are able to produce DMSP and in particular that *A. catenella* is a new DMSP-producing species of dinoflagellate. We also present new challenges and investigations on DMSP pathways in *Alexandrium*. Research for proposed key genes in a transcriptomic database of 18 *A. minutum* strains revealed that homologues to diatom and coral key genes are absent. However, homologues to the bacterial *dsyB* key gene that were recently identified in some dinoflagellates are also present in *A. minutum*. These results suggest that *Alexandrium* cells might use the “algal” DMSP biosynthesis pathway. Finally, concentrations of STXs are reduced at higher salinity while DMSP, as an osmolyte, is expected to increase suggesting a tradeoff regarding how the methionine precursor of these two compounds is used.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-090

Long term pattern of toxic phytoplankton species occurrence along the Eastern Adriatic

Ž. Ninčević Gladan [1,*], J. Arapov [1], S. Skejić [1], M. Bužančić [1], B. Grbec [1], A. Bakrač [1], M. Straka [1]
[1]Institute of Oceanography and Fisheries, Split, Croatia

Abstract: Time series data set of toxic phytoplankton species occurrence collected through the National monitoring program in a period from 2000 to 2017 along the eastern part of Adriatic Sea are analyzed in relation to environmental parameters (temperature, salinity) and changes in the phase of the North Atlantic Oscillation (NAO). The sequential t-test analysis of regime shift (STARS) method and locally-weighted scatter plot smoothing (LOWESS) method were applied to detect inter-annual variability in toxic species occurrence. The *Dinophysis* species were related to the NAO, temperature and salinity fluctuations. Low NAO winter index is associated with earlier *Dinophysis caudata* bloom occurrence. The influence of the NAO oscillation on temperature and salinity of the water column was different depending on the season and hydro-morphological characteristic of area. Significant correlation were recorded between NAO index with surface salinity throughout the year and in the seasons. Significant positive correlation between abundance of *Dinophysis caudata* and temperature at Istrian area is recorded and negative correlation between the abundances of *Dinophysis acuta* and *Alexandrium* species with temperature and salinity in the Šibenik Bay.

Although the majority of the recorded toxic phytoplankton species are determined in all monitoring areas, significant difference were recorded at spatial distribution. Šibenik Bay is separated from other areas due to *Prorocentrum cordatum* and *Dinophysis acuta* occurrence as well as more frequently occurrence of *Alexandrium* species.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-091

Climate changes effect on Algal Organic Matter and MC-LR production: lab culture experiments and in-situ monitoring

H. THURET BENOIST [1,*], G. FEUILLADE-CATHALIFAUD [1], V. PALLIER [1]
[1]PEIRENE, Université de Limoges, 87000 Limoges, France

Abstract: Eutrophication is a natural process describes as ponds' enrichment with nutrients conducting to their filling. Excessive inputs of nitrogen (N) and phosphorus (P) accelerate this phenomenon as the phytoplankton is no longer limited by N and P concentrations and can thus significantly proliferate. Their proliferations affect the aquatic life, the recreational activities and the water treatment processes and climate changes magnify the symptoms. Air temperature (T°C) rise increases water T°C and extends the stratification period, higher frequency and severity of storms increase runoff and N, P inputs and drought increases water residence time. T°C rise effect on cyanobacteria development and cyanotoxins production have already been studied and the evolution of AOM characteristics during eutrophication is now well known. Phytoplankton development increases Natural Organic Matter (NOM) quantity ($DOC_{oligotrophic\ pond}$=2ppm–$DOC_{eutrophic\ pond}$=10ppm) and modifies its characteristics. Algal Organic Matter (AOM) is less hydrophobic and aromatic than NOM ($SUVA_{Pigeard,winter2012}$=42L/cm/gC-$SUVA_{Pigeard,summer2012}$=28L/cm/gC). However, none study deals with the impact of climate changes on AOM characteristics and its interactions with toxins.

Climate changes' impact on both NOM modification and cyanotoxins production was studied in controlled conditions on lab cultures of *C.vulgaris* (CV) and *M.aeruginosa* (MA) and by *on-site* monitoring. Rainfall patterns reproducing drought, runoff and storm were simulated under 23, 15 and 5°C (average T°C recorded on the monitored ponds). Specific absorbance measurements and cells counting provided culture growth phases. OM was quantified by measuring DOC content and characterized by fractionation according to hydrophobicity and SUVA index. MC-LR concentration was analyzed by HPLC-UV/PDA.

Both T°C and rainfall did not affect significantly CV's growth. T°C drop only increased latency period: 2-days, 4-days and 12-days lag phases were observed at 23, 15 and 5°C respectively. On the contrary, MA's growth was highly impacted by a decrease of T°C to 15°C (at day 50, $Abs_{750,23°C}\approx0.85$-$Abs_{750,15°C}\approx0.51$) and did not start at 5°C. This T°C drop reduced AOM content (day 50, $COD_{23°C}\approx$28ppm–$COD_{15°C}\approx$11ppm) but increased hydrophilic fraction (HPI) (day 50, $HPI_{23°C}\approx$52%–$HPI_{15°C}\approx$72%). Rainfall did not affect significantly MA's growth and OM characteristics.

In Pigeard pond (France), when the water T°C increased, the phytoplankton density reached 2.10^6cells/mL with 99% of filamentous cyanobacteria. Both DOC content and HPI percentage subsequently increased (HPI_{summer}=60%>HPI_{winter}=37%) and SUVA index decreased. An average total concentration of MC-LR of 7µg/L (96% of intracellular) was quantified in 2017 during hot period unlike winter. OM characteristics and MC-LR production will be correlated to climate data to evaluate its impact.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-092

Adaptive response to global warming detected in a life cycle trait of revived Baltic Sea dinoflagellates

J. Hinners [1], I. Hense [1], A. Kremp [2,*]

[1]Institute for Marine Ecosystem and Fishery Science, University of Hamburg, Hamburg, Germany, [2]Leibnitz Institute for Baltic Sea Research Warnemuende, Roshtock, Germany

Abstract: Over the past century, the Gulf of Finland in the Northern Baltic Sea has already warmed by 3°C. To investigate if and how phytoplankton responded to this severe increase in temperature, we revived resting cysts of the dinoflagellate *Apocalathium malmogiense* from 100 year old and recent sediment layers. In laboratory experiments we compared temperature-dependent traits of historic and recent strains. The only difference we detected concerns a life cycle trait: The rate of resting cyst formation in recent strains was significantly lower compared to historic strains. Based on these experimental data, we developed a life-cycle-ecosystem model for the dinoflagellate *A. malmogiense*, which allows for evolutionary adaptation. Using this advanced ecosystem model we support the hypothesis that the observed changes in resting cyst formation, by affecting the phenology of *A. malmogiense*, can indeed represent an adaptive response to global warming. Here, we present results of both resurrection experiments and ecosystem modeling including evolutionary adaptation. The combination of these two approaches represents a powerful tool, which can be used further for analyses on the evolution of HABs.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-093

Occurrence and spatial distribution of typical lipophilic algal toxins in seawater of Pearl River Estuary and Hong Kong Waters, China

X. Li [1 2,*], Y.-L. Mak [1 3], M. Yan [1 3], P. T. Leung [1 3], J. Li [3], P. K. Lam [1 2 3]

[1]State Key Laboratory in Marine Pollution (SKLMP), [2]Department of Chemistry, City University of Hong Kong, Hong Kong, [3]Research Centre for the Oceans and Human Health, City University of Hong Kong Shenzhen Research Institute, Shenzhen, China

Abstract: Toxic algae are capable of producing toxins that can cause fish kills or severe intoxication in humans following consumption of contaminated seafood. They are viewed as an emerging threat to marine ecosystem. Previously, toxic algae that can produce lipophilic algal toxins were found in HK waters, but the information on the toxin levels in environmental samples, e.g. seawater and sediments, is largely unknown. This study aimed to investigate the distribution of typical lipophilic algal toxins in seawater samples collected from Pearl River Estuary and Hong Kong Waters, and to discuss the relationships between salinity and temperature on their toxin production. According to the analytical results, 5 types of lipophilic algal toxins (OA, DTX1, YTX, PTX2, GYMA) and putative CTXs were detected in seawater samples, and the distribution patterns varied among different toxins. The toxins were mainly detected in areas with salinity between 20-30‰ and temperature 24-26°C. This study suggests that the spatial distribution of lipophilic algal toxins in seawater may be related to environmental conditions. The effects of temperature and salinity on the growth of and toxin production by benthic and epiphytic toxic algae were investigated under laboratory conditions. Upcoming results of these laboratory experiments will be discussed as well.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-094

A 40 year dataset on the species composition and ecology of the genus *Dinophysis* in the open waters of the northern Adriatic

D. Maric Pfannkuchen [1,*], A. Baricevic [1], M. Smodlaka Tankovic [1], N. Kuzat [1], M. Pfannkuchen [1]
[1]Center for marine research, Rudjer Boskovic Institute, Rovinj, Croatia

Abstract: The marine dinoflagellate genus *Dinophysis* is an important component of HAB blooms in coastal ecosystems worldwide, however the knowledge on the ecology and species composition of this genus in the open waters of the Adriatic Sea is limited. Annual dynamics, seasonality and ecological characteristics of the genus *Dinophysis* spp. associated with shellfish toxicity were studied before but only in coastal areas where shellfish farms are present (Ninčević Gladan, 2008). Our aim was to analyze the *Dinophysis* species composition on a spatial temporal scale across the NA basin and look into details over the last four decades to see if there were some changes in species composition, abundances, frequency of appearance and contribution to the phytoplankton community over last 40 years. We looked at *Dinophysis* distribution over 46 stations spread over the whole northern Adriatic in more that 10000 samples. To get clear data on the vertical distribution, Niskin samples in 5 depths at each sampling station were analyzed. *Dinophysis* spp. was found in approximately 1800 times.

According to the checklist from 2002 there are 24 species from this genus in the Northern Adriatic. In our dataset we found 20 of them. Twelve species were very rare (*e.g. D. hastata*, *D. parvula, D. schroederi, D. sphaerica…*) while 6 species were significantly more frequent. The frequency of appearance for most frequently found species were: *D. sacculus* (3.8 %), *D. caudata* (3.75 %), *D. fortii* (2.65 %), *D. ovum* (1.3 %) and *D. tripos* (0.93%). Those species were found to be the most abundant as well. Maximal abundances were recorded during 1996 for *D. fortii* (8.8 x10^3 cells l^{-1}) and *D. caudata* (7.4 x10^3 cells l^{-1}) at stations at western part of the basin. While *D. ovum* showed maximal abundances in the offshore waters in the middle of the basin. In the 1970s *D. ovum* bloomed in May while in the 1980s the bloom switched to July. Similar behavior was observed also for *D. sacculus*. We also observed that *D. caudata* and *D. fortii* reached their maximum in late summer months while *D. tripos* was present from autum until January.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-095

Effect of Climate Change and *Cochlodinium* bloom in Korean coast

W. Lim [1,*], J. Park [1], T. Park [2], M. Son [3], G. Kim [4]

[1]Ocean climates & Ecology Research Divison , NIFS, Busan, [2]researcher, NIFS, Tongyeong , [3]researcher, NIFS, Yeosu, [4]School of Earth & Environmental Sciences, Seoul National University , Seoul, Republic of Korea

Abstract: *Cochlodinium polykrikoides* blooms in the Korean coastal waters have occurred for more than 20 years since 1995 and continue to cause damage to fisheries. In order to reduce fisheries damage caused by *C. polykrikoides* blooms, we have periodically investigated marine environmental factors and phytoplankton composition, as well as used real-time PCR to analyze free living cells and cyst of *C. polykrikoides*. Since 1995, surveys of *C. polykrikoides* appearance have been conducted through microscopic observation, and real-time PCR has been also used since 2014. The results allow us to predict the occurrence of *C. polykrikoides* blooms and give us more than a week to respond to the *C. polykrikoides* blooms. In addition, we could establish the three-stage Red Tide Alert System in 2014 by adding the preliminary warning stage to the previous Alert System in Korea. This result shows that the major mechanism of *C. polykrikoides* blooms is the invaded free living cells from the offshore water (Tsushima warm current). The influence of the Tsushima Warm Current with *C. polykrikoides* cells and the south wind are regarded as the main causes of *C. polykrikoides* blooms in the Korean coastal water. Diatoms dominate when rainfall increases the concentration of Dissolved Inorganic Matters(DIM) in summer, followed by (in a consecutive order) diatom increase, nutrient depletion and finally diatom depletion. Consequently, the concentrations of the DIM increase, resulting in the creation of a suitable environment for the growth of *C. polykrikoides* and the outbreaks of *C. polykrikoides* blooms (Kwon et al., 2002). In recent two years, *C. polykrikoides* blooms did not occur because the water temperature for the two years was 3-5 degrees Celsius higher than the average temperature, which is not suitable for the growth of *C. polykrikoides*. In addition, the constant east wind prevented the *C. polykrikoide* cells from accumulating on the coast, resulting in a low cell density (<1 - 20 cells/mL). In general, most of the *C. polykrikoides* seed population is carried by the Tsushima Warm Current. However, the super-strong Tsushima Warm Current could not flow into the southern coastal water, but through the Korean Straits. As a result, diatoms dominate due to the non-occurrence of *Cochlodinium*, as well as *Karenia mikimotoi, Chattonella*, and *Alexandrium affine* blooms occur.

Although the exceptionally high water temperature for the two years is not direct evidence of climate change, if the water temperatures are often high, it is highly likely to result in succession of HABs species.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-096

Impact of agro-industrial residues on the physico-chemical quality and phytoplankton diversity of some rivers of Cameroon

M. P. M. Banga Medjo [1,*], S. H. Zébazé Togouet [1]

[1]Biology, University of Yaounde 1, Yaounde, Cameroon

Abstract: Among phytoplankton's biological communities, phytoplankton occupy a prominent place. Indeed, it consists of all plant microorganisms suspended in water, able of developing photosynthesis their own organic substance, and is therefore the base of the trophic chain. These characteristics make this group an important link in the aquatic food chain. A possible imbalance of intrinsic origin (grazing pressure for example) and / or extrinsic due to an imbalance in the control of the nutritive resources can have a great impact on the quality of the water and lead to a modification of the structure of the biological communities subservient these water favor the proliferation of certain algal species known as efflorescence. These organism can have many health, ecological and economic consequences. This is the case of some species of cyanobacteria, which are likely to synthesize toxins causing more or less serious intoxications, representing significant risks to human and animal health. Thus, a good knowledge of the existing plant plankton would allow an assessment of the quality of water and its value for the production of drinking water.

In Cameroon, there is little research on phytoplankton organisms. Indeed some data are available on the diatoma some streams of the city of Yaounde (Ebang, 2016) and on the phytoplankton community of some lakes. These include those of Kemka (2000) on the Lake Municipal. Their work have revealed the immense diversity of the phytoplankton community. For better management of these hydrosystems, a study to evaluate the impact of agricultural and industrial residues on the physico-chemical quality and phytoplankton diversity of some rivers located in an agro-industrial zone (40 thousand ha) has been conducted for 15 months (January 2016 to March 2017) in central Cameroon.

These rivers feed Cameroon's longest river, thus crossing the agro-industrial zone which will allow drinking water to be rehabilitated in the Yaounde capital and the surrounding areas by the PAEPYS project.

Some identifications were carried out on the phytoplankton samples collected and showed a very great diversity of phylum cyanobacteria and diatoma. In add, the determination of toxic algae in these environments for a better evaluation of the type of toxins to study.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-097

Effects of lower pH on growth rate and toxin production in *Alexandrium catenella* cultures from southern Chile: Preliminary studies.

M. Frangopulos [1 2,*], G. Pizarro [3], M. Pinto [2 4], E. Iglesias [1], V. Sandoval [1], R. Torres [2 5], J. Iriarte [4 6 7 8]

[1]Centro de Investigación GAIA Antártica (CIGA), Universidad de Magallanes, [2]Centro FONDAP de Investigación Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL), [3]Centro de Estudios de Algas Nocivas (CREAN) grupo Magallanes, Instituto de Fomento Pesquero (IFOP), Punta Arenas, [4]Escuela de graduados, Universidad Austral de Chile, Puerto Montt, [5]Centro de Investigación en Ecosistemas de la Patagonia (CIEP), Coyhaique, [6]Centro FONDAP de Investigación Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL), Valdivia, [7]Centro COPAS Sur Austral, Universidad de Concepción, Concepción , [8]Programa de Doctorado en Ciencias de la Acuicultura, Universidad Austral de Chile, Puerto Montt, Chile

Abstract: In the last decades, climate change has been one of the key pieces in the increase of the frequency, intensity, spatial-temporal distribution and composition of the Harmful Algal Blooms (HABs) worldwide, affecting the physiology and toxicity of the microalgae involved in these outbreaks. The fjords and channels ecosystem from southern Chile (48-56ºS) has historically presented blooms of toxic species that produce a wide variety of toxins (PSP, DSP, ASP, among others), becoming a very vulnerable system to environmental changes. We present here preliminary studies of the potential effect of future increases in carbon dioxide ($p\mathrm{CO}_2$) on the growth rate and toxin production of the PST producer *Alexandrium catenella*. Batch cultures of *A. catenella* (strain M7 isolated from isla Crossover, 49ºS) were grown in short-term experiments under the combination of 2 $p\mathrm{CO}_2$ levels (415 and 1200 ppm) and pH ranges from 7.31 to 8.01, comparable values to present days and supposed future levels, respectively. We observed differences between the treatments on the growth rate and toxin production, with effects of both $p\mathrm{CO}_2$ and pH on the cultures. Our data showed a relationship between the specific growth rate and the impact of $p\mathrm{CO}_2$ on growth, were the *A. catenella* cultures were positively affected to increased $p\mathrm{CO}_2$. The toxin composition was also altered by the increased $p\mathrm{CO}_2$ supply, resulting in a promotion of PST production. This is the first evidence that toxin production by a sub Antarctic strain of *A. catenella* is sensitive to changes in atmospheric $p\mathrm{CO}_2$ levels. Further studies will be necessary to assess whether other strains of *A. catenella* and others toxic dinoflagellates species from southern Chile has similar responses to this climate stressors; the eventual consequences of an expansion of the blooms and increase in toxicity under increased $p\mathrm{CO}_2$, for the fjords and channels system of southern Chile, are also discussed.

Financial Support: Fondecyt 1140184, Fondecyt 1170174, FONDAP IDEAL 15150003

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-098

Diversity of cyanobacteria and their potential to produce toxins in microbial mats of the wetlands in cold desert of the Eastern Pamir.

I. Jasser [1,*], N. Khomutovska [2], M. Sandzewicz [3], L. Lach [4], M. Suska-Malawska [2], J. Kwiatowski [5] and Ecology and Toxicology of Cyanobacteria

[1]Plant Ecology and Environmental Conservation, University of Warsaw, Warsaw, [2]Plant Ecology and Environmental Conservation, [3]Plant Ecology and Environmental Protection, [4]Plant Ecology and Environmental Biotechnology, [5]Molecular Phylogenetic and Evolution, University of Warsaw, Warszawa, Poland

Abstract: The importance of arid areas increases globally as the temperature rises due to climate changes. Such areas are characterized by low precipitation, scarcity of water and often high salinity of soil and water. However, when not saline, these water bodies are frequently used as a source of drinking water by humans and farm animals. The question thus arises whether they are safe to use. We collected microbial mats from over 30 small water bodies in wetland in cold desert of Eastern Pamir Mountains in July 2017. These water bodies – little ponds, springs, kettles and pools – differed in salinity and temperature, as well as in microbial mats overgrowing their bottoms, edges and sometimes even surfaces. The study shows that abundance and variability of microbial mats in wetlands of cold Eastern Pamir desert exceeds by far those from other arid environments. The mats represent various morphological types, from thin and deliquescent, emerald in color, through leathery-like, to spectacular, thick and multilayer colorful forms. Morphological analyses showed that, besides diatoms and green algae, Nostoc, Trichormus, Calothrix, Nodularia, Phormidium, Microcoleus, Geitlerinema, Oscillatoria and Leptolyngbya were building these mats. The identified cyanobacteria belong to genera known for producing hepatotoxins and/or neurotoxins. However – similarly to other cyanobacteria communities – microcystins and microcystin encoding genes were the most common, although we found also a few samples of anatoxin and saxitoxin coding genes. We tested four sets of 16S rRNA gene primers for the study of cyanobacteria diversity in microbial mats by amplicon metagenomic analysis. The study shows that primers targeting v5-v7 region are the most promising for such analyses.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-099

Harmful Algal Bloom and Fish Kill Events in Singapore Coastal Waters

S. C. Y. Leong [1,*]

[1]Tropical Marine Science Institute, National University of Singapore, Singapore, Singapore

Abstract: Singapore coastal environments are increasingly affected by regular harmful algal blooms (HABs). The increase in frequencies of HABs have led to enhanced interest in monitoring and detecting of such blooms. In December 2009, a massive fish kill was observed in Singapore water along the Johor Strait due to HABs caused by toxic dinoflagellates. In Feb 2014, another massive fish kill was observed due to *Karlodinium* spp. In the year 2015, two major blooms were observed, the first bloom was caused by dinoflagellate *Karlodinium* spp. which caused massive fish kills and economic losses. The second bloom was caused by *Karenia mikimotoi*, however, no fish kill was observed. In 2016, prolonged *Karenia mikimotoi* blooms of more than three months were observed. Blooms were generally formed during the neap tide and under favorable physical settings. The eutrophic conditions could be the driving force for bloom to be sustained along Johor Strait. Moreover, the variability of phytoplankton abundance in this area was significantly driven by nutrient and rainfall.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-100

Morphological, ecological and toxicological aspects of *Cylindrospermopsis raciborskii* in a eutrophic urban lake in southern Brazil

H. D. Laughinghouse [1,*], A. Tucci [2], D. Berthold [3], L. Silva [4], J. Yunes [5], V. Werner [4]

[1]Ft. Lauderdale Research and Education Center, University of Florida, Davie, FL, USA, [2]Phycology, Institute of Botany, Sao Paulo, SP, Brazil, [3]Fort Lauderdale Research and Education Center, University of Florida, Davie, FL, USA, [4]Natural Sciences Museum, Zoobotanical Foundation of Rio Grande do Sul, Porto Alegre, RS, [5]Research Unit on Cyanobacteria, Federal University Foundation of Rio Grande, Rio Grande, RS, Brazil

Abstract: Agricultural and urban eutrophication is expanding with the growth in human population and changes in land use. With pervasive nutrients in our waters and changes in climate, toxic cyanobacterial blooms have intensified worldwide and are found ubiquitously in aquatic environments. Furthermore, certain cyanobacterial taxa have increased their occurrence and distribution and are now considered invasive in some areas. One of these taxa is the toxigenic *Cylindrospermopsis raciborskii*, which we observed in an urban artificial lake in southern Brazil (29°53’13”S & 51°09’29.9”W). In this study we morphologically analyze *C. raciborskii* and verify its spatio-temporal variation in order to identify the main factors for its development. Bloom toxicity was also analyzed. Monthly samplings from November-2009 to November-2010 were carried out at 2 locations in the lake: water inlet and outlet. *Cylindrospermopsis raciborskii* was found in all samples, dominating 77% of the samples, with highest densities at the end of summer (March: 199,550 ind. mL^{-1} at 95.3 % of the community total) and mid-autumn (May: 169,191 ind. mL^{-1} at 98.8 % of the community total). The lowest cell concentrations were found mid-summer (December: 482 and 737 ind. mL^{-1}, at the inlet and outlet, respectively), when diatoms dominated the community structure. Saxitoxins and gonyautoxins were found and interrelations with abiotic and biotic factors are discussed.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-101

Investigation of germination characteristics of Lingulodinium polyedra cysts from Loch Creran, Scotland

C. Winterton [1,*], J. Lewis [2], K. Davidson [3]

[1]GeoChem & Microbiology , SAMS, Oban, [2]University of Westminster, London, [3]Microbiology, SAMS, Oban, United Kingdom

Abstract: *Lingulodinium polyedrum*, is a dinoflagellate species, which, in some parts of the world, forms “red tides”. In common with other dinoflagellates, as part of its sexual life cycle, *L. polyedra* forms resistant cysts that accumulate in the sediments. The germination of these cysts results in the inoculation of overlying waters with motile cells in subsequent years. So a detailed understanding of the factors controlling cyst germination is key to improving our understanding of *L.polyedra* ecology. Laboratory studies have led to knowledge of general germination requirements for this species, including temperature, oxygen and light requirements, but nothing is known about the drivers of dormancy or quiescence in this species. In contrast, more is known for *Alexandrium catenella*, from the east coast of the United States, which is described as having an endogenous circannual rhythm controlling dormancy cycling. Recent experiments have illustrated the importance of temperature in controlling *A. catenella* patterns of dormancy and quiescence, in particular the role of ‘cold conditioning’ in entraining the breaking of dormancy. *L. polyedra* is commonly found in Loch Creran, on the west coast of Scotland where its ecology has been described and so the loch represents an ideal situation for further investigation of this important species. This study aims to test two hypotheses:
a) *L.polyedra* cysts from Loch Creran have an endogenous circannual system controlling dormancy and
b) *L.polyedra* cysts from Loch Creran have a chilling requirement for breaking dormancy.

At the end of October 2017, after the annual recruitment of cysts to the sediment had completed, sediment was collected from Loch Creran by Craib coring. The surface (0-3cm) of 18 cores was combined, and then portioned into 2ml vials under red light at 11°C. The vials are kept in sediment-filled, airtight containers in the dark in three treatments: 4°C, 8°C and 12°C. For the 4°C treatment, ~40 cysts are isolated from the sediment every two weeks and incubated at 20°C. Germination is monitored weekly over 4 weeks. For the other two treatments isolations are carried out monthly in a similar fashion. Initial results suggest that *L. polyedra* may have an endogenous mechanism controlling dormancy, with all treatments showing a substantial increase in quiescence after 14 weeks of storage. Results so far also suggest that there may not be a chilling requirement for breaking dormancy. As the experiment progresses, other patterns may emerge.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-102

EMERTOX - Emergent Marine Toxins in the North Atlantic and Mediterranean

V. Vasconcelos [1,*], L. Botana [2], B. Brkic [3], A. Tubaro [4], C. Elliot [5], L. Medlin [6], P. Hess [7], M. Weber [8], W. Medhioub [9], S. Mynott [10], T. Ocelka [11], G. Mengs [12], J. Neves [13], B. Sabour [14]

[1]Centro Interdisciplinar de Investigação Marinha e Ambiental and University of Porto, Porto, Portugal, [2]Universidade de Santiago de Compostela, Facultad de Veterinaria, Lugo, Spain, [3]Q Technologies LTD, Liverpool, United Kingdom, [4]Universita Degli Studi di Trieste, Trieste, Italy, [5]The Queen's University of Belfast, Belfast, [6]Marine Biological Association of the United Kingdom, Plymouth, United Kingdom, [7] Ifremer, Nantes, France, [8]Estação Litoral da Aguda, Águas de Gaia EM, Vila Nova de Gaia, Portugal, [9]Institut National des Sciences et Technologies de la Mer, Tunis, Tunisia, [10]Applied Genomics Ltd,, Brixham, United Kingdom, [11]E&H Services, Praha, Czech Republic, [12]Natural Biotech SL, Madris, Spain, [13]Universidade de Cabo Verde, Mindelo, Cape Verde, [14]Universite Chouaib Doukkali, El Jadida, Morocco

Abstract: Episodes of human poisoning caused by Harmful Algal Blooms have been commonly recorded in the last century mostly because of the lack of regular monitoring programs. Today, the cases of human poisonings are sporadic, usually because of violations of national health authorities' regulations imposing the closure of harvesting areas and seafood commercialization. Nevertheless, the occurrence of emergent toxins and the respective producing organism in the North Atlantic and Mediterranean, such as tetrodotoxins, ciguatoxins, palytoxins and a diversity of congeners, is being increasingly reported either by scientific studies or by events of human intoxications. The project EMERTOX - Emergent Marine Toxins in the North Atlantic and Mediterranean: New Approaches to Assess their Occurrence and Future Scenarios in the Framework of Global Environmental Changes, funded by H2020 under the RISE program, will create a robust and sustainable network of experts with excellent complementary competencies on marine algal toxins and the detection of the organisms producing these toxins. The network will collaborate not only with national authorities but also with European ones, such as the European Food Safety Authority (EFSA), for the assessment and management of risks associated to emerging toxins and the species that produce them. Current risks assessment relating to emerging harmful algae and predicting future scenarios will be fundamental for EFSA, which will recommend whether these emerging toxins should be monitored in Europe, and for the development appropriate strategies to protect human health. EMERTOX - aims to map the actual situation in emergent marine toxins and the producing organisms, develop new approaches to assess their occurrence and predict the possible future scenarios in the framework of global warming. The consortium, formed by a multidisciplinary team, will produce a joint research and innovation project that will exploit the complementary expertise of the participants and will create synergies among them. The main objectives are: i) to assess the current situation on potentially harmful algae and bacteria and the relevant emerging toxins in 9 countries belonging to different but geographically connected areas (Mediterranean Sea and North Atlantic); ii) to develop innovative approaches to sample, and analyze the producing organisms and their toxins by chemical and biological methods including immunoassays and sensors; iii) to predict different future scenarios based on molecular data (routes of dispersion) and modelling.

Disclosure of Interest: None Declared

Ecology - harmful algae and global change

P-103

The Status of Harmful Algal Blooms (HABs) in the Coastal Waters of Ghana.

D. Dzifa [1 2], M. Cabrini [3], D. K. Adotey [1 2], D. Gyingiri Achel [2], H. Kuranchie-Mensah [2], L. Palm [2], A. Beran [3], Y. Serfor-Armah [1 2,*]

[1]School of Nuclear and Allied Sciences, University of Ghana, Atomic, [2]Ghana Atomic Energy Commission, Legon-Accra, Ghana, [3]Section of Physical, Chemical and Biological Oceanography, National Institute of Oceanography and Experimental Geophysics, Trieste, Italy

Abstract: Studies of harmful algae have been on-going in many parts of the world since the last half of the century. However, in Africa for that matter West Africa including Ghana not much has been studied or reported on the status of HABs. An increasing demand for seafood products in Ghana implies that there is the need to study HABs in the coastal waters of Ghana, and its associated health and economic risks. The fisheries sector forms an integral part of the Ghanaian economy and employs a sizable number of people, especially women. It is therefore imperative that the safety of natural seafoods is ascertained through frequent food safety monitoring and assessment. Algal toxins can accumulate in fish and shellfish and consequently cause poisoning in consumers of seafood. Such toxins and the algae producing them are measured in many developed countries. Nevertheless, very little is known about the occurrence of such algae and their toxins in most African countries, such as Ghana. This study assesses the distribution of harmful phytoplankton along the coast of Ghana. The monitoring study so far has identified eleven harmful species which are mostly thecate dinoflagellates including such species as *Lingulodinium polyedra, Gonyaulax spinifera, Dinophysis caudata, Dinophysis fortii, Alexandrium spp* being toxin producers with the potential to cause diarrhetic shellfish, paralytic shellfish, azaspiracid and yessotoxin-like poisoning symptoms. Species showed a great variation both in size and form. High diversity of HABs species were observed for the rainy season with low cell abundances. There were seasonal variations in specie densities with high dominance for dry season. *Lingulodinium polyedra* showed the highest cell density of 5.0×10^4 cells/L and was observed in December 2016 and January 2017 followed by *Gonyaulax spinifera* (3.0×10^4 cells/L) and *Dinophysis caudata* (8.2×10^3 cells/L). Observations also have revealed possible presence of *Alexandrium spp*, one of the most toxic genus among the HABs species. This indicates that our shores are possibly prone to harmful algal blooms.

Disclosure of Interest: None Declared

Taxonomy

P-104

Isolation and taxonomic characterization of epiphytic dinoflagellates from Bahía de La Paz and Isla San José, Gulf of California

L. Morquecho [1,*], A. Reyes-Salinas [1], I. Gárate-Lizárraga [2], A. Mazariegos-Villarreal [3]

[1]Planeación Ambiental y Conservación, Centro de Investigaciones Biológicas del Noroeste, S.C. (CIBNOR), [2]Centro Interdisciplinario de Ciencias Marinas (CICIMAR-IPN), Instituto Politécnico Nacional, [3]Ecología Pesquera, Centro de Investigaciones Biológicas del Noroeste, S.C. (CIBNOR), La Paz, Mexico

Abstract: In Mexico, research into harmful dinoflagellates has focused primarily on planktonic species, and although the presence of toxic epiphytic and benthic species has been confirmed, the taxonomic and biological studies conducted so far are confined primarily to Veracruz and the Yucatán peninsula. The current study establishes the composition of species and strains from subtropical latitudes of the Mexican Pacific. Macroalgae were collected (free diving and SCUBA) in a lagoon of Isla San José (2011 and 2014), and several coastal areas of Bahía de La Paz (2016). Dinoflagellates were separated from the macroalgae and isolated with a micropipette, and the culture media GSe, f/2, and L1 were used to preserve strains. These were maintained in incubation with light/dark cycles of 12:12 h at temperatures of 20±2 and 25±2°C. Taxonomic characterization of dinoflagellates was accomplished by observing the arrangements of thecal plates using optical and scanning electron microscopy, while the internal and external morphology of the macroalgae was analyzed by cross-section. Of the 36 strains of epiphytic dinoflagellates that were isolated and identified, several stand out that are potentially toxic and/or may not have been observed in the region previously: *Amphidinium operculatum*, *Bysmatrum gregarium*, *Coolia canariensis*, *Fukuyoa yasumotoi*, *Gambierdiscus* cf. *carolinianus*, *G*. cf. *carpenteri*, *Prorocentrum concavum*, *P*. *fukuyoi*, *P*. *lima*, *P*. *rhathymum*, *Ostreopsis marina*, and *O*. *ovata*. These dinoflagellates were associated mainly with the macroalgae *Caulerpa peltata*, *C*. *sertularioides*, *Centroceras clavulatum*, *Ceramium* sp., *Codium* sp., *Halimeda discoidea*, *Laurencia* spp., *Padina* sp., *Sphacelaria* furcigera and *Spyridia filamentosa*.

Disclosure of Interest: None Declared

Taxonomy

P-105

Potentially bloom-forming dinoflagellates in Korean coastal area

Z. Li [1,*], H. H. Shin [1]
[1]Korea Institute of Ocean Science & Technology, Geoje, Republic of Korea

Abstract: The frequency of harmful algal blooms (HABs) has been increasing dramatically since the 1980's in Korean coastal waters, caused by the bloom-forming dinoflagellates. Water samples were collected in the southern coastal area, Korea. We isolated and successfully established 108 culture strains of potentially bloom-forming dinoflagellates. Here, we provided a list and descriptions of these potentially bloom-forming dinoflagellates in detail to help a better understanding of the morphological characteristics and molecular phylogenetic positions. Potentially bloom-forming dinoflagellates recorded during study periods included 6 *Alexandrium* species, 1 *Akashiwo* species, 2 *Ceratium* species, 1 *Cochlodinium* species, 3 *Gonyaulax* species, 3 *Gymnodinium* species, 4 *Heterocapsa* species, 2 *Karlodinium* species, 1 *Lingulodinium* species, 1 *Noctiluca* species, 4 *Prorocentrum* species, 1 *Protoceratium* species and 2 *Scrippsiella* species. The presence of potentially bloom-forming dinoflagellates in Korean coastal waters deserves more attention and efforts in HAB monitoring and in HAB management in the future. In addition, these live cultures could be also applied in more extensive scientific areas.

Disclosure of Interest: None Declared

Taxonomy

P-106

Alexandrium pacificum (Group IV) is causative species for past paralytic shellfish poisoning outbreaks in Jinhae-Masan Bay, Korea

H. H. Shin [1,*], Z. Li [1]

[1]Korea Institute of Ocean Science & Technology, Geoje, Republic of Korea

Abstract: Paralytic shellfish poisoning (PSP) caused the deaths of four people in coastal area of Korea, mainly Jinhae-Masan Bay and adjacent areas, in April 1986 and in 1996. The PSP outbreaks were caused by the consumption of mussels, *Mytilus* edulis. The organism that caused PSP was identified, from morphological data only, as *Alexandrium tamarense* which is recently renamed as *A. catenella*, however recent studies have shown that the morphological diagnostic characteristics used to identify *Alexandrium* species have uncertainties and molecular tools and other criteria should be considered as well. The organism that caused past PSP outbreaks and incidents in Korea therefore need to be carefully reconsidered. The aim of this study was to re-evaluate the species really responsible for past outbreaks of PSP in Jinhae-Masan Bay, Korea. The temporal production and fluxes of the resting cysts of *Alexandrium* species were investigated for one year (from March 2011 to February 2012) using a sediment trap, and the morphology and phylogeny of vegetative cells germinated from the resting cysts were analysed. The production of *Alexandrium* species peaked in August and November, when temporal discrepancies were found in the water temperature (22.4 and 22.7 °C in August, 19.1 and 19.6 °C in November) and salinity (29.5 and 26.1 psu in August, 30.5 and 31.8 psu in November). The morphological data revealed that *Alexandrium* species germinated from resting cysts collected in August have a ventral pore on the 1′ plate, whereas the 1′ plate in *Alexandrium* species germinated from resting cysts collected in November lacks a ventral pore. Molecular phylogenetic data for the vegetative cells from the germination experiments allowed the August and November peaks to be assigned to *Alexandrium catenella* (Group I) and *A. pacificum* (Group IV), respectively. This indicates that the production of resting cysts of *A. catenella* can be enhanced by relatively high water temperature. This result is not consistent with those of previous studies that *A. catenella* responsible for PSP outbreaks was found at relatively low water temperature. In addition, large subunit ribosomal sequences data revealed that *A. pacificum* isolates from Korea were closely related to those from Australia, Japan and New Zealand where the PSP toxicity of shellfish and blooms occurred in the 1990s, indicating that the introduction of toxic dinoflagellates were related to ballast water from bulk-cargo shipping. Based on these results, we concluded that past PSP outbreaks in Jinhae-Masan Bay of Korea could have been caused by *A. pacificum* rather than by *A. catenella*.

Disclosure of Interest: None Declared

Taxonomy

P-107

Discovery of novel “epiphytic” *Alexandrium* spp.: Molecular phylogeny, toxicity, and population dynamics in Japanese coastal waters

T. Nishimura [1,*], Y. Kuribara [1], R. Fukuzawa [1], K. Mimura [1], H. Funaki [1 2], Y. Tanii [1], K. Hashimoto [1], K. Miyachi [1], K. Tanaka [3], I. Yanagida [4], M. Adachi [1]

[1]Laboratory of Aquatic Environmental Science, Kochi University, Nankoku, [2]Ehime University, Matsuyama, [3]Usa Marine Biological Institute, Kochi University, Tosa, [4]NPO INO, Okinawa, Japan

Abstract: The dinoflagellate genus *Alexandrium* has been well studied worldwide. At least half of the *Alexandrium* species produces toxins that are responsible for the outbreaks of paralytic shellfish poisoning. On the other hand, >30 planktonic species of the genus are reported worldwide, whereas only one benthic species, *A. hiranoi*, has been reported from benthic habitats such as tidepools. However, to the best of our knowledge, no study has yet reported on the epiphytic species in the genus.

In this study, we identified the novel strains of epiphytic *Alexandrium* spp. from the Japanese coastal regions and investigated their molecular phylogenetic positions, toxicities, and population dynamics. Between 2013 and 2015, macroalgal samples were collected from temperate and subtropical areas (<3–30 m depths), and 49 clonal strains were recognized. Phylogenetic analysis based on the LSU rDNA D1/D2 region revealed that the strains could be separated into three apparent novel phylotypes that were sister taxon to *A. ostenfeldii*: *Alexandrium* sp. type 1 (n = 6 strains), *Alexandrium* sp. type 2 (n = 42), and *Alexandrium* sp. type 3 (n = 1). Most cells of all strains were spherical and non-motile, and the cell sizes of the types 1, 2, and 3 were 35.4 ± 3.8, 39.2 ± 6.5, and 36.2 ± 2.9 µm, respectively. The toxicities of crude cell extracts from the strains of the three phylotypes were assessed by mouse bioassay (intraperitoneal injection). The results revealed that the *Alexandrium* strains showed lethal toxicities to mice; the strains of the types 1 and 3 were more toxic than those of the type 2. Further, the population dynamics of the three phylotypes were assessed by a newly developed qPCR assay specific for the three types. Between 2015 and 2017, macroalgal samples were collected every four months from temperate and subtropical areas (<3 and 30 m depths). The results of the qPCR assay demonstrated that the types 1 and 2 occurred throughout the year in both areas. Conversely, the type 3 was rare; the cells occurred in summer and autumn only in the temperate areas. To the best of our knowledge, this is the first study that unveiled the existence of toxic epiphytic *Alexandrium*. Further studies comprising detailed morphological observations and toxin analyses are ongoing.

Disclosure of Interest: None Declared

Taxonomy

P-108

Morpho-molecular diversity of Amphidomataceae (Dinophyceae) in France and French territories and relation to azasparacid toxins

E. Nézan [1,*], K. N. Mertens [1], N. Chomérat [1], G. Bilien [1], A. Derrien [1], A. Duval [1], A. Terre-Terrillon [1], M. Gottschling [2], U. Tillmann [3]

[1]LER BO, Ifremer, Concarneau, France, [2]LMU Department für Biologie, Ludwig-Maximilians-Universität München, Munchen, [3]Biosciences, Ecological Chemistry , AWI, Bremerhafen, Germany

Abstract: The family Amphidomataceae includes *Amphidoma* and *Azadinium*, of which several species were shown to produce azasparacid toxins. These toxins were first characterized in 1998, and an organism producing them, namely *Azadinium spinosum*, was described in 2009. In France, azasparacids AZA-1 (dominant) and AZA-2 were detected in oysters from Île de Sein (Brittany, France) in 2010, and this sparked research into *Azadinium* and the related *Amphidoma*. Since then, non-toxic *Azadinium dalianense* and toxic *Azadinium poporum* (AZA-2) were observed in French territorial waters, as well as *Azadinium caudatum*, whose toxicity has not been fully investigated. Several other *Azadinium* species remain undescribed in French territorial waters, and the causative agent of AZA-1 production remains unknown. In Saint Pierre et Miquelon, cells of the toxigenic *Azadinium spinosum* was observed together with several undescribed species of *Amphidoma* and *Azadinium*. In Reunion Island (Indian Ocean), *Amphidoma nucula* was detected together with an undescribed *Amphidoma* species. The species were studied morphologically using light microscopy and/or scanning electron microscopy. LSU rRNA sequences were obtained from single cells of these species from Saint Pierre et Miquelon and Reunion Island, and the molecular phylogeny confirms that these species belong to the Amphidomataceae and their respective lineages. The toxicity of these species is not known, and will be the subject of future investigations.

Disclosure of Interest: None Declared

Taxonomy

P-109

Morphology and molecular characterization of planktonic *Prorocentrum* species from coastal waters of Buenos Aires Province (Argentina).

I. Sunesen [1 2,*], F. Rodríguez Hernández [3], J. Tardivo Kubis [1], A. Lavigne [1 4], E. Sar [1 2]

[1]División Ficología, Facultad de Ciencias Naturales y Museo, UNLP, [2]CONICET, La Plata, Argentina, [3]Departamento de Microalgas nocivas, Centro Oceanográfico de Vigo, Instituto Español de Oceanografía, Vigo, Spain, [4]Dirección Provincial de Pesca, Ministerio de Agroindustria de la Provincia de Buenos Aires, La Plata, Argentina

Abstract: In the framework of a monitoring program of toxigenic and noxious microalgae from coastal environments of Buenos Aires Province, we have focused our attention on the morphology, taxonomy, distribution pattern and molecular characterization of planktonic *Prorocentrum* species. The studied material was collected at nine sampling stations along the coast: San Clemente del Tuyú, Santa Teresita, Villa Gesell, Mar Azul, Necochea, Claromecó, Los Pocitos, Ría Jabalí and Bahía San Blas. Since March 2008, phytoplankton qualitative and quantitative samples were taken twice a month from October to April and once a month from May to September. Live aliquots were used for isolation of cells and the establishment of unialgal cultures, to facilitate the morphological identification and molecular analysis of the species. Samples and strains were analyzed using light microscopy with phase contrast and differential interference contrast, fluorescence microscopy and scanning electron microscopy and the counts were made with inverted microscope using the Utermöhl technique. DNA samples were obtained from culture pellets and/or individual cells, for PCR amplification of partial LSU rDNA gene (D1-D2 regions). *P. triestinum, P. shikokuense, P. cordatum, P. compressum* and *P. texanum* were identified, compared and discussed with allied taxa. We report the first occurrence in South Atlantic waters of *P. texanum* Henrichs, Steidinger, Scott & Campbell, an okadaic acid producing species recently described from the Gulf of Mexico. This taxon was compared with *P. obtusum* Ostenfeld previously reported for the Argentinean Sea and *P. micans* Ehrenberg sensu stricto, recently redescribed from Korean and Japan coastal waters. The necessary taxonomical changes are proposed.

Disclosure of Interest: None Declared

Taxonomy

P-110

First report of *Alexandrium affine* in Uruguay; molecular, morphological and toxicological study of a bloom during summer 2017

S. M. Mendez [1,*], F. Rodriguez [2], A. Martinez [3], P. Riobo [4], M. Vargas [5]

[1]Phytoplankton Laboratory, National Direction of Aquatic Resources, Montevideo, Uruguay, [2]Instituto Oceanográfico de Vigo, Vigo, Spain, [3]Oceanografia, National Direction of Aquatic Resources, Rocha, Uruguay, [4]Instituto de Investigaciones Marinas, Vigo, Spain, [5]Centro de Investigación en Estructuras Microscópicas, San José, Costa Rica

Abstract: A bloom of a thecate dinoflagellate, chain-forming *Alexandrium* species, was reported at the tourist beach of Punta del Este in January 2017 (austral summer). This area is regularly monitored by the harmful algae and phycotoxins programme since 1980. The bloom reached in this locality a density of $1,2x10^3$ cells L^{-1} estimated by Utermöhl method. Bloom-forming species of the genus *Alexandrium* previously reported for this area were the Paralytic Shellfish Toxins (PST) producer *Alexandrium tamarense/catenella* associated to high toxicity levels in mussels between 1991 and 1996, and *A. fraterculus* a non-toxic species in Uruguay. The responsible species for the bloom in 2017 was identified by light microscopy, SEM and molecular analyses (partial LSUrDNA gene). Genetic results confirmed its identity as *Alexandium affine*, which is the first report of this species for Uruguayan waters and the region of the South-western Atlantic Ocean. During the bloom, no Paralytic Shellfish Toxins (PSTs) were reported in mussels from the area by the official method of mouse bioassay. Neither were detected PSTs using liquid chromatography with fluorescence detection (LC-FLD) although possible traces of GTX3 were observed that need to be confirmed in future studies. The report of this cyst-forming species from the genera *Alexandrium* in an area with long term data series (1980-2018) from a monitoring programme, calls attention to the potential geographical expansion of new HAB species in this region.

Disclosure of Interest: None Declared

Taxonomy

P-111

Morphology and phylogeny of harmful dinoflagellate *Takayama* sp. associated with massive fish kills in Bolinao, Northwestern Philippines

G. A. Benico[1,*], K. Takahashi[2], R. Azanza[3], M. Iwataki[2]

[1]Graduate School of Agricultural and Life Sciences, [2]Asian Natural Environmental Science Center, University of Tokyo, Tokyo, Japan, [3]The Marine Science Institute, University of the Philippines, Quezon City, Philippines

Abstract: Mass mortality of cultured milkfish (*Chanos chanos*) in Bolinao, Northwestern Philippines in May 2016 has coincided with the bloom of *Takayama* sp. with maximum cell density of 1,700 cells/mL. To identify the fish kill causative *Takayama* species, four cultures were isolated from Bolinao in May and September 2017, and their morphology and phylogenetic positions were compared with other strains isolated from New Washington, Philippines and Nagasaki, Japan. Cells were observed by light microscopy, scanning and transmission electron microscopy, and their phylogeny were inferred from sequences of LSU rDNA and ITS region. Cells of all *Takayama* cultures shared morphological characters, with the typical sigmoid apical structure complex (apical groove), a large spherical pyrenoid located in the hyposome and a finger-like small sulcal intrusion in the episome. Cells from Bolinao measured 12.0–18.8 µm (mean 16.6 µm, n = 30) in length and 9.5–17.6 µm (mean 12.8 µm, n = 30) in width. The cell size was similar to that of New Washington strain (mean 15.3 µm long) and relatively smaller than the Nagasaki strain (mean 18.6 µm long). Cells had a relatively larger chloroplast connecting the spherical pyrenoid and 14–22 small chloroplasts. Nucleus was basically large and located in the epicone, but the shape was rather variable; elliptical laterally elongated, C-shaped along the dorsal side, or sometimes developed an extension into left in the hyposome. SEM revealed that the Bolinao strains have a tube-like structure in the sulcal region, which was also observed in *T. acrotrocha* from Singapore. TEM showed that the chloroplast was bounded by two membranes and all small chloroplast lacked pyrenoid. Phylogenetic trees showed the Philippine strains were related to *T. xiamenensis* from China, *T. acrotrocha* from Italy and Singapore, and *Takayama* cf. *pulchella* from New Zealand. However, the Bolinao strain was different from *T. acrotrocha* and *T. pulchella*, which have an embedded pyrenoid in each small chloroplast and lack the large spherical pyrenoid. It also differed *T. xiamenensis*, which is large (20.0–27.0 µm in length), having many chloroplasts (30–40) and several large pyrenoids.

Disclosure of Interest: None Declared

Taxonomy

P-112

Ultrastructure and phylogeny of *Asterodinium gracile* collected from Nagasaki, Japan

G. Benico [1,*], K. Takahashi [2], W. M. Lum [1], M. Iwataki [2]
[1]Graduate School of Agricultural and Life Sciences, [2]Asian Natural Environmental Science Center, University of Tokyo, Tokyo, Japan

Abstract: The genus *Asterodinium* is a rarely reported, planktonic dinoflagellate from deep water (up to 100 m depth), having three extensions in the episome and two in the hyposome. Because of their morphological resemblance, it has been classified to the family Brachidiniaceae which also includes *Brachidinium* having four extensions in the hyposome. Recently, the phylogenetic affinity of *Brachidinium* to the family Kareniaceae was demonstrated. To understand phylogenetic position and morphological variability of *Asterodinium*, a culture was established from Matsuura coast in Nagasaki, Japan and the morphology was observed by light, scanning, transmission electron microscopy, and phylogenetic position was inferred from ITS region and LSU rDNA (D1–D3) sequences. Under LM, cells were star-shaped typical in the genus, dorsoventrally compressed and having elongated extensions three in the episome and two in the hyposome. Nucleus was located in the lower left in the hyposome. It had 32–49 yellow-green flattened chloroplasts overlapping with one another and distributing also throughout the extensions. Cells ranged 62.5–105.6 µm (mean 83.7 µm, n = 41) in length (including extensions) and 21.2–39.5 µm (mean 29.2 µm, n=54) in cingular width, which coincided with the range reported in the original description of *A. gracile* from Mozambique Channel, Indian Ocean with a maximum length of approx. 80 µm and hyposome width of 30 µm. SEM revealed that the apical structure complex (apical groove) was straight with series of knob vesicles. TEM showed that that the chloroplasts were bounded by double membrane without pyrenoid. Phylogenetic tree showed the position of *A. gracile* in the genus *Karenia* with high support values of bootstrap and posterior probability. In the *Karenia* clade it was closely positioned with *Karenia papilionacea* both in LSU rDNA and ITS trees, but their relationship was not supported. It was not closely related to *Brachidinium* in the *Karenia* clade.

Disclosure of Interest: None Declared

Taxonomy

P-113

Morphology and phylogeny of two *Azadinium* species isolated from Japan

M. Iwataki [1,*], K. Takahashi [1], W. M. Lum [1], G. Benico [1], T. Suzuki [2], Y. Fukuyo [3]

[1]University of Tokyo, Tokyo, [2]National Research Institute of Fisheries Science, Yokohama, [3]Tokai University, Shimizu, Japan

Abstract: An unarmored dinoflagellate genus *Azadinium* contains azaspiracids producing species. In Asia, three *Azadinium* spp., *A. poporum*, *A. dalianense* and *A. zhuanum* have been reported from Korea and China. In the present study, strains of *Azadinium poporum* and *Azadinium* sp. were newly established from Japanese coasts, and cells were examined by light and scanning electron microscopy. Their phylogenetic positions were inferred from ITS and LSU (D1–D3) rDNA sequences. Cells of *A. poporum* were 11.1–14.5 µm long and 8.4–11.0 µm wide. Thecal tabulation was Po, cp, X, 4', 3a, 6'', 6''', 2'''' without antapical spine, and the 1'' plate touched with the 1a. The ventral pore was observed at left side of the apical pore plate. In ITS and LSU rDNA phylogenetic trees, three ribotypes A–C were recognized in this species; the Japanese *A. poporum* strain was clustered in ribotype C. Cells of *Azadinium* sp. were 13.0–22.6 µm long and 9.1–19.4 µm wide. Thecal tabulation was Po, cp, X, 3', 2a, 6'', 6''', 2'''' with an antapical spine. The ventral pore was observed at right side of the apical pore plate, and the anterior and posterior edges of cingulum formed cingular lists. In phylogenetic trees, two LSU rDNA sequences determined from *Azadinium* sp. strains were almost identical to *A. zhuanum*, while those of ITS formed a clade sister to a clade of *A. zhuanum* sequences. In comparison with *A. zhuanum*, morphological features of *Azadinium* sp. were basically identical to this species, with the exception of less number of apical plate series (3' vs. 4').

Disclosure of Interest: None Declared

Taxonomy

P-114

An undescribed marine woloszynskioid dinoflagellate *Dactylodinium* sp. (Borghiellaceae) with a curving apical groove and ejectile bodies

W. M. Lum [1,*], K. Takahashi [2], H. Takayama [3], M. Iwataki [2]

[1]Graduate School of Agricultural and Life Sciences, [2]Asian Natural Environmental Science Center, University of Tokyo, Tokyo, [3]Kure, Hiroshima, Japan

Abstract: A marine woloszynskioid dinoflagellate isolated from Manazuru, Japan in April 2017 was examined by use of light and scanning electron microscopy and molecular phylogeny inferred from SSU rDNA–ITS–LSU rDNA sequences. Cells in established culture ranged 14.4–34.2 µm (mean 24.7 µm) long and 10.9–25.7 µm (mean 18.3 µm) wide, and the sizes of epicone and hypocone were equal. Cells were slightly dorsoventrally flattened, with rounded conical epicone and hemispherical hypocone, entirely covered by swollen amphiesmal vesicles. The cingulum displaced its own width, and the sulcus extended also into the epicone. Nucleus was subspherical located in the hypocone or ellipsoidal in left side of the cell. Chloroplasts distributed peripherally in the cell, an eyespot located in right side of the sulcal region, and the pyrenoid-like round structure located in the center were observed. SEM revealed an apical structure complex (= apical groove) originated from right side of the sulcal extension toward the anterior, then curved to the left and encircling the apex in counterclockwise. A large peduncle was present in the sulcal extension in the epicone, similar to that of *Dactylodinium pterobelotum*. Four ejectile structures were observed under SEM, of which two in ventral side were always at the particular positions; one in the hypocone was left-hand side of the sulcus, and another in epicone was right of the sulcal extension. Two ejectile structures in dorsal side were located each in the epicone and hypocone, although the positions looked variable. Phylogenetic tree revealed this species positioned between the families Borghiellaceae and Suessiaceae, and closely related to a recently described species, *Dactylodinium pterobelotum*. Since the cell shape, number of amphiesmal vesicles, apical groove type and presence of ejectile structures are different from *D. pterobelotum*, we concluded this species is an undescribed species in the genus *Dactylodinium*.

Disclosure of Interest: None Declared

Taxonomy

P-115

Phylogenetic and morphological comparisons of *Chattonella* spp. collected from Southeast Asia

W. M. Lum [1,*], K. Takahashi [2], G. Benico [1], H. C. Lim [3], P. T. Lim [4], C. P. Leaw [4], R. Azanza [5], E. Furio [6], S. C. Y. Leong [7], T. Lirdwitayaprasit [8], H. Thoha [9], M. Iwataki [2]

[1]Graduate School of Agricultural and Life Sciences, [2]Asian Natural Environmental Science Center, University of Tokyo, Tokyo, Japan, [3]Department of Applied Sciences, Tunku Abdul Rahman University College, Johor, [4]Institute of Ocean and Earth Sciences, University of Malaya, Kelantan, Malaysia, [5]The Marine Science Institute, University of the Philippines Diliman, [6]National Fisheries Research and Development Institute, Quezon City, Philippines, [7]Tropical Marine Science Institute, National University of Singapore, 18 Kent Ridge Road, Singapore, [8]Department of Marine Science, Chulalongkorn University, Bangkok, Thailand, [9]Research Center for Oceanography, Indonesian Institute of Sciences, Jakarta, Indonesia

Abstract: Sixteen culture strains of the harmful raphidophyte *Chattonella* spp. were established from coastal waters of Indonesia, Japan, Malaysia, Philippines, Singapore and Thailand during 2012–2018, and morphology and ultrastructure were examined by use of light and transmission electron microscopy, and molecular phylogeny inferred from LSU rDNA and ITS sequences. Cells of these cultures possessed common features found in *C. marina* and *C. subsalsa*; i.e., ellipsoidal or teardrop-shaped with the posterior tail, two heterodynamic flagella anteriorly inserted, many brown chloroplasts with a pyrenoid facing inward, and two granules different in sizes covering the cell surface. Number of the larger granule was various, and not found in some cultures. Differences were found in the presence of mucocyst and ultrastructure of the pyrenoid matrix. Discharge of mucocysts was observed in Malaysian and Philippines strains. TEM showed differences in thylakoid number penetrating into the pyrenoid matrix; three appressed thylakoids were found in Indonesian, Japanese and Malaysian strains, while many profiles of single tubular thylakoid were found from Thailand strain. Phylogenetic trees inferred from sequences of LSU rDNA and ITS region revealed that *Chattonella* clade was mainly divided into two subclades; *C. subsalsa* and *C. marina*, but some cultures isolated from Southeast Asia were not fit into these subclades, e.g., cultures from Malaysia and Thailand.

Disclosure of Interest: None Declared

Taxonomy

P-116

An undescribed kareniacean dinoflagellate harboring peridinin-type chloroplast with an eyespot

K. Takahashi [1,*], G. Benico [2], W. M. Lum [2], M. Iwataki [1]

[1]Asian Natural Environmental Science Center, [2]Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

Abstract: In unarmored dinoflagellates of the family Kareniaceae, all reported species possessed chloroplasts that contain 19'-acyloxyfucoxanthins (19'-AFs) and lack peridinin. A culture strain of an undescribed kareniacean dinoflagellate was established from Japanese coast, and cells were examined by LM, SEM, TEM and HPLC. Phylogeny of the host was inferred from nucleus-encoded rDNAs (SSU rDNA, ITS and LSU rDNA), and the chloroplast from chloroplast-encoded *psbA* and *psbC* sequences. Under LM, cells were 5.9–9.5 µm long, with the smaller epicone. Nucleus was located in the hypocone and a chloroplast was positioned periphery in the hypocone. An eyespot, which has not been reported in any described kareniaceans, was observed in the sulcal region. SEM showed the straight apical groove that resembles to those in *Karenia* and *Karlodinium*. Under TEM, the number of chloroplast enveloping membranes was usually two, unlike the common three membranes found in the peridinin-type. An eyespot was composed of a single layer of osmiophilic globules in chloroplast (type A). HPLC revealed the main pigment was peridinin, with no trace of 19'-AFs found in kareniaceans. Phylogenetic trees based on rDNAs showed core position of this species in the Kareniaceae, but not included within clades of any described genera (*Karenia*, *Karlodinium* and *Takayama*). Chloroplast *psbA* and *psbC* phylogeny demonstrated that chloroplast of this species was derived from a peridinin-type chloroplast of dinoflagellate, whereas 19'-AF-type of other kareniaceans originated from a single endosymbiotic event of a haptophyte. These results suggested the chloroplast of this undescribed kareniacean was replaced from 19'-AF-type to peridinin-type.

Disclosure of Interest: None Declared

Taxonomy

P-117

Ultrastructure of an unarmored dinoflagellate *Kapelodinium* sp. possessing yellowish chloroplasts derived from a haptophyte

K. Takahashi [1,*], M. Iwataki [1]

[1]Asian Natural Environmental Science Center, The University of Tokyo, Tokyo, Japan

Abstract: The unarmored dinoflagellate genus *Kapelodinium* has a heterotrophic species *K. vestifici*, which has been commonly reported as *Katodinium glaucum*. Two cultures of *Kapelodinium* sp. having yellow chloroplasts were isolated from Japan, and cells were investigated under light and electron microscopy. Pigment profile of this species was analyzed by HPLC, and host and chloroplast phylogeny was inferred from nucleus- (SSU, ITS and LSU) and chloroplast- (SSU) encoded rDNA sequences. Cells were fusiform with the posteriorly located cingulum, and measured 15.3–22.4 µm in length. Chloroplasts were spherical to ribbon shaped. Under SEM, longitudinal striations on cell surface and cap-like apical groove at cell apex were observed. TEM showed features of the chloroplasts, including several embedded pyrenoids with electron dense matrix, each were penetrated by a tube-like structure. No trace of the nucleus from symbiotic alga was observed under TEM. In HPLC, fucoxanthin and 19'-hexanoyloxyfucoxanthin (19'-Hex) were detected as major accessory pigments, and peridinin was not detected. In host dinoflagellate phylogeny, two strains formed a clade sister to *K. vestifici*. Chloroplast phylogeny showed that of *Kapelodinium* sp. was clustered within *Chrysochromulina* in haptophytes, and the sister was *C. apheles* with <1% substitutions. Phylogenetic position of the chloroplast distant from those in the Kareniaceae, in haptophytes, suggested two different symbiotic events of chloroplast from haptophytes had taken place in dinoflagellates. This species was smaller than *K. vestifici* (16–57 µm long), and probably having permanent chloroplasts, because these cultures have been kept for >18 months since isolation, without any prey feeding.

Disclosure of Interest: None Declared

Taxonomy

P-118

Revisiting the taxonomy of the "*Dinophysis acuminata* complex" (Dinophyta)

J. Park [1,*], M. Kim [2], H. J. Jeong [3], M. G. Park [1]

[1]Department of Oceanography, [2]Research Institute for Basic Sciences, Chonnam National University, Gwangju, [3]School of Earth and Environmental Sciences, Seoul National University, Seoul, Republic of Korea

Abstract: Marine dinoflagellates of the genus *Dinophysis* are well-known to produce Diarrheic Shellfish Poisoning (DSP) toxins, which have significant impacts on public health as well as marine aquacultures. Of over 100 species of *Dinophysis* recorded so far, *D*. cf. *acuminata* has been most commonly observed in coastal area worldwide. However, there is the problem of difficulty in morphological discrimination among the *D*. cf. *acuminata* species such as *D. acuminata*, *D. okamurai, D. ovum, D. sacculus*, and *D. recurva* (=*D. lenticula*) under the light microscope due to high similarity in morphology among them, so those species have been referred to as the "*Dinophysis acuminata* complex" so far. Recent studies have distinguished *D. ovum* from *D. acuminata* complex with different morphological features such as cell size, shape of hypothecal plate, shape of small cells formed during life-cycle, development of left sulcal list and ribs, and length of right sulcal list. In this study, we tried to distinguish between *D. acuminata* and *D. ovum* from 60 isolates of *D. acuminata* complex collected in Korean coastal waters based on those morphological characters. Morphological data showed that those characters of the two species overlapped each other even within the same isolates. We also determined the mitochondrial COX I (cytochrome oxidase subunit I gene) gene sequences of all the isolates, but could not find genetically distinct difference between the *D. acuminata* and *D. ovum*. Our results suggest that *Dinophysis acuminata* complex may be resulted from morphological variations caused by various factors (e.g. different geographical location, seasonal changes, and different environmental condition), as well as suggests the possibility that *D. acuminata* and *D. ovum* are the same species.

Disclosure of Interest: None Declared

Taxonomy

P-119

Morphology and phylogeny of two new parasitoids (Perkinsozoa) of the marine dinoflagellates

B. S. Jeon [1,*], M. G. Park [1]
[1]Department of Oceanography, Chonnam National University, Gwangju, Republic of Korea

Abstract: Marine parasitic protists have been recognized as a key component of food web in marine ecosystems, as well as have long received particular attention as biological control organism against harmful algal blooms. Although many environment sequences analyzed show that the phylum Perkinsozoa has considerable diversity and a wide distribution in freshwater lake, marine water column and sediment, however, identities of them remain mostly still unknown. During the intensive samplings Korean coastal waters on June, August and September 2017, two different parasitoids were detected and were successfully established in culture. Based on morphological, ultrastructural and molecular data, both of the two parasitoids were included in the family Parviluciferaceae, which has three life-cycle stages consisting of free-living zoospore, trophocyte and sporocyte, but showed distinct differences from the Parviluciferaceae members. In this study, we will present the details of morphological characteristics and phylogeny of the newly discovered two species.

Disclosure of Interest: None Declared

Taxonomy

P-120

Morphological and phylogenetic characterization of *Amphidinium* (Dinophyceae) strains from the Bay of Biscay and the Mediterranean Sea

L.-J. Lavier-Aydat [1,*], A. Laza-Martínez [1], S. Seoane [1]
[1]Plant Biology and Ecology, University of the Basque Country, Leioa, Spain

Abstract: Members of *Amphidinium* are among the most abundant and diverse benthic dinoflagellates worldwide, as they range from fresh-water to marine sandy sediments in tropical, sub-tropical, and temperate ecosystems. Some *Amphidinium* species can still be qualified as cryptic. This issue proves to be of great importance, as diverse reports have demonstrated the potential toxicity of some species, with the dangers it can result from such as fish mortality. The correct identification at species level is therefore critical for a deepen insight into the ecology of the different species, which could help to understand the causes and mechanisms of the toxin production. The current study focuses on the identification of strains isolated from diverse areas from the Bay of Biscay (European Atlantic Coast; 11 strains) and the Mediterranean Sea (Spanish, Maltese and Slovenian coasts; 9 strains). Five morphospecies were identified from the Bay of Biscay. Most of them appeared to belong to the *A. operculatum* complex (4 strains), followed in occurrence by *A. carterae* (3 strains), *A. thermaeum* (2 strains), *A. massartii* (1 strain) and *A. steinii* (1 strain). In the Mediterranean Sea the morphospecies *A. carterae* (8 strains) and *A. operculatum* (1 strain) were identified. The semicryptic nature of some of the species hampered their morphological identification, highlighting the need of the molecular identification of the strains. For this purpose, we proceeded to the amplification of the D1-D3 region of the 26S Large Sub-Unit ribosomic DNA by PCR, followed by a phylogenetic analysis. *Amphidinium carterae, A. massartii* and *A. operculatum* are widely considered cosmopolitan species, although it is apparently the first case for *A. steinii* being observed on Spanish coasts and *A. thermaeum* had only been reported in the Aegean Sea, Australia and New Zealand.

Disclosure of Interest: None Declared

Taxonomy

P-121

Preliminary results from an investigation into sedimentation rates of preserved harmful algal species

S. C. Swan [1,*], E. MacLeod [1], C. Whyte [1], K. Davidson [1]
[1]SAMS, Oban, United Kingdom

Abstract: The analysis of fixed seawater samples by light microscopy is a commonly used method for the detection of harmful marine phytoplankton. The volume of sample analyzed using the Utermöhl method typically ranges from 5 mL to 50 mL, depending on the density of phytoplankton, and the amount of sediment and detritus present. Rapid reporting of cell counts is central to regulatory monitoring of harmful algae. This study therefore set out to determine how factors such as the settling duration and the concentration of fixative used influence the sedimentation rate for a range of harmful algal species. Filtered seawater was spiked with cultured dinoflagellates and a chain-forming diatom and fixed with acidic Lugol's iodine, to obtain a final concentration of 1%. The algal species in 50 mL sub-samples were then allowed to settle onto the base plate of a sedimentation chamber for between 16 and 24 hours, prior to enumeration. For a settling period of 20 hours, cell counts were slightly higher when the concentration of Lugol's fixative was increased to 2%, although the effect was not significant. However, rapid settlement of phytoplankton during the sub-sampling process had considerable influence on the results obtained, highlighting the importance of standardized sample preparation.

Disclosure of Interest: None Declared

Taxonomy

P-122

Diversity of species of the *Pseudo-nitzschia* genus (Peragallo, 1900) in the subantartic ecoregion of the southern Pacific, Chile

M. A. Pinto Torres [1 2 3,*], M. Frangópulos [3 4], G. Pizarro [5 6], R. York [7], J. L. Iriarte [1 2 8 9 10]

[1]Escuela de Graduados, Instituto de Acuicultura , [2]Programa de Doctorado en Ciencias de la Acuicultura, Universidad Austral de Chile, Los Pinos S/N. Puerto Montt, [3]Centro FONDAP IDEAL, Universidad Austral de Chile, Av. El Bosque 01789, Punta Arenas, [4]Centro de Investigación GAIA Antártica (CIGA), Universidad de Magallanes, Av. Bulnes 01855, Punta Arenas, [5]División de Acuicultura, Departamento de Medio Ambiente, [6]Centro de Estudios de Algas Nocivas (CREAN), Grupo Magallanes, Instituto de Fomento Pesquero (IFOP), Enrique Abello 0552, Punta Arenas, Chile, [7]Algal Resources Collection, University of North Carolina Wilmington, Marine Biotechnology Facility, 5600 Marvin K. Moss Ln., Wilmington, NC 28403, USA, [8]Centro FONDAP IDEAL, Universidad Austral de Chile, Calle Gral Lagos 1194, Valdivia, [9]Centro COPAS-Sur Austral, Universidad de Concepción , Víctor Lamas 1290, Concepción, [10]Centro de Investigación de Ecosistemas de la Patagonia (CIEP), José de moraleda 16, Coyhaique, Chile

Abstract: The *Pseudo-nitzschia* species are a usual component of phytoplankton of the fjords and channels system of southern Chile where producing recurrent blooms, and are considered a toxic or potentially toxic genus in the HABs monitoring program. However, its taxonomic identification has been scarcely developed and it is based in the external morphology to classify the species mainly within the *seriata* and *delicatissima* complexes. Here, the identification of the species present in the sub Antarctic ecoregion (45 ° to 56 ° S) of Southern Pacific, is showed. We used a total of 75 samples distributed within the study area obtained between 2006 and 2016. Morphological characterization of the species was performed first with optical microscopy and Nomarski interference contrast (NIC) to observe the contour, end- shape and overlap length of the valves, and extension of the chain. Later the samples were digested to eliminate the organic material. Silica frustules were observed with Scanning Electron Microscopy (SEM), considering the more conspicuous morphological characteristics used for diagnosis at level of *Pseudo-nitzschia* species. These are: length, width, striae, fibulas (10 µm), poroids (1 µm), presence or absence of central interspace (CIS), row number of poroids and type of hymen pores for the *delicatissima* complex. Five species were identified: *P. australis, P. pungens, P. subpacifica, P. calliantha* and *P. delicatissima*. The results of this study are the first taxonomic identification of the species that make up the genus *Pseudo-nitzschia* in sub-Antarctic waters of the southern Pacific.

Financial support: CONICYT-PCHA Scholarship / National PhD -2016-folio number 21160047 / Operational Expenses 7332/2017 / FONDECyT code 1170474 / PhD Program in Aquaculture Sciences.

Disclosure of Interest: None Declared

Taxonomy

P-123

Characterisation of *Gambierdiscus lewisii* sp. nov. and *Gambierdiscus holmesii* sp. nov. from the Great Barrier Reef, Australia.

A. L. Kretzschmar [1], M. Larsson [1], M. Hoppenrath [2], M. Doblin [1], S. Murray [1,*]
[1]C3, University of Technology Sydney, Sydney, Australia, [2]DZMB, Forschungsinstitut Senckenberg, Wilhelmshaven, Germany

Abstract: Ciguatera fish poisoning is a human illness caused by the consumption of seafood contaminated with neurotoxins produced by some species from the benthic dinoflagellate genus *Gambierdiscus*. Despite the widespread occurrence of this illness in tropical Australia, very little is known of the causative organisms in this region. Toxicological investigations of *Gambierdiscus* isolates established from Heron Island, in the Great Barrier Reef revealed two currently unclassified species as potential candidates. In this study, we characterise these isolates using (i) light microscopy; (ii) scanning electron microscopy; (iii) DNA sequencing based on the nuclear encoded ribosomal 18S as well as D1-D3 and D8-D10 28S regions. Morphology and phylogenetics confirmed these isolates represent two new, toxic species of *Gambierdiscus*. *Gambierdiscus lewisii* sp. nov. (Po, 3', 0a, 7'', 6c,?s, 5''', 0p, 2'''') is distinctive due to strong reticulate-foveate ornamentation of two different specifications and genetically distinct from the sister species *G. pacificus*. *Gambierdiscus holmesii* sp. nov. (Po, 3', 0a, 7'', 6c, 6s?, 5''', 0p, 2'''') is genetically distinct from the sister species *G. silvae* and is separated morphologically due to a strongly ventrally displaced apical pore complex and a distinct fold at the anterior edge of the sulcus. Characterisation of these new *Gambierdiscus* species provides essential knowledge to more accurately estimate the risk of human exposure to ciguatera fish poisoning in Australia while contributing to the global classification of species within the genus.

Disclosure of Interest: None Declared

Taxonomy

P-124

First detection of domoic acid produced by *Pseudo-nitzschia multistriata* in Peruvian waters

C. Tenorio [1 2], G. Alvarez [3 4,*], M. Araya [5], F. Alvarez [3], J. Blanco [6], E. Uribe [3]

[1]Banco de Germoplasma de Organismos Acuáticos, Instituto del Mar del Perú, Lima, Peru, [2]Doctorado en Acuicultura, Programa Cooperativo Universidad de Chile, Universidad Católica del Norte, Pontificia Universidad Católica de Valparaíso, [3]Departamento de Acuicultura, Universidad Católica del Norte, [4]Centro de Investigación y Desarrollo Tecnológico en Algas (CIDTA), Facultad de Ciencias del Mar, [5]Centro de Investigación y Desarrollo Tecnológico en Algas (CIDTA), Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile, [6]Centro de Investigacións Mariñas , Xunta de Galicia, Vilanova de Arousa, Spain

Abstract: *Pseudo-nitzschia*is a marine diatom genus that comprise 49 species, with at least 28 species that can produce the neurotoxin domoic acid (DA), that causes amnesic shellfish poisoning.Bivalve molluscs, can accumulate high DA levels by suspension-feeding on toxic *Pseudo-nitzschia*species, therefore, harvesting shellfish from the natural beds and aquaculture sites could be banned. In July 2017, phytoplankton samples were obtained from Bahía Paracas one of the most important scallops (*Argopecten purpuratus*) aquaculture sites in Peru. Phytoplankton analyses revealed the presence of *Pseudo-nitzschia*, that were isolated and cultivated under laboratory conditions. Morphological examination of the culture carried out with SEM, presented a good fit to the descriptions of *P. multistriata*. The cells were symmetrical and broad lanceolate in valve view. The apical axis ranges from 40 to 48 µm. The transapical axis of the valves was between 4.3 and 3.2 µm. A large central nodule was absence. The fibulae and striae number 24 and 40 in 10 µm, respectively. Each stria has two or three rows of poroids 1 µm. Toxin analysis of the culture extract, using high resolution mass spectrometry (HRMS) revealed the presence of domoic acid. The estimated amount was between 0,004 and 0,01 pg cell^{-1}. Even when, DA has not been reported in shellfish from natural beds or culture scallops in this geographical area, their detection in *P. multistriata*indicates that they are a potential risk and suggests that it is necessary consider to routinely monitor these species in order to warrant public health.

Disclosure of Interest: None Declared

Taxonomy

P-125

Diversity and bioactivities of cyanobacteria in the BCCM/ULC public collection

A. Wilmotte [1,*], K. Beets [1], Y. Lara [2 3], M. Santoro [1], V. Simons [4], B. Durieu [2], V. Savaglia [2], H. D. Laughinghouse IV [5], E. Stenico [6], M. Fiore [6], L. Cornet [3 7], D. Baurain [7]

[1]Life Sciences, BCCM/ULC, In-Bios Centre for Protein Engineering, [2]Life Sciences, In-Bios Centre for Protein Engineering, [3]Geology, University of Liège,, Liège, [4]BCCM/MUCL, Université Catholique de Louvain, Louvain-la-Neuve, Belgium, [5]Applied Phycology, University of Florida / IFAS, Fort Lauderdale, USA, [6]CENA, University of Sao Paulo, Piracicaba, Brazil, [7]Life ScienceInBioS—PhytoSYSTEMS, Eukaryotic phylogenomics, University of Liège,, Liège, Belgium

Abstract: The BCCM/ULC public collection funded by the Belgian Science Policy Office (BELSPO) aims to gather a representative portion of the cyanobacterial diversity with a focus on Polar biotopes (e.g. limnetic microbial mats, soil crusts, cryoconites, endolithes). It makes characterized cyanobacterial strains available for researchers to study the taxonomy, biogeography, evolution, synthesis of secondary metabolites, adaptation to harsh environmental conditions, and genomic make-up. It presently includes 177 cyanobacterial strains, including 120 of Polar origin (catalogue: http://bccm.belspo.be/catalogues/ulc-catalogue-search). Continuous maintenance of living cultures, most of which are also cryopreserved (as back-up), ensure the preservation and the rapid delivery of strains to clients for fundamental and applied research. Genomic DNA is also available on request. The collection has obtained the ISO 9001 certification for deposition and distribution of strains, as part of the multi-site certification for the Belgian Coordinated Collections of Microorganisms (BCCM) consortium.

Morphological and molecular identifications (based on SSU rRNA sequences) show that the strains belong to the orders Synechococcales, Oscillatoriales, Pleurocapsales, and Nostocales. This broad diversity makes the BCCM/ULC collection particularly interesting for taxonomic, biogeographic and phylogenomic studies. Hence, the sequencing of the genomes of several strains is underway.

In addition, cyanobacteria are known to produce a range of secondary metabolites (e.g. alkaloides, cyclic and linear peptides, polyketides) including cyanotoxins, with different bioactive potential. Bioassays have shown antifungal activities of the cell extracts of strains *Plectolyngbya hodgsonii* ULC009, *Phormidesmis priestleyi* ULC026 as well as the recently sequenced *Phormidesmis priestleyi* ULC007 (Lara et al. 2017, Genome Announcements, 01546-16). Litterature also has shown that cyanobacterial mats in Polar Regions contained microcystins and other cyanotoxins but, till now, no known toxin-producing organisms were observed in polar samples and these have yet to be identified. Due to the geographic isolation and the strong environmental stressors of the habitat, the exploration of these metabolites in Polar cyanobacterial strains seems promising for biotechnology or biomedical applications.

Disclosure of Interest: None Declared

Taxonomy

P-126

First Report of *Vulcanodinium rugosum* and Pinnatoxins in the Arabian Gulf

A. Al Muftaha [1,*], A. I. Selwood [2], M. Yilmaz [3]

[1]Department of Biological and Environmental Sciences, Qatar University, Doha, Qatar, [2]Cawthron Institute, Nelson, New Zealand, [3]Department of Bioengineering, Bursa Technical University, Bursa, Turkey

Abstract: The dinoflagellate *Vulcanodinium rugosum* has been previously observed in the Mediterranean Europe, North America, Asia, Australia and New Zealand. Various isolates of this species were shown to produce the cyclic imines pinnatoxins and portimine in previous studies. In this study, non-motile cells of this species were observed for the first time in the Arabian Gulf in samples collected off the coast of Qatar in 2013 (n=2). These samples also contained pinnatoxins (PnTxs) -G, -H and portimine as determined by liquid chromatography tandem mass spectrometry (LC-MS/MS). While PnTx-G concentrations were 0.006 ng.mg dw^{-1} for both sites, PnTx-H concentrations were 0.043 and 0.046 ng.mg dw^{-1} in the two sites analyzed. Four mono-algal *V. rugosum* cultures were established from these environmental samples. Large subunit rRNA gene and internal transcribed spacer region sequences of *V. rugosum* cultures from Qatar, Japan and Florida were identical for the analyzed gene regions. On the contrary, a *V. rugosum* strain from China showed only 97% and 86% identities for LSU rRNA gene and ITS region sequences, respectively, to sequences of strains from Qatar in this study. Isolates from Qatar produced PnTx-H (603 ng mg dw^{-1} to 908 ng mg dw^{-1}), and portimine as determined by LC-MS/MS. Absence of PnTx-G in cultures suggested that there might be other strains capable of producing this toxin in the Gulf or that local isolates did not produce this PnTx variety under the culture conditions used.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-127

Biochemical characterization of hemolytic toxin of dinoflagellate *Karenia mikimotoi*

L. Wencheng[1], D. Kim[2], Y. Yamasaki[3], Y. Matsuyama[4], T. Oda[1,*]

[1]Graduate School of Fisheries Science & Environmental Studies, Nagasaki University, Nagasaki, Japan, [2]Daegu Center, Korea Basic Science Institute (KBSI), Kyungpook National University, Daegu, Republic of Korea, [3]Department of Applied Aquabiology, National Fisheries University, Shimonoseki, [4]Seikai National Fisheries Research Institute, Fisheries Research and Education Agency, Nagasaki, Japan

Abstract: *Karenia mikimotoi* is one of the most noxious dinoflagellates causing HABs in many coastal waters worldwide. HABs due to this species have been reported in Japan, North Atlantic region, and other coastal areas, and frequently associate with severe damage to wild finfish, cultured finfish, and shellfish. Previous studies have demonstrated that *K. mikimotoi* produces various toxic agents such as hemolytic toxins, cytotoxic polyethers, and reactive oxygen species (ROS). In addition to finfish and shellfish, *K. mikimotoi* shows lethal effect on microzooplankton rotifer (*Brachionus plicatilis*). Comparative studies using two strains of *K. mikimotoi* isolated from different localities in Japan demonstrated that hemolytic activity and rotifer toxicity are well correlated, suggesting that hemolytic toxin is a major factor responsible for the rotifer toxicity. Since the cell-free culture supernatant and the ruptured cell suspension prepared from the exponentially growing cell suspensions showed no toxic effects on rotifer, it is considered that the flagellate cell mediated direct contact with rotifer through the hemolytic toxins located on the cell surface is essential for the toxicity. Furthermore, when the direct contact between *K. mikimotoi* and rotifer was interrupted with the cell-impermeable membrane with 3 mm-diameter pores, the toxic effect of *K. mikimotoi* on rotifer was completely inhibited. On the other hand, *Heterocapsa circularisquama*, which is highly toxic to shellfish but not to fish, also has hemolytic toxins and rotifer toxicity as well. In fact, both *K. mikimotoi* and *H. circularisquama* show hemolytic activity against rabbit erythrocytes and rotifer toxicity in a cell-density dependent manner. To gain further insight into biochemical features of the hemolytic toxins of *K. mikimotoi*, in this study, we investigated the hemolytic activity of different strains of *K. mikimotoi* against fish erythrocytes in addition to rabbit erytjrocytes. Interestingly, all the three strains of *K. mikimotoi* tested showed potent hemolytic activities against yellowtail (*Seriola quinqueradiata*) and japanese flounder (*Paralichthys olivaceus*) erythrocytes with different extents depending on the strains. In spite of the several similarities between *K. mikimotoi* and *H. circularisquama*, the biochemical features of the hemolytic toxins may be somewhat different.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-128

Cyclic imines (CIs) in mussels from north-central Adriatic Sea: first report of gymnodimines in Italy

S. Bacchiocchi [1,*], M. Siracusa [1], S. Evangelista [1], G. Scortichini [1], A. Piersanti [1]
[1]Centro di Referenza controllo chimico e microbiologico molluschi bivalvi, Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati", Ancona, Italy

Abstract: Cyclic imines (CIs) are a group of emerging lipophylic marine toxins, originating from dinoflagellates. They include three main types of compounds: spirolides (SPXs), gymnodimines (GYMs) and pinnatoxins (PnTXs) and other minor sub-groups. The cyclic imine moiety is responsible for their fast-acting neurotoxicity in mice upon intraperitoneal administration. CIs occur in microalgae and/or shellfish worldwide but no human intoxication has been related to their presence in seafood yet and no regulatory limits are set in Europe and in the world. Recently the EFSA requested more data in order to assess the risk CIs pose to shellfish consumers.

In North Adriatic Sea (Italy), a bloom of the SPXs producer *A. ostenfeldii* and high levels of SPXs in mussels were reported at the end of 2003. The toxin profile in *A. ostenfeldii* and mussels was mainly characterized by 13-desmethyl and 13,19 didesmethyl spirolide C (13-desMe SPX C; 13,19 didesMe SPX C).

This work aimed to evaluate the presence of CIs in mussels farmed along the north-central Adriatic Sea (Italy) in 2014 and 2015. A total of 23 compounds belonging to SPXs, GYMs and PnTXs were analysed by the Official method for Marine Lipophilic Biotoxins.Two SPXs, 13-desMe SPX C and 13,19 didesMe SPX C, were found at low concentrations in more than 80% of the samples. The highest levels were reported during the first half of the years in all the areas, reaching their maximum (29 µg/Kg) in January/March and then decreasing until June. Only GYM-A, belonging the GYMs group, was found, at low concentrations, in more than 70% of the mussels analysed. At the best of our knowledge, for the first time GYMs were detected in Italy while few cases were already reported in Europe. The GYM-A was detectable only in few mussel samples in the months in which SPXs reach the highest concentrations, afterwards they start to increase in early summer with a maximum (12 µg/Kg) during summer or autumn. PnTXs were never detected. The low levels of CIs found in mussels, compared to the reference of 400 µg sum of SPXs/kg in shellfish, proposed by the EURL for marine biotoxins, don't yet represent a risk for public health. However samples contain SPXs and GYMs for the large part of the year. Chronic and sub-chronic toxicity data on CIs are still missing, but in case of (sub)chronic toxicity evidences, CIs exposure should be taken into consideration even at low levels. The Adriatic toxicological scenario is continuously changing, therefore, in the future, those toxins levels in mussels may increase posing a specific risk for humans. Monitoring should be recommended even if action limits are not yet set. The identification of the SPXs and GYMs biogenic origin, eventually classifying the producer algae, would be helpful to better assess the phenomenon.

This work was funded by the Italian Ministry of Health (project n. RC007/2016)

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-129

Miniaturisation of yessotoxin-analysis of cultures of *Protoceratium reticulatum* and related species

K. N. Mertens [1,*], A. Terre-Terrillon [1], A. Duval [1], A. Derrien [1]

[1]Researcher, Ifremer, Concarneau, France

Abstract: Yessotoxins are a group of lipophilic, structurally related, polyether toxins that are produced by some Gonyaulacoid dinoflagellates: *Protoceratium reticulatum, Lingulodinium polyedra* and some strains of *Gonyaulax spinifera* that have been identified as such.

Strains were established of *Protoceratium reticulatum* and *Lingulodinium polyedra*. LC-MS-MS was used to test the reproducibility of measurements of yessotoxin content (pg/cell) in low cell and cyst counts of the studied strains (10, 20, 30, 40, 50, 100 cells/cysts). The effect of fixation on cultured strains was investigated as well: cells and cysts were fixed with lugol, ethanol and formol. Subsequently, the yessotoxin content in cells and cysts of the same strain was compared, to assess the difference in yessotoxins present in cysts and the corresponding motile stage. The method was applied as well directly to cells isolated from plankton samples and cysts isolated from surface sediment samples.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-130

Selective isolation of GTX 1,4 from the dinoflagellate *Alexandrium minutum* based on molecularly imprinted solid-phase extraction

Z. Lian [1,*], J. Wang [2]

[1]Marine college, Shandong University, China, Weihai, [2]Ocean university of China, Qingdao, China

Abstract: Harmful algal blooms are a global threat to coastal marine ecosystems, with consequences for fisheries and shellfish production. *Alexandrium* is among the most common bloom forming toxic dinoflagellate genera and is generally held responsible for the outbreak of paralytic shellfish poisoning. In the study, Gonyautoxins 1,4 (GTX1,4) from *Alexandrium minutum* samples were isolated selectively and recognized specifically by an innovative and effective extraction procedure based on molecular imprinting technology. Novel molecularly imprinted polymer microspheres (MIPMs) were prepared by double-templated imprinting involvement of both caffeine and pentoxifylline as the pseudo co-templates. The morphology and structure information of imprinted microspheres were observed and the selective recognition ability was investigated by adsorption experiments. The synthesized polymers displayed good affinity to GTX1,4 and applied as a special sorbent for selective recognization of GTX1,4 in marine microalgal extract samples. Further, an off-line molecularly imprinted solid-phase extraction (MISPE) protocol was optimized and an effective approach based on the MISPE coupled with HPLC-FLD was developed for selective isolation of GTX1,4 from the cultured *A. minutum* samples. The separation method showed good extraction efficiency (73.2-81.5%) for GTX1,4 and efficient removal of interferences matrices was also achieved after the MISPE process for the microalgal samples. The outcome demonstrated the superiority and great potential of the MISPE procedure for direct separation of GTX1,4 from marine microalgal extracts. The presented MISPE extraction method had the characters of simplicity, rapidity and selectivity, while the new imprinted material could provide a new promising alternative for selective separation of paralytic shellfish toxins.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-131

Neuro-2a Cell Bioassay for STX-derivates: TEF and Complex samples

A. E. Aballay González[1,*], M. Silva[1], J. J. Gallardo[2], L. Delgado[3], A. Rivera[4 5], G. Rios[6], A. Allisson[7 8]

[1]Laboratory of Cell Culture and Marine Genomics, Faculty of Natural and Oceanographic Sciences, [2]Department of Chemical Engineering, Faculty of Engineering, Concepción, [3]Instituto de Salud Pública, Santiago, [4]COPAS Sur-Austral, Universidad de Concepción, [5]Department of Oceanography, Faculty of Natural and Oceanographic Sciences, [6]Facultad de Farmacia, [7]COPAS Sur-Austral, [8]Laboratory of Cell Culture and Marine Genomics, University of Concepción, Concepción, Chile

Abstract: Paralytic shellfish poisoning (PSP) includes more than 57 STX-derivatives. Until recently there were two official methods for seafood samples (Mouse Bioassay and HLPC-FLD) with application in most of the countries. However, ethical considerations regarding Mouse Bioassay, have led to regulations being adopted in some countries that limit or prohibit the application of these tests. Analytical methodologies (e.g. HPLC or LC-MSMS), despite being highly sensitive, have the disadvantage of not being able to detect new toxins or analogues or provide an overall toxicity of the sample. A proposed method is the Neuro-2a cell-bioassay. The presence of ouabain and veratridine enhance sodium influx in the mouse neuroblastoma cell line, causing cellular swelling and subsequent death, PSP toxins block the sodium channel of excitable membranes antagonizing this effect and enabling cell growth to continue (cell rescue). In this work Neuro2a cells were exposed to commercial PSP standards and natural samples contaminated with PSP and other toxins. Here we demonstrate that from Neuro-2a cell-bioassay Toxicity Equivalency Factors (TEF) can be obtained. These TEFs were similar to those from the European Food Safety Authority. Neuro-2a cell-bioassay allowed calculating *in vitro* potencies of the PST standards in the following order: Neosaxitoxin (NeoSTX) > gonyautoxin 1,4 (GTX1,4) > saxitoxin (STX) >decarbamoylsaxitoxin (dcSTX) > decarbamoylgonyautoxin 2,3 (dcGTX2,3) > gonyautoxin 2,3 (GTX2,3) >decarbamoylneosaxitoxin (dcNeoSTX) > gonyautoxin 5 (GTX5) > N-sulfocarba-moyl-gonyautoxin-2 and -3 (C1,2). Spiked samples of mussel *Mytilus chilensis* with 40 and 80 µg STX/100 g were positive by cell bioassay, without false negatives. By analyzing spiked samples, we defined a cut-off of 30% of cell rescue for detection of contaminated natural samples with concentrations over the regulatory level. Then, we analyzed 17 natural complex samples of *Aulacomya ater*, *Mytilus chilensis*, *Venus antiqua* and *Choromytilus chorus* obtained from south of Chile and we were able to confirm the presence of STX-like analogues in 16 of the 17 samples. The semi-quantifitation of complex samples with bioanalytical methods requires demonstrated parallelism between dose-response curve standard and the contaminated sample curve, which is sometimes difficult, due to the nature of the sample that could interfere the cell response. The analysis of a greater number of complex samples will allow us the characterization and parameterization of the cell response, to establish correction parameters that allow estimating the concentration of naturally contaminated samples, as well as establishing controls to avoid false negatives and prevent human poisoning with new toxins. We can suggest that this type of functional bioassays is useful for toxin screening (positive/negative) in control programs in the future.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-132

Using a validated and accredited real time PCR method for the detection of *Azadinium spinosum* in seawater for rapid routine monitoring

D. Clarke [1,*], R. Gallardo Salas [1], P. Hynes [1], A. McCarthy [1], D. Walsh [1], J. Kelly [1], L. Devilly [1]
[1]Phytoplankton Unit, Marine Institute, Galway, Ireland

Abstract:

The Marine Institute’s Phytoplankton Unit have developed, validated and tested a number of in-house and published real time PCR (Polymerase chain reaction) molecular methodologies which detect specific target DNA regions of toxin producing phytoplanktonic species and the screening of specific genera. Specific DNA targeting has proved to be a valuable research tool in the identification of both toxic and non-toxic species of the same genus, which are traditionally difficult to distinguish and identify such as *Alexandrium, Pseudo-nitschia* & *Azadinium*.

Azaspiracid (AZP) toxin events are often observed in Irish aquaculture producing areas and have devastating impacts on the shellfish industry leading to long term closures and resultant economic losses. The known causative organism *Azadinium spinosum* is difficult to identify with any certainty to species level via light microscopy, therefore the molecular detection and confirmation of this organism in water samples prior to the onset of AZA's in shellfish is vital as a risk management tool. As part of the Astox II project, the Alfred Wegner Institute, Germany, designed a number of Taqman hydrolysis probe assays for the method detection of 3 *Azadinium* species, *A. spinosum, A. obesum* and *A. poporum.*

An in-house validated method for the detection of *A. spinosum* in seawater samples is used as a part of the routine phytoplankton monitoring programme. This method has recently been accredited to ISO 17025 standards, the first laboratory internationally to have a real time PCR method accredited for the detection of a phytoplanktonic species. The key areas of extraction efficiency, specificity, sensitivity, cross-reactivity, inhibition and reference genes have been addressed by using different strains of *A. spinosum* cultures, field samples and standard curves for detection. This method has a high sample throughput with rapid turnaround times which is highly specific and sensitive to low cell numbers in field samples. The method has been recently expanded as a multiplex assay for the simultaneous detection of both *A. spinosum* and *A. poporum.*

This method has provided information on the likelihood of the onset and subsequent monitoring of AZP events in aquaculture areas (Killary - data from 2012 onwards), and also during research surveys (2012 - 2017), the geographical distribution of this cosmopolitan species (high presence observed from Ireland to Mid-Atlantic), its occurrence at different depths in the water column (mainly at surface and also at *Chl.* max layer) and also what fraction size it occurs in. Results show that whilst *A. spinosum* is observed mainly at the 3 - 20 µm fraction, it has also been shown to occur in lower cell densities in the 60 - 120µm and > 120µm fractions indicating its predation by heterotrophic vector species, i.e. *Protperidinium, Favella.*

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-133

Portable System for Early Detection of Harmful Algal Bloom Toxins

M. Lochhead [1], S. Bickman [1,*]
[1]MBio Diagnostics, Inc., Boulder, USA

Abstract: Harmful Algal Blooms in freshwater and marine environments are increasing in frequency and duration and constitute a growing public health threat while also carrying substantial economic, ecologic, and food supply implications. Recent development of guidelines for microcystin (MC) and cylindrospermopsin (CYN) in drinking water has increased the need for rapid onsite testing for these toxins. MBio Diagnostics had developed a simple yet powerful portable system that enables users in the field to perform laboratory-quality, cost-effective cyanotoxin testing that will help protect drinking water, monitor commercial food resources, and provide critical data for ecosystems management. MBio's cartridge and reader system provides an unprecedented combination of speed, ease-of-use, quantitation and results multiplexing in a field-portable device. Here, we present results from a one-step, 10-minute assay that simultaneously measures microcystin and cylindrospermopsin toxin, with quantitative output centered at the WHO guideline value of 1 microgram/L for MC-LR. Data are presented for 40 natural lake water samples with and without known *Microcystis* and *Planktothrix* blooms; MBio data show concordance with laboratory immunoassay methods. Data are also presented for water samples fortified with eight different MC congeners, demonstrating broad performance for total microcystin. A one-step, 10-minute, field-portable mechanical cell lysis method will also be described in this presentation. By adding the portable lysis module, a fully integrated, field-portable kit is enabled.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-134

Case of sea turtle mortality in El Salvador in October 2017, analysis by receptor binding assay confirms saxitoxin findings

O. A. Amaya [1,*], M.-Y. Dechraoui Bottein [2], R. Quintanilla [1], G. Ruíz [3]

[1]Professor, LABTOX-UES, San Salvador, El Salvador, [2]Department of Nuclear Science and Application, International Atomic Energy Agency, Monaco, France, [3]LABTOX-UES, LABTOX-UES, San Salvador, El Salvador

Abstract: At the end of October 2017, once again the coasts of El Salvador in Central America were affected by hundreds of dead sea turtles and in order to provide a rapid response to the phenomenon the receptor binding assay was carried out (AOAC method 2011.27); More than 21 analyzes were carried out on 25 samples of dead sea turtles (*Chelonia mydas aggassizzi*) in different organs, mainly stomach contents. Maximum concentrations of approximately 300 µgSTX eq / kg were found. In the absence of threshold levels of toxicity in marine turtles it is difficult to ensure that these species died from saxitoxin contamination. It is presumed that the turtles acquired saxitoxins from a harmful algae bloom located far from the coastal area where they were stranded. In the past, dead sea turtles in El Salvador have been reported during 2005, 2006, 2009, 2010 and 2013 with saxitoxins found in dead sea turtle organs. In 2017, the Ministry of the Environment of El Salvador sent NOAA samples of dead sea turtles who confirmed the presence of saxitoxins. In the marine turtle mortality that occurred in 2013, the receptor binding assay was run, determining high concentrations of paralyzing toxins, corroborating the results with the marine environment laboratory of the IAEA international atomic energy organism, located in Monaco. This study provides information on the distribution of toxins in marine turtles to determine thresholds of toxicity that could help in the future.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-135

Quantitation of ichthyotoxic compounds produced by *Prymnesium parvum* and linking their presence to fish mortality

D. K. Svenssen[1], E. Varga[1 2,*], S. B. Binzer[3], N. R. Andersen[4], S. A. Rasmussen[1], T. O. Larsen[1], P. J. Hansen[3]
[1]Department of Biotechnology and Biomedicine, Technical University of Denmark (DTU), Kgs. Lyngby, Denmark, [2]Center for Analytical Chemistry, Department of Agrobiotechnology, University of Natural Resources and Life Sciences, Vienna (BOKU), Tulln, Austria, [3]Marine Biological Section, Department of Biology, University of Copenhagen, Helsingør, [4]National Institute of Aquatic Resources, Technical University of Denmark (DTU), Kgs. Lyngby, Denmark

Abstract: Harmful blooms formed by planktonic microalgae (HABs) in both freshwater and coastal marine waters regularly lead to severe mortalities of fish and invertebrates causing substantial economic losses of commercial marine products worldwide. The mixotrophic haptophyte *Prymnesium parvum* is one of the best-studied HAB species. In a previous study we could show that all 26 tested *P. parvum* strains produced prymnesins, the suspected causative allelochemical agent. The prymnesins are currently divided up in three different groups (A-, B- and C-type prymnesins) based on the carbon-number in the backbone. Each strain produced only one type of prymnesin, but different analogues were observed.

Following the identification of the causative agent, it is important to know how much is produced by the microalgae and what the effective (toxic) dose to fish is. We therefore tried to develop a quantitation method and faced many challenges along the way. First of all, the huge number of different analogues (51 identified so far) and the lack of authentic standards make an accurate determination impossible. Secondly, reproducibility problems were observed which partly are attributed to the “sticky” nature of these large ladder frame polyethers. Thirdly, the majority of the toxins are found intracellular and the concentration of the toxins in water is very low (low fmol L^{-1}, pg L^{-1} range) which results in sensitivity issues. We successfully developed a quantitation method for the sum of prymnesins in the biomass with fluorescence detection after derivatisation, but are still working on measures to determine the toxins in water.

With this method the toxin production of two B-type prymnesin producing strains along the growth curve was assessed. The highest prymnesin concentration was observed in exponential growth. Furthermore, a fish trial using rainbow trout and five different strains of *P. parvum* (one A-type, two B-type and two C-type prymnesin producers) at one cell concentration was conducted. A clear trend between the molar amounts of prymnesins in the biomass of the used strains and the survival time of the fish was observed. Not all questions were answered though and further investigations will be necessary.

Concluding, along with the challenges in the quantitation of prymnesins, we will present the current status of the work and its application to quantitate prymnesins along the growth curve and in a fish trial. The presented results bring us one step closer to the above mentioned research questions.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-136

Toxin profiles of cultured *Dinophysis* spp. as determined using low and high resolution mass spectrometry

M. Sibat-Dubois [1,*], M. Garcia Portela [2], V. Sechet [1], V. Raimbault [1], P. Rial [2], F. Rodriguez [2], B. Reguera [2], P. Hess [1]
[1]Ifremer, Phycotoxins Laboratory, Nantes, France, [2]Oceanographic center, IEO, Vigo, Spain

Abstract: Dinoflagellates of the genus *Dinophysis* produce toxins involved in diarrheic shellfish poisoning (okadaic acid (OA) and dinophysistoxins (DTXs)), and pectenotoxins (PTXs). In this study, toxin profiles from cultures of several species of *Dinophysis* spp. from French and Spanish coastal waters, were determined using liquid chromatography coupled to low resolution and high resolution mass spectrometry (LC-LRMS and LC-HRMS).

Cultures of *Dinophysis sacculus* and *D. acuminata* isolated from Arcachon Bay (France) and *Dinophysis acuta* and *D. acuminata* isolated from the Galician Rias (Spain) were harvested and their extracts obtained for toxin analyses in two laboratories (Ifremer and IEO). The results of LC-LRMS analysis in *D. sacculus*, revealed the presence of a high level of PTX2 and to a lesser extent, of OA. In contrast, OA was the dominant compound detected in both *D. acuminata* strains. In turn, toxin profile of *D. acuta* contained DTX2, in addition to OA and PTX2. To complete and confirm toxin profiles found using LRMS, full scan HRMS analysis was carried out to screen contents against an in-house phycotoxins database. This workflow identified several candidates of OA analogues or derivatives in *Dinophysis* spp. Tandem high resolution mass spectral acquisition was applied to confirm the nature of the compounds present. This approach permitted to reveal the presence of an OA-C9-diol ester, reported for the first time in *Dinophysis acuta.*

This study outlines the complexity of algal metabolites related to okadaic acid and raises further questions in terms of ecological relevance of the metabolites of this toxin group.

Acknowledgements:

This study was funded by CoClime project (ERANET, ERA4CS) and Spanish project MARBioFEED (ERANET Marine Biotechnlogy, PCIN-2015-252).

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-137

The first finding of azaspiracids from *Azadinium poporum* collected in coastal seawaters in Japan and Vietnam

H. Uchida [1,*], M. Ozawa [1 2], R. Watanabe [1], R. Matsushima [1], H. Oikawa [1], K. Takahashi [3], M. Iwataki [3], H. V. Dao [4], T. Suzuki [1]

[1]National Research Institute of Fisheries Science, Japan Fisheries Research and Education Agency, Yokohama, [2]Tokyo University of Marine Science and Technology, Department of Marine Bioscience, [3]Asian Natural Environmental Science Center, The University of Tokyo, Tokyo, Japan, [4]Institute of Oceanography, Vietnam Academy of Science and Technology, Nha Trang, Viet Nam

Abstract: Azaspiracid (AZA) analogues are the causative toxins of AZA poisoning. In 1995 human poisonings were caused by the consumption of mussels, *Mytilus edulis*, harvested from Killary Harbor, Ireland. The human symptoms are nausea, vomiting, severe diarrhoea and stomach cramps. The symptoms are similar to those caused by diarrhetic shellfish toxin okadaic acid and dinophysistoxin analogues. Approximately 60 analogues including stereoisomers have been identified by MS/MS and NMR studies. AZA1 and AZA2 are produced by a small dinoflagellate *Azadinium spinosum*. AZA36 and AZA37 are produced by *Azadinium poporum*. Many other AZA analogues are shellfish metabolites or chemically degraded artefact. There have been no reports on the capability to produce AZAs in Azadinium spp. in coastal seawaters in Japan whereas trace levels of AZA2 were detected in bivalves in a previous AZA survey in Japan. In the present study, several strains of A. poporum was isolated from coastal seawaters in Japan and Vietnam, and were cultured with the f/2 medium for 20 days. AZAs extracted from algal cells by a solid phase extraction (SPE) were analyzed by linear iontrap LC/MS/MS and qTOF LC/MS/MS. AZA2 was detected in the algal cells of Japanese strain as a dominant toxin along with a putative AZA11 and another novel AZA analogue. Novel AZA1 isomer was detected in the strain from Vietnam. Identification of AZA2 from the algal cells was carried out by the retention time, MS/MS spectra, and accurate mass data against the CRM AZA2 purchased from National Recearch Council Canada. Structural information on the putative AZA11 and the novel AZAs were also obtained by several MS/MS experiments. This is the first finding of the capability producing AZAs in *Azadinium* spp. in Japan and Vietnam. Because AZA2 was detected in Japanese bivalves in a previous study, the present finding of AZA2 in *A. poporum* strongly suggests that *A. poporum* could be a causative specie in contaminations in bivalves in Japan. Furthermore, clarifying the toxicities of AZA analogues will be important for food safety. Further research will be required to consider a monitoring system for AZAs in Japan and Vietnam.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-138

SEARCH FOR POTENTIAL BIOMARKERS FOR SAXITOXIN DETECTION

A. A. Rivera [1 2,*], A. Aballay [1], K. Vega [1], E. Tarifeño [3], V. Ulloa [1], J. J. Gallardo [4], A. Astuya [1 2]

[1]Laboratory of Cell Culture and Marine Genomics, Department of Oceanography, Faculty of Natural and Oceanographic Sciences, [2]COPAS Sur-Austral, [3]Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, [4]Department of Chemical Engineering, Faculty of Engineering, University of Concepción, Concepción, Chile

Abstract: The cytotoxicity in Neuro-2a cell assay has been shown as a promising in vitro alternative for the detection of saxitoxin (STX)-like toxins, however its application in contaminated samples has not been carried out due to interferences caused by shellfish meat or “matrix effect”. In the search for alternative procedures that reduce or avoid this interference, we evaluated the modulation that STX produces, after 24 hours, on the transcriptome of Neuro-2A cells, under 3 conditions: exposure to STX (33 nM), matrix (12.5 mg meat/mL) and a spiked sample or STX-fortified matrix (STX-matrix). Massive sequencing (Illumina) and bioinformatic analysis were used to identify genes showing differences in expression between 4 conditions. Interestingly, a broad modulation of the expression was not observed between control cells and the other conditions tested; however, control and matrix conditions form a group which was different from the STX-matrix and STX groups. Based on this analysis, 7 genes (Fgf-1, Adgrb2, Tfpt, Zfr2, Gm4353, Fam195a and Dusp7), which showed greater expression differences in both conditions with STX were selected and analyzed by q-PCR, confirming a tendency to down-regulation. Particularly, gm4353 (involved in nucleo-cytoplasmic transporter activity) was significantly down-regulated by the STX-matrix and STX. In a second step, we evaluated the early response of these genes (1 to 6 hours) by q-PCR under identical conditions. Our results showed that tfpt and fgf-1 mRNA (involved in cellular proliferation and apoptosis signalling, respectively) are up-regulated and zfr-2 mRNA (involved in nucleic acids binding) is significantly down-regulated in STX and STX-matrix conditions. Therefore, we can assume that these gene expression variations are directly related to STX response and to suggest these genes as potential biomarkers for the development of alternative screening method for the detection of STX-like toxins.

Acknowledgement: This work was supported by the FONDEF Project [grant number D11I1140], the Vicerrectoría de Investigación y Desarrollo from University of Concepción, Chile (VDRI-ENLACE 217.114.003-1.0) and the COPAS Sur Austral (PFB31/2007) Program.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-139

ELISA for the Anabaenopeptins and its use for the monitoring of source waters

T. Glaze [1], M. Aubel [2], A. Foss [2], F. Rubio [1,*]
[1]R & D, Abraxis, Inc., Warminster, [2]R & D, Green Water Lab, Palatka, USA

Abstract: Cyanobacterial harmful algal blooms occur in freshwater lakes, ponds, rivers, and reservoirs, and in brackish waters throughout the world. The wide variety of cyanotoxins and their congeners can lead to frequent exposure of humans through consumption of meat, fish, seafood, blue-green algal products and water, accidental ingestion of contaminated water and cyanobacterial scum during recreational activities, and inhalation of cyanobacterial aerosols. Cyanotoxins can also occur in the drinking water supply. In order to monitor human exposure, sensitive analytical methods such as enzyme linked immunosorbent assay and liquid chromatography-mass spectrometry are often used.

Anabaenopeptins (APs) are cyclic peptides comprised of a ring of five amino acid residues connected to an exocyclic residue through an ureido linkage. Anabaenopeptins were first isolated from the cyanobacteria *Anabaena flos-aquae*. To date at least 96 anabaenopeptins have been reported, the various congeners are structurally related. *Planktothrix*, *Nodularia*, *Microcystis*, *Lyngbya*, and *Schizothrix*, have also been reported as producers of anabaenopeptins. APs have been shown to be inhibitors of protein phosphatases and carboxypeptidase A.

An ELISA for the monitoring of anabaenopeptins was developed and utilized for the detection of these toxins on 109 source water samples obtained from 30 midwestern sites during the 2016 to 2017 time period. The samples were also characterized for microcystins concentration by ELISA; and genes assays for mcyE, 16S, and sxtA by PCR. Nineteen of the samples were analyzed by PP2A, and by a LC/MS/MS method developed by Greenwater Laboratories. The results obtained indicate that anabaenopeptins are found at high concentration (up to 103 ppb) and at a high incidence rate. Details of the methods and data obtained will be presented.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-140

Synthesis of immunosorbents supports for the selective extraction of MCs and the detection of free vs bound form in biological matrix

A. AÏCHABOU [1], A. COMBES [2], V. PICHON [2], K. COMTE [1], A. Méjean [3,*]

[1]AVIV, UMR7245 CNRS-MNHN, [2]Analytical, Bioanalytical Sciences and Miniaturization (LSABM), ESPCI- UMR CBI 8231 , [3]LIED, Paris, France

Abstract: The increasing occurrence and proliferation of cyanobacteria worldwide are serious issues as their bloom-forming ability impairs water quality in many ways (*i.e.* turbidity, anoxia…) and because some of common species are able to produce various harmful metabolites, including hepatotoxins like microcystins (MCs) which can affect all living aquatic organisms. Most of the current researches are focused on the microcystin toxic effects on individual species, but large knowledge gaps exist when considering the complex transfer pathways of MCs in the food webs. This major issue is due to the presence of two fractions of MCs (*i.e.* free *vs* protein bound-MC) and the leak of efficient methods to detect and quantify the bound-MC fraction.

A new analytical approach based on the specificity of two monoclonal antibodies (one that target the amino acid in position 2 of MC, the Mdha, and the second specific to Adda group) was developed. These antibodies were grafted on solide phase thus providing two immunosorbents (IS) that were used to extract specifically the MCs from extracts. In order to characterize ISs, these standard of free-MC and GSH-MC conjugate (used as model *of* bound-MC) were used. A LC-HRMS method was developed to allow the separation and the quantification of three MC variants and of the GSH-MC conjugate. The two ISs allowed an efficient and repeatable immunoextraction of the three variants of free MCs in pure media. Different extraction profiles were observed between the both ISs for GSH-MC conjugate with a higher retention on the IS grafted with the antibody that target Adda group. These two ISs constitute a valuable perspective for the further detection of bound-MC fractions in real biological matrices.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-141

Quantitation and ichthyotoxicity of karmitoxin, a *Karlodinium armiger* metabolite

S. B. Binzer[1], A. J. C. Andersen[2 3], L. S. de Medeiros[4], S. A. Rasmussen[3], D. K. Svenssen[3], E. Varga[3 5,*], P. J. Hansen[1], K. Jørgensen[2], T. O. Larsen[3]

[1]Marine Biological Section, Department of Biology, University of Copenhagen, Helsingør, [2]National Food Institute, [3]Department of Biotechnology and Biomedicine, Technical University of Denmark (DTU), Kgs. Lyngby, Denmark, [4]Departamento de Química, Universidade Federal de São Paulo (UNIFESP), Diadema-SP, Brazil, [5]Center for Analytical Chemistry, Department of Agrobiotechnology, University of Natural Resources and Life Sciences, Vienna (BOKU), Tulln, Austria

Abstract: Dinoflagellates of the genus *Karlodinium* are known to be involved in fish-killing events worldwide. We previously reported the isolation and characterisation of karmitoxin, the suspected allelochemical agent of the mixotrophic microalga *Karlodinium armiger*. Being able to quantify ichthyotoxic metabolites of microalgae allows for the determination of ecologically-relevant concentrations that can be simulated in laboratory experiments.

Therefore, we developed a quantitative method for the determination of karmitoxin in whole culture samples using high-performance liquid chromatography (HPLC) coupled to electrospray ionisation high-resolution time-of-flight mass spectrometry (HRMS) (Andersen *et al.* (2017) Marine Drugs 15 (9), 278, DOI: 10.3390/md15090278). The karmitoxin used as external standard was purified from *K. armiger* cultures (80 L) and was quantified by fluorescent detection after derivatisation of the primary amine. Various sample preparation methods for whole culture samples were assessed. The karmitoxin concentrations were determined using the *Bacillus subtilis* metabolite iturin A2 as internal standard due to the very similar retention time. Matrix effects were assessed by using *K. armi*ger cultures grown with ^{13}C-labelled bicarbonate as the primary carbon source.

The final method requires only 3 mL whole sample culture and uses Strata-X as stationary phase in the solid phase extraction. Although the apparent recovery (57%) is not ideal, it is reproducible (< 10% RSD) and the best compromise. The achieved limit of detection (0.03 $\mu g\ mL^{-1}$) and limit of quantification (0.11 $\mu g\ mL^{-1}$) are below the ecologically-relevant concentrations. If required increasing the injection volume from initial 2 to 6 µL will further lower those limits. The method was applied to assess the concentration of karmitoxin in laboratory grown cultures over 23 days, including the stationary phase, as well as to determine mixotrophic effects on the karmitoxin production in the presence of *Rhodomonas salina* as food source. Furthermore, we tested the toxicity of karmitoxin to fish larvae of sheepshead minnow (*Cyprinodon variegatus*) and the karmitoxin concentration at the start and the end of the experiment.

Concluding, we will present a quantitative method for the determination of karmitoxin in whole culture samples and shed some light on the ichthyotoxicity of this *K. armiger* metabolite.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-142

Application of molecular assays for rapid diagnosis of shellfish toxin producing and fish-killing micro-algae in seawater

L. Mackenzie [1,*], J. Banks [1], K. Smith [1], K. Steiner [1]
[1]Cawthron Institute, Nelson, New Zealand

Abstract: The establishment of large scale offshore shellfish aquaculture presents new challenges for harmful algal bloom (HAB) monitoring, especially in areas where blooms of toxic species (e.g. *Alexandrium pacificum*) are a common occurrence. Monitoring methods will need to provide identification and enumeration of problem species from multiple samples collected over large areas in as near real-time as possible. To accomplish this, several quantitative polymerase chain reaction (qPCR) assay formats (conventional qPCR, Aquila Hydrogel, Phytoxigene DinoDTec STX gene assay) were evaluated for the routine screening of seawater samples for the presence and abundance of several species of toxic and noxious micro-algae (*Alexandrium pacificum, Alexandrium minutum, Karenia brevisulcata, Heterosigma akashiwo, Pseudochattonella verruculosa*) that are a particular hazard to New Zealand aquaculture. The aim was to carry out trials of fast low-cost methods that can be applied by industry in real-world aquaculture settings. Samples collected from natural blooms and cell cultures were used to evaluate the assays. All the molecular methods tested showed good correlations with cell counts though their sensitivity varied. The results of validation trials comparing the different methods will be presented.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-143

Optimized post-column oxidation method for the determination of PSP toxins

K. Kapp [1,*], L. Heinze [1], A. Hiller [1], A. Preiss-Weigert [1]
[1]German Federal Insitute for Risk Assessment (Bundesinstitut fuer Risikobewertung), Berlin, Germany

Abstract: Paralytic shellfish poisoning (PSP) toxins are potent neurotoxins which are produced by several species of dinoflagellates. These toxins can accumulate in shellfish when they feed on the PSP toxin producing phytoplankton. Human consumption of contaminated shellfish can lead to a range of different gastrointestinal and neurologic symptoms; and in worst case it can lead to death. Therefore, it is mandatory to determine the presence of PSP toxins in shellfish with robust, precise, sensitive, specific, and rapid detection methods.

There are few internationally recognized methods available. The Mouse Bioassay (MBA) was the first method applied to determine the PSP total toxicity. The MBA is still the regulatory reference method in the EU. PSP toxins have no natural UV absorption or fluorescence activity, which is necessary for spectroscopic detection after chromatographic separation. Therefore, PSP toxins have to be oxidized to fluorescent products before detection. This oxidation can be implemented in two ways: before or after the HPLC separation of the toxins. The pre-column oxidation method, also named AOAC 2005.6 is very challenging and time consuming. The main drawback is the complex interpretation of the chromatograms and the lack in separation of the epimeric isomers. The post column oxidation method (PCOX), published as AOAC 2011.02, is simpler and faster, however in two cases two PSP toxins cannot be distinguished. The epimeric isomers can be determined separately, by applying two different chromatographic systems.

In our laboratory the AOAC 2011.02 was implemented with the following optimization steps:

- Sensitivity increased by changing the oxidation reagents and their flow rates, as well as the gradient of the mobile phase
- Extraction with acetic acid to retain the toxin profile
- Implementation of a hydrolysis step to enable quantification of GTX6, C3 and C4 as well as separation of GTX4 and GTX6 by using only one chromatographic system

The method was in-house validated regarding recovery rates, precision, linearity of the calibration range, limits of detection, and quantification. Validation data proved the methods performance and its fitness for purpose of qualitative and quantitative determination of PSP-toxins in mussels using our optimized PCOX method. Results were comparable to those of the AOAC 2011.02.

Over three years samples were analysed in our laboratory with pre- and post-column oxidation methods. The correlation is very good if the toxicity equivalent factors are used for both methods in the same way.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-144

Toxicokinetic evaluation of the diarrheic shellfish toxins administered orally to mice

M. C. Louzao [1,*], P. Abal [1], T. Suzuki [2], R. Watanabe [2], N. Vilariño [1], M. R. Vieytes [3], L. M. Botana [1]

[1]Farmacologia, Universidad de Santiago de Compostela, Lugo, Spain, [2]National Research Institute of Fisheries Science, Fisheries Research and Education Agency, Yokohama, Japan, [3]Fisiologia, Universidad de Santiago de Compostela, Lugo, Spain

Abstract: Okadaic acid (OA) and its main structural analogs dinophysistoxins (DTXs) are marine lipophilic phycotoxins produced by dinoflagellates of the genera Prorocentrum and Dinophysis and worldwide distributed. When Harmful Algal Blooms (HABs) occur, these toxins accumulate in shellfish and cause diarrheic shellfish poisoning (DSP) upon human consumption.

This is the first comparative study of absorption and excretion kinetics of OA, dinophysistoxin-1 (DTX1) and dinophysistoxin-2 (DTX2). DSP toxins were administered orally to mice and quantified in urine, feces and intracardiac blood by LC-MS/MS. Furthermore; the toxins were also examined in organs such as liver, kidney, stomach and intestine. DTX1 was quickly detected in urine but remained present in feces even in blood at the end of the experiment (24 h). OA was eliminated in feces since the beginning of the assay while DTXs excretion was increasing towards the end. These results suggested a different toxicokinetic pathway between OA, DTX1 and DTX2.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-145

NeuroTorp: Fast & Early Warning Device for the Detection of Aquatic Neurotoxins by end-users.

R. ARAOZ [1 2,*], F. NOIRMAIN [2], J. WYSOCKI [3], H. VOLLAND [3], S. SIMON [3], D. SERVENT [2]

[1]Neuro-PSI, UMR9197, CNRS, [2]CEA/ DRF/ JOLIOT/ SIMOPRO/ Toxines Récepteur et Canaux Ioniques, [3]CEA/ DRF/ JOLIOT/ SPI/ Laboratoire d'études et de recherches en immunoanalyse, CEA-Saclay, Gif sur Yvette, France

Abstract: Nicotinic acetylcholine receptors are cation selective pentameric transmembrane proteins that mediate fast neurotransmission at the neuromuscular junction and at the autonomous nervous system necessary for respiration, muscle contraction and escape from predation, among other important physiological functions. Therefore, they constitute a primary target for a wide array of toxins from diverse origin including dinoflagellates and cyanobacteria. On one hand, cyclic imine toxins (CiTx) produced by marine dinoflagellates are antagonists of nicotinic acetylcholine receptors. Shellfish accumulate cyclic imine toxins following filter feeding on toxic dinoflagellates vectoring them to humans. Harmful algal blooms dominated by CiTx-producing dinoflagellates have prompted the closure of shellfish farms worldwide. On the other hand, the fast-acting cyanobacterial anatoxins are agonists of nicotinic acetylcholine receptors and are responsible for animal poisonings by ingestion of toxic cyanobacteria in lakes and ponds worldwide. Herein is presented a lateral flow test based on two new concept for test strips: the high affinity of neurotoxins for their receptor targets and the use of high porosity filter membranes as support for the fabrication of the lateral flow test “NeuroTorp” (WO2017108115)[1]. We immobilized *Torpedo marmorata* electrocyte-membranes rich in nicotinic acetylcholine receptors on the test-line and use Biotin-α-Bungarotoxine conjugate as toxin tracer for the detection of neurotoxins acting on nicotinic acetylcholine receptors. When a toxic sample is applied, the nicotinic toxin will displace the biotinylated toxin tracer enabling its visual detection by using Streptavidine-Alkalyne Phosphatase conjugate or Streptavidine nanogold coated. Unlike immunochromatographic lateral flow tests that detect a given toxin, NeuroTorp could detect cyanobacterial anatoxins, dinoflagellate pinnatoxins, spirolides, gymnodimines or pteriatoxins, or snake and cone snail α-toxins. NeuroTorp is a ready-to-use low-cost point-of-care warning device for rapid and early detection of nicotinic neurotoxins in freshwater and marine environments by end-users.

[1] WO2017108115. Device for detecting neurotoxins and process for manufacture thereof. Aráoz R. et al. (2017).

Acknowledgements. The authors acknowledge the funding support from the LABEX LERMIT to DETECTNEUROTOX project and to Interreg/ Atlantic Area for ALERTOX-NET EAPA_317/2016.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-146

Neuroblastoma cell-based assay optimization for harmonized marine biotoxin detection: reducing ouabain and veratridine oversensitivity.

C. R. Loeffler [1,*], D. Bodi [1], A. Preiss-Weigert [1]

[1]Contaminants, Bundesinstitut für Risikobewertung, Berlin, Germany

Abstract: The neuroblastoma cell-based assay (N2a assay) is a sensitive, high-throughput, ouabain (O) and veratridine (V) dependent *in vitro* method for detecting sodium channel-specific toxins. This assay is used to screen for voltage-gated sodium channel-specific effects on cell membranes, such as toxins that activate (e.g., brevetoxins (PbTx), ciguatoxins (CTX)) or block (e.g., tetrodotoxins, saxitoxins) the target voltage-gated sodium channels. Additionally, the assay is used to differentiate between toxins that affect the sodium channel specifically and toxins with other modes of action. The concentration of toxins which have an effect on the sodium channel can be estimated semi-quantitatively using the N2a assay. Toxins investigated in this study specifically activate the voltage-gated sodium channel, which results in cell death. Assay sensitivity is dependent on the pre-treatment of the cells with O to inhibit the sodium-potassium pump (which would otherwise permit cells to compensate for excessive sodium ion flux) and V (which increases sodium channel permeability through a blockage of the voltage-gated sodium channel into an open position). The presence of O\V increases the concentration of intracellular sodium and the addition of sodium channel activating toxins (i.e., PbTx and CTX) further exacerbating the intracellular sodium concentration, resulting in dose dependent cell mortality. A mitochondrial dye (MTT) is used for end-point development where viable cells with an active metabolism convert MTT into a color byproduct that can be measured by absorbance, for analysis of cell viability. This assay is more sensitive than the traditional mouse bioassay and other current analytical methods and presents a valuable tool for toxin analysis in food and environmental samples.

In this study we describe a method for desensitizing neuroblastoma cells to O\V for the N2a assay. Because O\V themselves cause cellular death it can be difficult to distinguish the source of cellular toxicity; and without desensitization, mortality rates from O/V may exceed the range of utility for the assay. Therefore, desensitizing new Mouse (*Mus musculus*) N2a cells (Neuro-2a (ATCC® CCL131™)) purchased from the American Type Culture Collection will enable a more standardized practical use of this assay in laboratories. We demonstrate the toxicity of O/V to newly purchased cell lines and how to desensitize them for use in existing CTX and PbTx testing protocols. Here we demonstrate that without desensitizing new cells, the addition of O/V, at levels described in the existing literature, can result in 60-80% cell mortality; and post desensitization cell mortality can be 10-30%, well within the range of acceptable utility for this assay. This study is the first step towards standardizing a method for routine analysis of CTX and PbTx.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-147

Culture of *Azadinium spinosum* (strain 3D9) in small scale and large scale bioreactors provides new information on toxin production

R. Salas [1,*], P. McCarron [2], C. Rafuse [2], M. MacArthur [2], I. Rajotte [2], U. Tillmann [3], G. Gaiani [4], C. Bailey [5]

[1]Scientific and Technical Officer, Marine Institute, Oranmore, Ireland, [2]Biotoxin Metrology, Measurement Science and Standards, National Research Council of Canada, Halifax, Canada, [3]Chemische Ökologie, Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven, Germany, [4]Research assistant, [5]Laboratory technician, Marine Institute, Oranmore, Ireland

Abstract: A study was conducted to test cellular growth and azaspiracid (AZA) toxin production of the marine dinoflagellate *Azadinium spinosum* (strain 3D9). Under controlled environmental conditions, experiments were carried out to select parameters for large scale culturing in a 300L closed photobioreactor (PBR).

The experiment comprised of 500 mL cultures in two temperature and photoperiod treatments (18 or 12°C and 14:10 or 10:14 L:D, respectively) with three nested factors (non-aerated, aerated and reduced irradiance), for a total of 12 cultures. The data shows that best results in terms of cell growth was achieved at 18 °C in aerated cultures at a 10:14 L:D photoperiod. Cultures subjected to a 50% irradiance level did not achieve any substantial growth (>20000 cells/ml) during the culture cycle, which was further exacerbated by the lower temperature. Aeration also had a negative effect on cell growth at the lower temperature by extending the lag phase.

Toxin analysis of AZA1, -2, -3 and -33 via LCMS/MS showed that AZA33 production in all treatments with positive cell growth began in the early exponential phase and that levels increased with cell growth. At senescent phase, AZA33 disappeared from the water when cell densities decreased in culture, in contrast, AZA-1 and 2 accumulate during the stationary and senescence phase. Analysis of the deposited material sampled from the bottom of the culture flasks did not account for the drop of AZA33 in the water. This suggests that AZA33 might not be stable when dissolved or exudated from decaying cells in culture, although a similar stability of AZA33 compared to AZA1 reported in previous work provide little chemical evidence that AZA33 should be more prone to degradation than AZA1 in the dissolved phase. In any case, a rapid loss of AZA33 could explain why this compound is not generally found in shellfish samples. The most unexpected results of the study was that the relative ratios of AZA33 to AZA1 were approximately 5:1 and thus substantially higher than published in previous work using the same strain, where this ratio was favourable to AZA 1 (0.3:1) Strain 3D9 has been kept in laboratory conditions since 2009 and it is possible that over time the toxin production of this organism has changed or entered a stage of quiescence. For example, another *Azadinium spinosum* strain (SM2) isolated from Irish waters stopped producing known AZA analogs in 2015 after 5 years in culture.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-148

From development to implementation of a new LC-MS/MS approach to quantify 43 lipophilic toxins within the French monitoring program.

A. Derrien [1], A. Terre-Terrillon [1], A. Duval [1], C. Martin [1], M. Sibat [2], G.-A. Rovillon [2], D. Reveillon [2], Z. Amzil [2,*]
[1]ODE, IFREMER, Concarneau, [2]ODE, IFREMER, Nantes, France

Abstract: Lipophilic toxins are a large family of hundreds of compounds produced by several marine dinoflagellates (e.g. *Dinophysis* sp., *Karenia* sp. …) and freshwater cyanobacteria (e.g. *Microcystis* sp., *Planktothrix* sp. …). As filter-feeders, bivalve mollusks tend to accumulate these toxins making them unsafe for human consumption. Thus, lipophilic toxins are considered as an important food safety issue. In Europe, four groups of toxins are currently regulated: okadaic acid (OA) and dinophysistoxins (DTXs), pectenotoxins (PTXs), yessotoxines (YTXs) and azaspiracids (AZAs). However, many other families of toxins exist including emerging toxins. Emerging toxins can be considered as both toxins recently detected in a specific area but not regulated so far (e.g. cyclic imines, palytoxins, ovatoxins) or toxins detected out of France (e.g. brevetoxins, gymnodimins). To anticipate the introduction in France of these emerging toxins, via the transfer of mollusks between countries or ballast waters, a monitoring program EMERGTOX has been set up along the French coasts. In order to detect these emerging toxins, a new analytical approach has been developed to quantify 43 lipophilic toxins in digestive glands of mussels and oysters. This approach required the use of three LC-MS/MS methods (i.e. three elution gradients and two ionization modes) but only one optimized extraction protocol and one RPLC column (Kinetex XB-C18). The single-laboratory validation of this approach was performed according to the NF V03 110 guideline by construction of an accuracy profile. We obtained excellent performance with acceptance limits between 15 – 25%. Our specific, reliable and sensitive approach allowed us to detect dmMC-RR [up to 31 ng/g in an oyster sample collected in February in Ronce (Atlantic Ocean, France)] and PnTX-G [up to 361 ng/g in a mussel sample collected in April in Ingril (Mediterranean lagoon, France)]. Note that this is the first time in France that the presence of freshwater lipophilic cyanotoxins in marine mussels has been demonstrated, which suggests a physical transfer of *Microcystis* from freshwater to marine environment. These results revealed that emerging toxins are present in bivalve shellfish collected on the French coasts, rising question about the future needs for French safety regulatory monitoring program.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-149

Assessment of the receptor binding assay performance for analysis of PSP toxins in shellfish from Moroccan south Atlantic coastlines

J. Naouli [1,*], R. Abouabdellah [2], A. Bennouna [2], A. laissaoui [3], A. Mesfioui [4], H. Aitbouh [3], M.-Y. Dechraoui Botein [5], S. Benbrahim [6]

[1]Etudiant, Faculty of Sciences Ibn Tofail Kenitra, Kenitra, [2]Institut de recherche halieutique, Casablanca, [3]Centre des Sciences et des Techniques Nucleaires, Rabat, [4]Faculty of Sciences Ibn Tofail Kenitra, Kenitra, Morocco, [5]Laboratoire d'Environement de Monaco, AIEA, Vienna, Austria, [6]INRH, CasaBlanca, Morocco

Abstract: Paralytic shellfish poisoning events regularly occur along the Mediterranean and Atlantic coast of Morocco and have been responsible of several severe cases of human intoxication. In the 90's a regulatory monitoring program was established, conducted by the National Institute of Marine research (INRH), which minimise consumers' risk intoxication and warrant seafood safety. The South Atlantic coast, site of intensive artisanal harvesting by local coastal population as well as of aquaculture, is particularly impacted. From Agadir to Dakhla, blooms of toxic species have been increasing both in intensity and frequency over the last decade, with *Gymnodinium catenatum* and *Alexandrium minutum* being the most commonly implicated in PSP events. Shellfish species commercialized and monitored in this region include cockle, solen, oysters and mussels. The regulatory monitoring conducted weekly include toxic phytoplankton enumeration and identification in parallel to toxin detection in these four shellfish species using the mouse bioassay (MBA). In the aim of evaluating alternative methodology for future replacement of the animal based assay, a Receptor Binding Assay (RBA) for the paralytic shellfish toxins was established in Morocco, and its performance assessed, in comparison with the MBA, for Moroccan shellfish species surveillance. Over 50 samples collected as part of the regulatory monitoring programme along South Morocco coast were analysed and included monthly samples for 2017 year, as well as all the samples identified as toxic by MBA through the regulatory monitoring, stored since 2011. The good performance of the RBA was confirmed through use of certified reference material, internal quality control and control of critical parameters (slope of calibration curve, QC and IC50). With this method STX concentrations detected in samples ranged from 33 to 8,500 µg STX equivalent/kg. RBA data were statistically significantly correlated ($P<0.0001$) with MBA data (Pearson $r = 0.96$, $R^2=0.92$). This method allowed the detection and quantification of PSP toxins at concentrations not detectable by the mouse bioassay. The limit of quantification of the RBA was calculated in our laboratory condition and found 10-fold lower than that of the MBA, respectively 35.24±5.99 and 325 µg STX equivalent/kg. In addition, the RBA presented a higher ease of use and rapidity to deliver results than the MBA without use of live animals.

Considering the increasing risks associated with HABs in Morocco, together with the development of aquaculture production, and the need to plan the removal of live animal testing, these preliminary findings identify the RBA as a potential method of choice to replace the MBA.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-150

Immunoaffinity columns for clean-up and concentration of algal toxins

I. Samdal [1,*], I. Van Nieuwenhove [2], K. Løvberg [3], S. Uhlig [3], M. Sandvik [3], C. O. Miles [4]

[1]Toxinology Research Group, Norwegian Veterinary Institute, Oslo, Norway, [2]Laboratory of Food Analysis, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium, [3]Section of Chemistry, Norwegian Veterinary Institute, Oslo, Norway, [4]Measurement Science and Standards, National Research Council Canada, Halifax, Canada

Abstract: Immunoaffinity columns are widely used in sample preparation for the analysis of mycotoxins. However, despite the availability of antibodies with broad specificity to many families of algal toxins, little use has been made of these antibodies in immunoaffinity columns. We have developed immunoaffinity columns using antibodies originally produced for ELISA at the Norwegian Veterinary Institute. Columns targeting the microcystin/nodularin, okadaic acid, azaspiracid, yessotoxin, and pinnatoxin/spirolide families were produced and then tested on a range of sample matrices including algal blooms, cyanobacterial "health food" supplements, algal cultures and shellfish. Results showed high recoveries with remarkable clean-up of samples, removing contaminants from the sample matrix and reducing the matrix effects in subsequent LC-MS analyses. The columns will make it possible to improve analytical capacity in labs without the most expensive LC-MS-equipment. They could also simplify semi-targeted metabolomics studies of various types of samples.

As an example, the microcystin-antibodies resulted in columns capturing standards of MC-LR, MC-RR, MC-YR, MC-LA, MC-LY , MC-LW, MC-LF, [D-Asp^3]MC-LR, [D-Asp^3]MC-RR and NOD-R with recoveries of 78-83% eluted within the first 3 mL and 92-104% within 9 mL. The total capacity was ~700 ng for a column with ~1.4 mL gel and preliminary attempts suggest the columns to be reusable with similar recovery at least five times. These columns were highly effective for cleaning up natural bloom, culture, and food supplement samples for LC-MS analysis.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-151

SCREENING OF TETRODOTOXIN IN BIVALVE MOLLUSCS FROM GALICIAN RIAS: A SECOND ATTEMPT ALONG SPRING-SUMMER 2018

A. Gago-Martinez [1], J. Giraldez [2], A. Lozano-Leon [3], O. Vilariño [4,*]

[1]Analytical and Food Chemistry , University of Vigo and EU Reference Laboratory for marine biotoxins , [2]Analytical and Food Chemistry, University of Vigo , [3]Analytical and Food Chemistry , University of Vigo and Laboratory ASMECRUZ , [4]Analytical and Food Chemistry, University of Vigo and EU Reference Laboratory for marine biotoxins , Vigo , Spain

Abstract: The incidence of Tetrodotoxins in EU waters linked to the presence of high concentrations of Vibrio affecting to several species of bivalves and gastropods is being an issue of particular relevance for the official control of marine biotoxins in the EU. As a result of the European Food Safety Authority (EFSA) recommendation regarding the need of data on the occurrence of TTX in bivalves and gastropods, from different EU coastal areas to decide whether these toxins should be or not included in the EU Legislation, a preliminary work was carried out from May to September 2017 focused on the evaluation of the presence of TTX in different bivalve samples from Galician Rias (NW of Spain). To carry out this evaluation the detection and isolation of the major Vibrio spp and other enterobacterial populations were carried out with the aim of determining the pathways genes (PKS and NRPS) involved in the biosynthesis of these toxins. The collected samples were screened for Vibrio spp, and the positive samples were analyzed by biochemical and genetic test. The selected samples were first screened for TTX-like toxicity by Neuroblastoma (N2a) cell assay and the positive samples were further analyzed by LC-MS/MS to confirm the presence of TTX. The results obtained in the first evaluation showed some positive response to the biochemical and genetic tests, and also a positive response after N2a was found for some samples, the positive TTX-like toxicity was further confirmed by LC-MS/MS but in very low concentrations (< LOQ=1.03µg/Kg). The main finding was that the positive samples were infaunal samples while no TTX was found in any of the mussel samples evaluated. To continue with the study, a second evaluation was planned for 2018, during the period May-September, based on the previous results, this second study on the occurrence of TTX has been focused on infaunal samples from different Galician Rias (clams, cockles, razor clams oysters and scallops) where the water circulation rate is low which is justify longer periods of toxicity. Results obtained on the evaluation of this occurrence will be presented and discussed in this work.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-152

Tetrodotoxins occurrence in bivalve molluscs collected along the French metropolitan coasts

V. Hort [1,*], T. Bertin [1], C. Desbourdes [1], N. Arnich [2], T. Guérin [1], G. Lavison-Bompard [1], M. Nicolas [1]

[1]Laboratory for Food Safety, [2]Risk Assessment Department, ANSES - French Agency for Food, Environmental and Occupational Health & Safety, Maisons-Alfort Cedex, France

Abstract: Tetrodotoxin (TTX) and its analogues are produced by marine bacteria and have been detected in marine bivalves and gastropods from European waters. The EFSA scientific opinion on the risks for public health related to the presence of tetrodotoxin (TTX) and TTX analogues in marine bivalves and gastropods (EFSA Journal 2017;15(4):4752) points out the need for more occurrence data in edible parts of molluscs from different EU waters. The opinion strongly recommends that data on concentrations of TTX and its analogues should be obtained using EU approved and validated chemical-analytical methods. Very few French data on molluscs contamination by TTXs are currently available and no data were published so far. This study aims to provide such data and consequently to contribute to a more reliable exposure assessment.

To integrate spatial variability, the sampling is carried out on the eleven geographical points of the French monitoring system for emerging toxins in order to cover all the French metropolitan coasts (from the English Channel, passing through Atlantic Ocean and the Mediterranean Sea). Samples were collected throughout the year 2018, on the basis of one sample per point and per month. Eight points were sampled for mussels, two points for oysters and one for both molluscs – i.e. 132 samples in all.

Toxins are extracted from the samples and purified through graphitized carbon SPE according to the harmonized protocol of the European Reference Laboratory (EULR) for the determination of tetrodotoxin. The analysis is performed by hydrophilic interaction chromatography coupled to a triple-quadrupole mass spectrometer (HILIC-MS/MS). This protocol is implemented for the detection and quantification of TTX, 4-epi-TTX, 11-deoxy-TTX, 5-deoxyTTX, 4.9 anhydro TTX, 6,11-dideoxy-TTX, 5,6,11-trideoxy-TTX. We present here analytical performances of the fully in-house validated method and the first results obtained for TTX and its analogues in mussels and oysters samples.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-153

Cytotoxic constituents from the Korean benthic Marine Dinoflagellate *Prorocentrum lima*

J.-R. Rho [1], S. Lee [1,*], W. Yih [1], Y. D. Yoo [1]

[1]Kunsan National University, Gunsan, Republic of Korea

Abstract: Marine dinoflagellates have been paid attention because they produce a variety of structurally complex and biologically toxic secondary metabolites. Among them, *Prorocentrum* is a respresentative genus that produces diarrhetic shellfish poisoning (DSP) toxins, such as okadaic acid(OA) and dinophysistoxin-1(DTX-1) and their analogues. Apart from these compounds, skeletally diverse bioactive constituents have also been isolated from cultured *Prorocentrum* species, including prorocentrolide, spiro-prorocentrimine, hoffmanniolide, prorocentrun, formosalides A and B, belizentrin and prorocentrol.

Recently, in our search for bioactive compounds from cultured Korean *P. lima*, a new polyketide, limaol and two new prorocentrolide derivatives, along with several OA/DTX-1 analogues, were isolated and their structures were determined by a combination of NMR spectroscopy and mass spectrometry. Furthermore, the configurations of new compounds were elucidated on the basis of ROESY correlations, *J*-based configuration analysis and chemical reactions. They showed strong cytotoxicity against HepG2, HCT-116 and Neuro2a cancer cell lines.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-154

In-house method validation of radio-receptor binding assay for paralytic shellfish toxins using porcine brain

C. O. Mendoza [1,*], R. S. Tabbada [1], M. L. Ranada [1], M. L. Romero [2]

[1]IAEA Collaborating Center on Harmful Algae, Chemistry Research Section, Philippine Nuclear Research Institute – Department of Science and Technology (DOST-PNRI), [2]Bureau of Fisheries and Aquatic Resources – Department of Agriculture (DA-BFAR), Diliman 1101 Quezon City, Philippines

Abstract: Radio-receptor Binding Assay (RBA) for the analysis of paralytic shellfish toxins in shellfish is an AOAC - International official method of analysis (AOAC Official Method 2011.27) as of 2011. However, the method uses rat brain membrane as receptor source. In this study, the RBA method was modified and validated using the commercially available porcine brain synaptosome which comes with the Saxitoxin kit for RBA testing. Assay parameters describing accuracy, precision, recovery, dynamic range, linearity, limit of detection and limit of quantitation, were established by a single analyst obtaining a variability of $\leq$ 30% (n=5 days). The standard concentrations used in the validation were chosen based on the Philippine's regulatory limit for closure of harvest areas which is at 60 µg Saxitoxin-equivalent per 100 grams shellfish meat. Naturally contaminated mussels and cockles were subjected in the validation for matrix analysis. The uncertainty budget of the assay was also determined using Kragten method.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-155

The occurrence of and effects of processing on 40+ azaspiracids in British shellfish

A. O'neill [1,*], B. Maskery [1], A. Turner [1]

[1]Food Safety, Cefas, Weymouth, United Kingdom

Abstract: Azaspiracids (AZAs) were first reported in 1998 and found to be a food safety issue after being linked to an intoxication event in the Netherlands following the consumption of mussels originating from the West coast of Ireland. In compliance with EU regulations of live bivalve molluscs from active shellfish harvesting beds, three specific azaspiracid analogues (AZA 1, 2 and 3) are monitored in Great Britain (GB) for routine official control monitoring. Since implementation of the LC-MS/MS method for lipophilic toxins in replacement of the biological assay in July 2011, analysis has been conducted throughout GB to assess the levels of AZA1, 2 and 3. However, it is well known that over 50 different AZA analogues have been discovered, some of which have been shown to be of a higher toxicity than AZA1, 2 or 3.

Without methods for monitoring these additional analogues, there is the potential for under-estimation of total AZA toxicity. In this study, we analysed official control shellfish samples, all of which contained total AZA toxicity above the regulatory limit of 160 µg/kg AZA1 equivalents, for 42 of these additional AZA analogues. Standards for AZA4-10, 33 and 34 allowed for the development of a quantitative method for these analogues. The method was also extended to include the semi-quantitation of AZA11-32, 35-39, 41 and 43-47.

This work will present the findings from this study and discuss the potential impact on food safety due to the potential increase in toxicity. 21 of the 42 analogues were detected in the samples with AZA17 having the highest concentration of all the new analogues at >8% of the total AZA. The effects of processing on the profile of these analogues will also be discussed with focus on the conversion of toxins, including the effect processing time has on conversion rate within the bivalve tissue. During processing there was a change in the concentrations of several of the analogues, with some including AZA17 not detected following 10 minutes of processing at 100°C whereas there was an increase in AZA6 and some of the other analogues.

The project has highlighted the potential need for the routine monitoring of some of these additional analogues, as well as highlighting the effect processing could have on the potential difference in toxicity between raw and cooked shellfish. Further toxicity studies are needed before the risks associated with AZA's can be fully assessed.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-156

Identification of [Asp3]MC-ER in a culture of *Planktothrix prolifica*

V. Mallia [1], S. Uhlig [1,*], C. M. Rafuse [2], C. O. Miles [2]

[1]Toxinology Research Group, Norwegian Veterinary Institute, Oslo, Norway, [2]Measurement Science and Standards, National Research Council Canada, Halifax, Canada

Abstract: Microcystins are a group of more than 250 hepatotoxic heptapeptides produced by a range of cyanobacterial species. Although the 2-and 4-positions of microcystins are highly variable, microcystins containing glutamic acid at position-2 have only been reported in a single strain of *Anabaena* that produced a range of MC-EE congeners. Recently, during LC-MS analysis of a culture of *Planktothrix prolifica*, we detected a minor candidate microcystin with $[M+H]^+$ 997.5, in addition to the major microcystin [Asp^3]MC-RR that had been reported previously. LC-HRMS/MS studies involving derivatization with mercaptoethanol, esterification, and ^{15}N-labelling, together with detailed examination of its product ion spectra, revealed the minor compound to be the previously unreported microcystin [Asp^3]MC-ER. This is the first report of a microcystin containing glutamic acid and arginine at positions 2 and 4, underscoring the biosynthetic flexibility of microcystin biosynthesis in cyanobacteria, and the power of combining LC–MS/MS techniques with functional-group-specific derivatizations.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-157

Multiclass Analysis of Polar Marine Toxins by Capillary Electrophoresis-Tandem Mass Spectrometry

D. Beach [1], E. Kerrin [1], K. Thomas [1], M. Quilliam [1], P. McCarron [1,*]

[1]National Research Council Canada, Halifax, Canada

Abstract: Natural toxins in the marine environment can be broadly classified as either lipophilic or polar. The latter includes three classes of potent neurotoxins; paralytic shellfish toxins (PSTs), tetrodotoxins (TTXs) and domoic acid (DA). Because of poor retention in reverse phase LC, low *m/z* and ionization suppression, polar toxins have been more challenging to analyze by LC-MS than lipophilic toxins. Due to its suitability for separating polar, charged analytes, a capillary electrophoresis-tandem mass spectrometry (CE-MS/MS) method was developed for simultaneous quantitative analysis of PSTs, TTXs and DA in seafood and environmental samples.

A custom sheath-flow interface was designed to couple the CE instrument to electrospray ionization-MS/MS. A novel, highly acidic background electrolyte (5 M formic acid) was designed to give the best combination of resolution, sensitivity and compatibility with the largest number of toxins. This CE method was combined with previously developed MS/MS parameters and applied to a wide range of standards, mussel tissue matrix reference materials and spiked samples.

The developed methodology shows excellent resolution of toxins, including all potentially interfering or isomeric PSTs and TTXs examined. Good resolution was also observed between analytes, salts and amino acids present in the matrix. Good performance and reproducible relative migration times were observed for standards and in mussel and pufferfish tissue extracts where only a 1:3 dilution with acetonitrile was required as part of sample preparation. Matrix effects were found to be minimal as long as a combination of internal standards was used to correct for differential analyte loading depending on mobility. Good linearity ($R^2 > 0.99$) and LODs (1.8 to 160 µg/kg) were observed in mussel matrix, making the method suitable for analysis of toxins in real samples below current regulatory limits. Precision and accuracy was assessed using mussel tissue matrix certified reference materials for PSTs and DA and control pufferfish tissue spiked with calibration solution CRMs for TTX and PSTs.

Initial work on transferring the developed methodology to a commercial CE-MS/MS system has been promising and a more in-depth optimization is under way. The orthogonality of the CE separation to both hydrophilic interaction LC and reverse phase LC separations used traditionally for these analytes has made the technique useful in characterizing new multi-class mussel tissue reference materials for polar marine toxins under development at the National Research Council Canada.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-158

Characterization of novel halogenated natural products in *Prymnesium polylepis*

Y. Lu [1,*], S. Rasmussen [2], S. B. Binzer [3], L. Soman de Medeiros [4], P. Juel Hansen [3], T. O. Larsen [2]

[1]Department of Biotechnology and Biomedicine, Technical University of Denmark, [2]Department of Biotechnology and Biomedicine, Technical Univeristy of Denmark, Kgs. Lyngby, [3]Department of Biology, University of Copenhagen, Helsingør, Denmark, [4]Departamento de Química, Universidade Federal de São Paulo , São Paulo , Brazil

Abstract: Several *Prymnesium* species are known to cause harmful algal blooms in marine ecosystems worldwide. *P. parvum* is by far the most common species associated with massive fish-kills. However several incidents, such as the extensive bloom in Skagerrak (waters in between Denmark, Sweden and Norway) in 1988, has been associated with blooms of *P. polylepis* (formerly *Chrysochromolina polylepis*). This species is known to produce lytic substances, however these compounds have not been chemically characterized yet, which has been our motivation for this study. A strain originating from the 1988 bloom in Scandinavian coastal waters was cultured and extractions were been made for general chemical analysis as wells as for the use in bioguided screening for lytic ichthyotoxins using a fish gill bioassay. Extracts of *P. polylepis* cell material exhibited toxicity in the RTgill-W1 cell bioassay, however the toxicity was scattered to several fractions, making it impossible to pinpoint a major single toxic compound. Spectroscopic analysis based on LC-HRMS identified several polychlorinated metabolites due to their distinct isotopic patterns. One of these metabolites was purified in enough quantities to obtain NMR data allowing for a full structure elucidation of a penta-chlorinated metabolite currently named polylepsin A. Altogether our work for the first time report on new chemistry from *P. polylepis*, however further studies are needed to clarify whether several different types of compounds act in concert for *P. polylepis* to have lytic and ichthyotoxic effects.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-159

ENHANCING HARMFUL ALGAL MONITORING USING LOW COST REAL-TIME SENSING TECHNOLOGIES

T. R. Miller [1], M. Smith [2,*]

[1]Zilber School of Public Health, [2]School of Freshwater Sciences, University of Wisconsin - Milwaukee, Milwaukee, USA

Abstract: Harmful algal blooms (HAB) are responsible for deteriorated water and ecosystem quality and may also impact public health through the production of algal toxins. In situ aquatic sensors coupled with data telemetry enable near real-time detection of a variety of physical, chemical or biological parameters that may influence bloom formation and toxin production. Typically, these sensor systems have high investment cost which results in the reduction of the sensor node coverage in an area, or the expansion of sensor systems into new geographical locations. We have applied a low cost embedded electronics platform with inexpensive sensors to report water quality parameters in real time. The inexpensive sensor system is capable of measuring temperature at multiple depths and can be configured to interface with numerous commercial sensors. The system is capable of both local data storage and data transmission over cellular networks. The resulting system can be constructed at costs an order of magnitude less than a comparable commercially available system. Using a citizen science driven effort to monitor HAB events in Tug Lake (Wisconsin, USA), we have coupled high resolution data acquisition from the system to laboratory based analysis (HAB toxins, phosphorous). It is anticipated that this research will be of direct benefit to existing in situ sensor systems, particularly for end users with limited budgets.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-160

Survey of Historical Irish Shellfish Samples for Tetrodotoxin (TTX).

C. Bailey [1], C. Duffy [1,*], J. Silke [1]
[1]Marine Institute, Rinville, Oranmore, Co., Galway, Ireland

Abstract: Tetrodotoxin (TTX) is a potent marine neurotoxin, produced by bacteria including Vibrio spp. TTX can be found in certain fish species but also marine gastropods and bivalves. There are no health-based guidance values for TTX worldwide and also no legislated maximum levels of TTX in seafood in the European Union (EU).TTX has been detected in shellfish in European countries as far north as the South Coast of England. This recent discovery and also the publishing of the EFSA opinion on TTX, lead the Irish Authorities to conduct a survey of Irish shellfish.Archived shellfish samples that were collected as part of the National Biotoxin Monitoring Programme were tested for TTX. The samples were analysed for the main TTX analogue by LC-MS/MS following acetic acid (1%) extraction and clean up by SPE cartridges. Samples were selected from the majority of production sites around the coast of the Republic of Ireland. Where samples were available, one sample for the months of June, July, August and Sept was selected for analysis from each site. All available gastropods were also tested. In total 508 samples were tested spanning the sampling period 2012 to 2016, with the majority of sample belonging to 2015 and 2016 period. No samples were positive for TTX in any of the samples tested.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-161

MS/MS-Based Molecular Networking Approach for the Detection of Aplysiatoxin-Related Compounds in Environmental Marine Cyanobacteria

C. Y. G. Ding [1], L. M. Pang [2], Z. X. Liang [2], K. K. K. Goh [3], E. Glukhov [4], W. H. Gerwick [4], L. T. Tan [1,*]

[1]Natural Sciences and Science Education, National Institute of Education, [2]School of Biological Sciences, [3]Singapore Phenome Centre, Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore, [4]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, USA

Abstract: Cyanobacteria produce a wide array of cyanotoxins, such as microcystins, lyngbyatoxin A and aplysiatoxins, that are associated with human health issues. In this pilot study, the combined LC-MS/MS and molecular networking platform approach was employed as a rapid method to detect aplysiatoxins from four environmental marine cyanobacterial samples in Singapore. Based on 16S-ITS rRNA gene sequences, marine cyanobacterial strains, collected from Pulau Seringat Kias and P. Hantu Besar, were identified as Trichodesmium erythraeum while those from P. Hantu Besar were revealed as Oscillatoria sp. PAB-2 and Okeania sp. PNG05-4. Organic extracts of the microalgal samples were prepared and analysed on LC-HRMS/MS and Global Natural Product Social Molecular Networking (GNPS) for the presence of aplysiatoxin-related compounds. From molecular networking, six known compounds, including debromoaplysiatoxin (1), anhydrodebromoaplysiatoxin (2), 3-methoxydebromoaplysiatoxin (3), aplysiatoxin (4), oscillatoxin A (5) and 31-noroscillatoxin B (6), as well as potential new analogues were detected in these samples based on HRMS data. In addition, differences and similarities in molecular networking clusters related to the aplysiatoxin molecular family were observed in extracts of T. erythraeum collected from two different locations and from different microalgal species found at Pulau Hantu, respectively. The use of the MS-based molecular networking approach in this study has shown to be an effective and quick method for the detection of aplysiatoxins. Such method could potentially be extended to the detection of other classes of cyanotoxins from environmental samples. (Message from the system admin: Co-authers will be added soon)

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-162

Determination of Gonyautoxin-4 in Echinoderms and Gastropod Matrices by Conversion to Neosaxitoxin

M. Silva [1 2], V. Rey [3], A. Botana [3], L. Botana [4], V. Vasconcelos [1 2,*]

[1]Department of Biology, Faculty of Sciences, University of Porto, Porto, [2]Interdisciplinary Center of Marine and Environmental Research–CIMAR/CIIMAR, University of Porto, Matosinhos, Portugal, [3]Department of Analytical Chemistry, Science Faculty, University of Santiago of Compostela, [4]Department of Pharmacology, Veterinary Faculty, University of Santiago de Compostela, Lugo, Spain

Abstract: Harmful Algal Blooms (HABs) are complex to manage once the conditions that promote them are yet to be fully understood, though climate change and anthropogenic intervention are pointed as significant factors. These phycotoxins can accumulate along the food-web, causing human poisoning incidents, affecting the fishery and tourism sectors and generating anoxic zones resulting in huge marine life fatalities. Regarding to public health and due to the intermittent nature of blooms the establishment of international regulations and guidelines based on periodic monitoring in shellfish areas through phytoplankton counting and the testing of bioaccumulation of toxins in bivalves has led to poisoning incidents becoming scarce, and mainly due to non-compliance with the rules on prohibition of consumption and harvest by the competent authorities' national health systems. However, vector species that are not normally monitored, such as gastropods, echinoderms, crustaceans, and fish, should be included in risk assessments since other toxin uptake routes, apart from filter feeding, as previous work proved the bioaccumulation and biomagnification of phycotoxins in the groups not normally monitored.In this work paralytic shellfish toxins (PSTs) were screened in a total of 29 different species of benthic vectors (echinoderms, bivalves, gastropods, crustaceans and fish), in Azores and Madeira archipelagos and the Moroccan Coast. These toxins can be monitored by a variety of techniques, and the post-column oxidation liquid chromatography with fluorescence detection method (PCOX), validated in 2011 by the Association of Official Analytical Chemists (AOAC International), has been shown to be a reliable method for monitoring PSTs in bivalve species. Nevertheless, due to its high sensitivity to naturally fluorescent matrix interferences, when working with unconventional matrices, there may be issues in identifying toxins because of naturally fluorescent interferences that co-elute with the toxin peaks, leading to erroneous identification and quantification. In this study, we faced matrix interferences that hinder detection of GTX4 in both echinoderm and gastropod matrices. In order to overcome this challenge, and based in the assumption that some of the GTXs have been shown to be transformed reductively into STX or NEO by biologically available thiol reagents, we tested and optimized the conversion of Gonyautoxins 1 and 4 to Neosaxitoxin with 2-mercaptoethanol. We present a new, economic and less time-consuming method with a good recovery (82.2%, RSD 1.1%, n = 3), requiring only a single reaction step.

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-163

Analysis of PSP toxins in Moroccan shellfish by MBA, HPLC and RBA Methods: monitoring and research

R. Abouabdellah [1,*], A. Bennouna [1], M.-Y. Dechraoui Bottein [2], J. El Attar [1], S. Benbrahim [3]

[1]National Institute of Fisheries Research, Aghsdis New Port, Agadir, Morocco, [2]International Atomic Energy Agency, Environment Laboratories Monaco, Monaco, Monaco, [3]National Institute of Fisheries Research, Casablanca, Morocco

Abstract: Paralytic shellfish poisoning toxins (PSTs) are secondary metabolites of some toxic species of phytoplankton, the consumption of bivalve shellfish having accumulated these toxins can lead to neurotoxic food poisoning or even death.

Cases of toxicity by PSP toxins occur regularly along the Moroccan and have been responsible for severe cases of intoxication early 90s. in recent years, the southern Atlantic coasts have been particularly affected by strong episodes of toxicity and more frequent occurrence of phytoplankton toxic species. Within the Moroccan statutory shellfish biotoxin monitoring programme established by National Institute of Fisheries Research (INRH), a study of PSTs was conducted from 2004 to 2018 in south Moroccan's shellfish, two species were studies mussels (*Perna perna*) sampled from south Agadir region and razor clam (*Solen marginatus*) from Dakhla bay. In parallel, monitoring of toxic phytoplankton in sea water was conducted. The aim of this study was to investigate the agreement between three different methods; liquid chromatography with post column derivatization and fluorescence detection (HPLC-FD), Receptor Binding Assay (RBA) and AOAC mouse bioassay (MBA) for determining PSP toxicity in Moroccan shellfish.

The PSP toxicity was associated with *Alexandrium cf. minutum* identified in seawater of south Moroccan costs. These toxic episodes were widely distributed in time and space and mainly detected during the summer and fall season, toxicity was exceeded in several time the sanitary threshold (800 µg eq STX /kg). The PSP toxin profile identified by HPLC was dominated by gonyautoxins 2, 3 and a minority of saxitoxin. Comparison between the different methods used in PSP toxin analysis revealed that MBA data were statistically significantly correlated with RBA (Pearson r = 0.809) and with HPLC data (Pearson r = 0.79).

Disclosure of Interest: None Declared

Toxin analysis – Novel detection methods

P-164

SURVEY OF ALGAL TOXINS IN THE ARABIAN GULF OFF THE COAST OF QATAR- STATUS IN 2017

A. Al Muftaha [1,*], Y. Nasr [1], M. Yilmaz [2]

[1]Department of Biological and Environmental Sciences, Qatar University, Doha, Qatar, [2]Department of Bioengineering, Bursa Technical University, Bursa, Turkey

Abstract: Arabian Gulf provides important ecosystem services to the surrounding countries such as drinking water through desalination and seafood supply. However, it is also under extreme stress due to coastal dredging, oil pollution, reduced freshwater input and intense maritime traffic. Harmful algae species are common and massive algal blooms were previously recorded in this stressed ecosystem. In this study, two cruises were performed in October and December of 2017 and samples were collected from 8 sites off the coast of Qatar in the Arabian Gulf. These samples were analyzed for 48 different algal toxins with liquid chromatography tandem mass spectrometry and 11 toxins were detected in the analyzed samples. Domoic acid were present in three sites from October concomitant with the presence of *Pseudo-nitzschia* species. Gymnodimine was present in seven sites in October and two sites in December. *Karenia selliformis* was not observed in any sample, therefore the source of gymnodimine in the Arabian Gulf is not known. Pectenotoxin 2 was observed in 3 sites in October and in 1 site in December. On the other hand, pectenotoxin seco acids were present in 5 sites in October and 7 sites in December. Potential producers of pectenotoxins, *Dinophysis* species, were present in these samples. While pinnatoxin G was present in 7 sites, pinnatoxin H was present in all sites of the October samples. Pinnatoxin G was absent in December samples, however pinnatoxin H was observed in all sites of December samples. The only known producer of pinnatoxins, *Vulcanodinum rugosum*, was observed in all samples. Paralytic shellfish toxins (PSTs) were not observed in December samples. On the contrary 4 PSTs including gonyautoxin 5, decarbamoylsaxitoxin, saxitoxin and neosaxitoxin (NEO) were present in all samples of October, except for one site for NEO. Potential PST producers *Pyrodinium bahamanse* and *Alexandrium* sp. were present in these sites.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-165

Cytotoxic cyanobacterial lipopeptides puwainaphycins and minutissamides: from outsiders towards widespread potential toxins

A. Kust [1 2,*], J. Mareš [1 2], M. Bártová [1], K. Čapková [1], D. Long Vu [2], D. P. Fewer [3], A. Mattila [3], O. M. Skulberg [4], I. Mysterud [5], K. Sivonen [3], P. Hrouzek [2]

[1]Biology Centre, Institute of Hydrobiology, The Czech Academy of Sciences, České Budějovice, [2]Center Algatech, Institute of Microbiology CAS, Třeboň, Czech Republic, [3]Department of Microbiology, University of Helsinki, Helsinki, Finland, [4]Norwegian Institute for Water Research (NIVA), [5]Department of Biosciences, University of Oslo, Oslo, Norway

Abstract: Puwainaphycins (PUW) and minutissamides (MIN) are structurally analogous cyclic lipopeptides, which exhibit toxicity against human cancer cells. We have identified the biosynthetic gene clusters in six cyanobacterial strains, characterizing the MIN gene cluster for the first time.

A comparison of the biosynthetic gene clusters indicated common origin of the hybrid nonribosomal peptide synthetase and polyketide synthase. Intriguingly, the PUW/MIN biosynthetic gene clusters encode two alternative biosynthetic starter modules. The first starter module is a standalone fatty acyl-AMP ligase (FAAL), while the second starter module contains a FAAL domain fused to an acyl carrier protein. We further detected a putative novel halogenase gene and an *O*-acetyltransferase gene corresponding to chemical modifications in the detected products. All gene clusters were detected in soil-inhabiting cyanobacteria, including one of the producer strains suspected to play a role in the development of the serious haemolytic Alveld disease among outfield grazing sheep in alpine mountains of coastal Norway. However, with the designed universal primers we have detected the PUW/MIN genes in samples of freshwater cyanobacterial blooms in fishponds in Czech Republic. The results of the PCR detection were proved by a specific liquid chromatography-mass spectrometry method developed in our lab. With these results we can suggest that the prevalence of cyanobacterial lipopeptides such as PUWs and MINs in freshwaters and soil is likely wide and deserves further attention.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-166

Usefulness of column switching HILIC-MS and 15N-labeled inorganic nitrogen for the study of STX biosynthesis in dinoflagellates

Y. Cho [1,*], S. Tsuchiya [1], T. Omura [2], K. Koike [3], H. Oikawa [4], K. Konoki [1], Y. Oshima [5], M. Yotsu-Yamashita [1]
[1]Graduate School of Agricultural Science, Tohoku University, Sendai, [2]Laboratory of Aquatic Science Consultant Co.,LTD., Tokyo, [3]Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, [4]National Research Institute of Fisheries Science, Yokohama, [5]Graduate School of Life Sciences, Tohoku University, Sendai, Japan

Abstract: The biosynthetic pathway toward saxitoxin (STX) and its analogues in cyanobacteria was proposed by genetic study and was chemically confirmed by the incorporation study using the stable isotope labeled biosynthetic intermediates, Int-A’, Int-C’2, 11-hydroxy-Int-C’2 and Int-E’, prepared by chemical synthesis [1]. The presence of the same biosynthetic intermediates as those found in cyanobacteria in the toxic sub-clone and their absence in the non-toxic sub-clone of dinoflagellate, *A. tamarense* suggested the similarity of the STX biosynthetic pathway in dinoflagellates to that in cyanobacteria [1]. However more evidences were needed to confirm these results. Column switching and two-step gradient elution using hydrophilic-interaction chromatography combined with high resolution mass spectrometry was developed for the simultaneous analysis of the primary STX analogues and their biosynthetic intermediates in dinoflagellates [2]. In this study, it was applied to two experiments; 1. the analysis of the biosynthetic intermediates in the two toxic and two non-toxic dinoflagellate species other than *A. tamarense* and 2. the observation of ^{15}N incorporation in the biosynthetic intermediates and STX analogues using ^{15}N-labeled sodium nitrate as the nitrogen source for dinoflagellates. As a result, STX biosynthetic intermediates and the shunt product were detected only in the extracts of the toxic species (*A. catenella* and *Gymnodinium catenatum*) and were not detected in those of the non-toxic species (*A. insuetum* and *Prorocentrum triestinum*). By the ^{15}N-labeled sodium nitrate incorporation study, arginine showed the highest ^{15}N incorporation followed by Int-A’, GTX5, GTX4 and C2 on 3rd and 6th day after the addition of ^{15}N-labeled medium. These results supported the biosynthesis of STX analogues in dinoflagellates would proceed as same as in cyanobacteria. The change of arginine metabolism between 6th and 10th day was suggested by the analysis of isotopomer distribution. The usefulness of the incorporation study using ^{15}N-labeled inorganic nitrogen in combination with the column switching HILIC-MS for the study of STX biosynthesis was indicated.

Reference

1. Tsuchiya, S. and Yotsu-Yamashita M. *et al. Angew. Chem. Int. Ed.* **56,** 5327–5331 (2017).
2. Cho, Y. *et al. J. Chromatogr. A* **1474,** 109–120 (2016).

Y. Oshima: Professor emeritus

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-167

Innovative metabolomic workflows to study growth kinetics dinoflagellate cysts revived from modern and ancient sediment

F. Mondeguer [1,*], F. Souard [2 3], Y. Guitton [4], P.-M. Allard [5], M. Sibat [1], M. Latimier [6], J. Quere [6], J.-L. Wolfender [7], R. Siano [6], P. Hess [1]

[1]Ifremer, DYNECO/PHYC, Centre Atlantique, Nantes, [2]Département de Pharmacochimie Moléculaire, CNRS UMR5063, Université Grenoble Alpes, St Martin d'Heres, France, [3]Laboratoire de Pharmacognosie, de Bromatologie et de Nutrition Humaine, Université Libre de Bruxelles, Brussels, Belgium, [4]LABERCA, Oniris, INRA, Université Bretagne-Loire, Nantes, France, [5]School of Pharmaceutical Sciences (EPGL), University of Geneva, University of Lausanne, Centre Médical Universitaire (CMU), Geneva, Switzerland, [6]DYNECO/Pelagos , Ifremer - Centre de Bretagne, Plouzané, France, [7](5) School of Pharmaceutical Sciences (EPGL), University of Geneva, University of Lausanne, Geneva, Switzerland

Abstract: Aquatic ecosystems worldwide have been negatively affected by eutrophication, many of them driven by increasing nutrient inputs from untreated domestic sewage and industrial and agricultural wastewater. During their life cycle, *Alexandrium minutum* and *Scrippsiella donghaienis* can produce resistant and revivable cysts that can accumulate in the sediments of the coastal marine environment for hundreds of years. As a working hypothesis, we presumed that these two genera have preserved their adaptation to such ecosystem changes in the form of cysts in the old sediments. From revivified cysts, some cultures of toxic and non-toxic dinoflagellates (*A. minutum* and *S. donghaienis*) were analyzed by a metabolomic approach to study the possible adaptive responses of phytoplankton to these modifications of coastal ecosystems.

Strains of *Scrippsiella donghaienis* were obtained from sediments isotopically dated to 1986 (ancient) and 2006 (modern) while those of *A. minutum* dated from 1996 (ancient) and 2006 (modern). In total 84 samples were obtained, with each experimental condition (combinations of species, age, growth phase) being cultured in triplicate in phosphate depleted conditions. Extracts of different growth phases of the ancient and modern strains of *A. minutum* and *S. donghaienis* were analyzed by liquid chromatography coupled to high resolution mass spectrometry and retreated using a workflow on a wide variety of hydrophilic and lipophilic metabolites. More specifically, two workflows have been used for univariate hypothesis testing and PCA multivariate modelling on: (i) a chemometrics platform, Agilent Mass Profiler Professional (MPP). Metabolic fingerprints of the various stages made it possible to highlight the distribution of the common and / or age-specific metabolites of the culture but also those common and specific to the different growth phases (exponential or stationary and supplemented or limited in phosphorus), and (ii) a collaborative portal dedicated to metabolomic data processing, analysis and annotation "Workflow4Metabolomics" consolidate the metabolites of the physiological and adaptive responses obtained. It is interesting to note that independent of the workflow used (MPP or Galaxy), metabolomic profiles of cultures revived from different ages were more different than those of different growth phases. While *A. minutum* did not show specific metabolites for the different growth phases, *S. donghaienis* contained specific metabolites for all growth phases, including P deficiency linked metabolites. A molecular network analysis gave an increased understanding of these metabolites produced in phosphate deficiency condition.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-168

A metabolomics approach to investigate the response of *Navicula mollis* P-91 to salinity and irradiance stresses

D. Réveillon [1 2,*], A. Tunin-Ley [3], J. Turquet [3], J.-F. Briand [1], G. Culioli [1]

[1]MAPIEM EA 4323, Université de Toulon, 83957 La Garde Cedex, [2]Permanent address: Ifremer, Phycotoxins laboratory, F-44311 Nantes, [3]Hydrô Réunion, 97490 Sainte Clotilde, France

Abstract: In this study we used an LC-MS-based metabolomics approach to determine the response of *Navicula mollis* P-91 to changes in salinity and irradiance levels. This strain, isolated from a tropical biofilm (Phytobank, Hydrô Réunion), was chosen for its interesting antifouling activity within the BioPainTrop project (ANR-12-CFII-0008). We cultivated *N. mollis* in controlled conditions using five different irradiance levels (10, 40, 100, 150, and 200 μmol m^{-2} s^{-1}) and five salinities (10, 22, 33, 44 and 55) for 15 days (n = 3). Methanolic endometabolome extracts were analyzed by ultra-performance liquid chromatography coupled to a high resolution mass spectrometer (UPLC-ESI-QToF, Bruker Impact II) using both full scan (*m/z* 50-1200) and auto-MS/MS acquisition modes. Data were processed using XCMS under R, filtered and analyzed with MetaboAnalyst 3.0. Non-supervised analysis (PCA) for the effect of irradiance showed five clusters of samples corresponding to each irradiance level. They were separated on the first component of the PCA (accounting for 38.3% of the total variance). Concerning the salinity, only three clusters were noted. On the PCA score plot, while the first component (78.8% of the total variance) separated the lowest level of salinity, the second one (8.2% of the total variance) separated the other two clusters (one for salinities 22, 33 and 44 and one for salinity 55). Subsequent PLS-DA analyses for the two factors showed both excellent explained variation and prediction performance of the models ($R^2 > 0.94$ and $Q^2 > 0.90$). We were then able to highlight potential biomarkers that could explain the progressive physiological acclimation of *N. mollis* in response to these two different stresses. Their identification for further biological interpretation is in progress (using classical dereplication and molecular networks). Diatoms of the genus *Navicula* are particularly known as fouling species (*i.e.* formation of biofilms on any submerged surface). However, the LC-MS-based metabolomics approach we have implemented here can serve as a proof-of-concept for its use in the study of benthic toxic microalgae responses to abiotic or biotic stresses (e.g *Ostreopsis* or *Gambierdiscus* spp.).

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-169

Transcriptomic responses of bay scallop (*Argopecten irradians*) larvae exposed to harmful algae and other coastal zone stressors

A. Griffith [1,*], M. Harke [2], D. Berry [1], C. Gobler [1]

[1]School of Marine and Atmospheric Sciences, Stony Brook University, Southampton, [2]Lamont-Doherty Earth Observatory, Columbia University, Palisades, USA

Abstract: While there is a large body of knowledge regarding the negative impacts of harmful algal blooms with regard to the growth and survival of marine life, less in understood concerning sub-cellular (i.e. biochemical/physiological pathways) responses. Here, we identify and compare unique and common global transcriptomic responses of *Argopecten irradians* (bay scallop) larvae exposed to two species of harmful algae, *Cochlodinium* (*Margalefidinium*) *polykrikoides* (rust tide) and *Aureococcus anophagefferens* (brown tide) using high-throughput sequencing (RNA-seq). In addition, subsets of larvae were exposed to acidification and thermal stress to compare responses between abiotic and biotic stressors. Global transcriptome profiles from each larval treatment revealed distinct expression profiles with larvae exposed to both species of harmful algae displaying broader responses in terms of unique transcripts significantly and differentially expressed (44,922 and 4,973; respectively), than larvae exposed to either low pH or elevated temperature (559 and 467; respectively). Patterns of expression between the two algal treatments, however, were strikingly different with larvae exposed to *A. anophagefferens* displaying large, significant declines in the expression of multiple transcripts (n = 3,615) whereas exposure to *C. polykrikoides* stimulated substantial increases in expression of more transcripts than all other treatments combined (n = 43,668). Strong up-regulation of biochemical pathways associated with defense against reactive oxygen species (ROS) in larvae exposed to *C. polykrikoides* affirmed this as a prime toxigenic pathway of this alga. In contrast, the down-regulation of multiple gene pathways upon exposure to *A. anophagefferens* suggest metabolic suppression in larvae which may ultimately contribute to mortality. Beyond these unique treatment responses, larvae within all stressor treatments up-regulated a common set of genes associated with protein synthesis, cellular metabolism, shell growth, and membrane transport. Collectively, this study reveals the common and unique biochemical responses of bivalve larvae exposed to harmful algae and other stressors common to coastal zones, providing insight into the means by which they promote mortality.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-170

Metabolomics applications for the chemotaxonomy of non-toxic *Ostreopsis* strains

M. Garcia-Altares [1], J. Diogene [2], M. Fernandez [2], I. Keith [3], O. Carnicer Castaño [4,*]

[1]Department of Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knöll-Institute (HKI), Jena, Germany, [2]Institut de Recerca i Tecnologia Agroalimentària, Sant Carles de la Ràpita, Spain, [3]Charles Darwin Fundation, Galápagos, [4]Escuela de Gestión Ambiental, Pontificia Universidad Católica del Ecuador, Esmeraldas, Ecuador

Abstract: The correct identification of toxic dinoflagellates is crucial for public health protection. The taxonomy of the toxic genus *Ostreopsis* has been traditionally based on morphological features, such as shape, plate pattern and cell sizes. However, the similarity and overlap of these traits among different *Ostreopsis* species has led to ambiguities and possible misidentifications. The comparative analysis of metabolic features of dinoflagellates could provide a valuable tool to assist species characterization and taxonomical classification, but chemotaxonomy has not been fully explored in dinoflagellates and has neglected non-toxic species and strains. We investigated the chemical diversity of 16 *Ostreopsis* strains from the Galapagos Marine Reserve: 13 samples of *O.* cf. *ovata* and three strains of *O. lenticularis*. The strains were analyzed in full scan mode by liquid chromatography-high resolution mass spectrometry (LC-HRMS) to confirm the absence of palytoxin-like compounds (since the strains were non-toxic in haemolytic assays). The metabolic features detected in the full scan LC-HRMS analysis were processed by an untargeted metabolomics workflow applying chemometrics methods such as principal component analysis. We found that the clustering of the strains based metabolic similarity was congruent with the species taxonomical classification. We studied the non-palytoxin compounds in the range of 700 to 2,000 Da that drive the chemical difference between *O.* cf. *ovata* and *O. lenticularis* and attempted the identification of two metabolites that have been not reported before. We conclude that metabolomics can assist the revision of *Ostreopsis* taxonomy, as well as lead to the discovery of new secondary metabolites from dinoflagellates from non-studied strains, such as non-toxin producers.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-171

Evolution of the Oxidative phosphorylation pathway in Dinoflagellates and sister taxa

J. Szymczak [1,*], E. Corre [2], L. Guillou [1], E. Kayal [1]
[1]UMR-7144, [2]ABiMS-FR2424, Station Biologique de Roscoff, Roscoff, France

Abstract: The mitochondrion is the site of chemical energy production (ATP) through the highly conserved oxidative phosphorylation pathway (OXPHOS). In recent years, variations in the OXPHOS pathway has been documented in a variety of organisms. Thanks to recent micro-eukaryotic transcriptomics and genomics projects, including the Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP), a large amount of data is now available for the analysis of various metabolic pathways in marine protists. The alveolates represent a major group of protists with a great diversity of lifestyles (phototrophy, mixotrophy, heterotrophy and symbiosis) and include, among others, ciliates, parasitic apicomplexes and dinoflagellates, some of which are responsible for some harmful algal blooms. Recent studies have suggested the modification or even the loss of key complexes of the OXPHOS pathway in Apicomplexa, and more recently, in Dinozoa. Here we characterized the main complexes of the OXPHOS pathway in more than 70 species of alveolates representing Dinozoa, Apicomplexa and less explored lineages such as Perkinsozoa (*Perkinsus marinus*) or Syndiniales (*Hematodinium, Amoebophrya*). To do so, we explored the annotated transcriptomes of the MMETSP project that we complemented with all the publicly available alveolate genomes. We cross referenced the presence/absence of various complexes of the OXPHOS pathway with organismal traits such as lifestyle, size and phylogeny in order to better understand the evolutionary mechanisms leading to the modification of this key metabolic pathway in Alveolata.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-172

Single-cell population genomics in harmful algal bloom species

R. Gollnisch [1,*], D. Ahrén [1], K. Rengefors [1]
[1]Department of Biology, Lund University, Lund, Sweden

Abstract: The invasive microalga *Gonyostomum semen* (Raphidophyceae) is known to cause nuisance blooms and has recently expanded in abundance across Europe by colonizing new habitats of diverse ecological characteristics and trophic status. In contrast, populations in North America appear to be less invasive and display less disruptive phenotypes despite presumably similar environments. By using a single-cell population genomic approach, we aim to explore detailed biogeographical dispersal patterns of *G. semen* and to discover the genetic basis of differentiation among populations in Europe compared to North America. Our understanding of habitat expansion and geographical dispersal patterns of harmful algal bloom species, analyzed using population genetic methods, is severely hampered by the dependency on culturing of clonal isolates. Many species cannot be cultured at all, and when possible, difficulties remain in acquiring sufficient samples, and the isolates may not accurately represent the sampled population. To investigate the genomic diversity within HAB species, we are developing a method to by-pass cultivation and thereby making direct genotyping of natural single-cell isolates possible. Specifically, we focus on techniques that are based on reduced representation sequencing (i.e. RADseq) used in population studies. This single-cell genomic approach will allow to assess the full width of genetic diversity and differentiation in natural samples, while circumventing the effort and bias of algal culturing, and thus obtain de novo genomes of uncultured lineages. Here, we present the results of alternative single-cell approaches and compare these to standard RADseq based on DNA extraction from cultured strains. While our focus for this work is on the freshwater raphidophyte *G. semen*, the method should be applicable to a range of HAB species, including dinoflagellates. Like the dinoflagellates, *G. semen* has a huge genome size (80-120 pg DNA per cell), making reduced-representation sequencing essential for a population genomic study.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-173

Systems-level analysis of the metabolic responses of the benthic dinoflagellate *Prorocentrum lima* to nitrogen limitation

D.-Y. Hou [1], X.-T. Mao [1], S. Gu [1], H.-Y. Li [1], J.-S. Liu [1], W.-D. Yang [2,*]

[1]Jinan University, Guangzhou, China, [2]Professor, Jinan University, Guangzhou, China

Abstract: *Prorocentrum lima* is a typical diarrheic shellfish poisoning (DSP) toxin-producing benthic dinoflagellate usually found attached to or associated with macrophytes, floating detritus, debris, or other substrates. In this paper, to gain a deeper understanding of the molecular and cellular responses to N limitation in *P. lima*, transcriptome profiling was performed. We found that most genes related to photosynthesis, porphyrin and chlorophyll metabolism were down-regulated following N limitation, while some genes concerning fatty acid biosynthesis, starch synthesis and ubiquitin pathway were up-regulated. Meanwhile, ABC transports ABCB1 and ABCG2 and glutathione S-transferase were up-regulated under N-limited conditions. These transcriptomic data were further corroborated by the biochemical analyses which revealed that starch, lipid content was increased, while photosynthetic efficiency was decreased. In addition, TEM analysis demonstrated that chloroplasts appeared smaller and were less abundant, while a major fraction of the cell volume was occupied by lipid bodies and starch granules, and thylakoid was twisted in the cells. Taking together, we proposed that N limitation could induce the accumulation of neutral lipid and starch in *P. lima* cells for carbon fixation trough recycling chloroplast membranes by autophagy. ABCB and ABCG transporters might be involved in the transport of DSP toxins. Our findings might provide global information for the response or adaptation of *P. lima* to N limitation.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-174

How are related the content in DMSP, saxitoxin and gene copies of sam and sxtA4 in *Alexandrium* spp strains?

S. Geffroy [1,*], M.-M. Lechat [1], F. Hervé [1], G.-A. Rovillon [1], F. Malo [1], L. Guillou [2], Z. Amzil [1], A. Caruana [1]

[1]Ifremer, Laboratoire Phycotoxines, Nantes, [2]CNRS, UMR 7144, Adaptation et diversité en milieu marin, Station Biologique de Roscoff, Roscoff, France

Abstract: Microalgae of the genus *Alexandrium* (Halim, 1960) are able to form blooms and may have health and climate impacts because they produce paralytic shellfish toxins (PSTs or saxitoxin and analogues) and dimethylsulphonioproprionate (DMSP). These two key molecules would come from the same precursor, methionine. Currently, the lack of genomic information on the dinoflagellate group seriously impedes a comprehensive understanding on how these compounds are synthesized. We selected two genes in these pathways: (i) the *sam* gene is a highly conserved gene involved in the production of the SAM protein, which represents a metabolic crossroads between the PSTs and the DMSP and (ii) the gene cluster *sxt*A with its domain 4 that would be discriminating in the PSTs production. In this study, we investigated the relationship between the content in PSTs, in DMSP and the genetic variability existing for the two genes in two *Alexandrium* toxic species, species occurring along the French coasts: *Alexandrium minutum* and *Alexandrium catenella*. To do this, saxitoxins and DMSP were analyzed by LC-FLD and LC-MS/MS respectively and gene copy numbers were determined by qPCR on cultures sampled in late exponential growth phase. This study provide a first comparison of intra- and interspecies variability in *sxtA4* and *sam* gene copy numbers as well as in the production of PSTs and DMSP and will contribute to a first necessary screening on several *Alexandrium minutum* and *Alexandrium catenella* strains.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-175

Transcriptomic study to unravel molecular mechanism of ciguatoxin production in *Gambierdiscus balechii*

Z. Wu [1 2,*], H. Luo [3], L. Yu [3], W. H. Lee [1 4], L. Li [3], Y. L. Mak [1 5], J. Wu [1 5], L. L. Chan [1 4 5], S. Lin [3 6], P. K. S. Lam [1 2 5]

[1]State Key Laboratory in Marine Pollution, [2]Department of Chemistry, City University of Hong Kong, Hong Kong, [3]State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, [4]Department of Biomedical Sciences, City University of Hong Kong, Hong Kong, [5]Shenzhen Key Laboratory for the Sustainable Use of Marine Biodiversity, Research Centre for the Oceans and Human Health, City University of Hong Kong Shenzhen Research Institute, Shenzhen, China, [6]Department of Marine Sciences, University of Connecticut, Groton, CT, USA

Abstract: Many benthic dinoflagellates of the genus *Gambierdiscus* are capable of producing ciguatoxins (CTXs) with polyether structures, which can be potentially bio-accumulated, bio-transformed and bio-magnified in marine food chains. Humans consuming coral reef fishes that have accumulated CTXs from ingested algal food sources might suffer ciguatera fish poisoning. In order to unravel the molecular mechanism of CTXs synthesis, we conducted transcriptome sequencing for both CTX-producing and non-CTX-producing *Gambierdiscus balechii*. Cultures of CTX-producing *G. balechii* (M1M10) strain and non-CTX-producing *G. balechii* strain (T6PRGD03N) were set up in L1 medium. Next generation sequencing of their transcriptomes and preliminary bioinformatics yielded 114,314 unigenes. Among them, 84642 (19.61% in total) genes showed differential expression between the two strains. Polyketides-related sequences were found in the differentially expressed gene dataset, including genes encoding keto reductase, acyltransferase, dehydratase, enoyl reductase and acyl carrier protein. Differential expression patterns of these transcripts were also analyzed. Furthermore, suppression subtractive hybridization was performed and the sequencing results also revealed differentially expressed genes between the two strains. These data suggest that polyketide synthase might contribute to polyketide synthesis and potentially CTXs synthesis in this dinoflagellate species. The dataset will also be a valuable genomic resource for research on the genus of *Gambierdiscus*.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-176

Molecular detection of *Azadinium spinosum* in Irish waters

A. Mccarthy [1,*], D. Clarke [1], S. mcgirr [1], J. silke [1], R. salas [1], P. hynes [1]

[1] Marine Institute, Galway, Ireland

Abstract: Azaspiracid toxins (AZAs) have been detected in Irish shellfish since 1995, and can lead to long term closures of shellfish farms around Ireland. A small armoured dinoflagellate, *Azadinium spinosum* is a known source of AZAs and is very difficult to distinguish taxonomically between *Heterocapsa* and other *Azadinium/ Amphidoma* species in water samples under a light microscope. Identification to species level is possible using a molecular method with species specific probes which were developed by the Alfred Wegener Institute in Germany. This method was used in the following two case studies.

Coastal surveys aboard the Celtic Voyager along the south, south west and west coast of Ireland were carried out in 2012, 2016 and 2017, to access the biological oceanography of *Azadinum*. A widespread distribution of *Azadinium spinosum* was identified when water samples gathered on these surveys were analysed using a real time polymerase chain reaction method (PCR).

A case study of Killary Harbour on the west coast of Ireland was carried out in 2013. To determine the number of *Azadinium* cells present in the water, samples were analysed under a light microscope and also by real time PCR with *Azadinium spinosum* probes. Liquid chromatography mass spectrometry was used to access the levels of AZAs present in samples of blue mussels, *(Mytilus edulis*). The results showed a correlation between an increase in the toxin levels and an increase in cell counts of *Azadinium.*

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-177

Transcriptome analysis of harmful algae *Alexandrium tamarense* and its algicidal bacteria during co-culture

Y. Jeong [1 2], H. Shin [1 2], S. Cho [1 2], E. Lee [1 2], S.-R. Ko [3], C.-Y. Ahn [3,*], H.-M. Oh [3], B.-K. Cho [1 2 4], S. Cho [1 2]

[1]Department of Biological Sciences, [2]KI for the BioCentury, KAIST, [3]Biological Resource Center, KRIBB, [4]Intelligent Synthetic Biology Center, Daejeon, Republic of Korea

Abstract: Harmful algal blooms (HABs) cause severe environmental problems in oceans and damage marine industries worldwide. *Alexandrium tamarense*, in particular, is one of eukaryotic marine dinoflagellates that produce neurotoxins that are accumulated in fish and shellfish leading to harm human. We found that *A. tamarense* is associated with numerous bacteria based on full-length 16S rRNA analysis using MinION nanopore sequencing. Further, we screened algicidal bacteria from South sea of Korea, which leads to lysis of *A. tamarense* upon co-culture. To understand the biological relationships between *A. tamarense* and the algicidal bacteria, we analyzed the changes in their metatranscriptome in response to their culture conditions. Since the genome sequence of *A. tamarense* is unknown yet*, de novo* transcriptome assembly was performed for the gene enrichment analysis. The discovery of novel algicidal bacteria and the comprehensive transcriptomic analysis on interactions with harmful algae can provide biological insights to solve the HAB problems.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-178

PHOSPHOPANTETHEINYL TRANSFERASES IN DINOFLAGELLATES: ONE BEGINNING WITH MANY ENDS

E. P. Williams [1,*], T. Bachvaroff [1], A. Place [1]
[1]IMET, University of Maryland, Baltimore, USA

Abstract: The phosphopantetheinyl transferase (PPTase) performs the first step in fatty acid and polyketide/non-ribosomal peptide synthesis (PKS/NRPS) by covalently attaching the phosphopantetheinyl arm of CoA to a protein. This provides the substrate upon which specific molecules can be sequentially added and is generally specific for synthesis of either lipids or other metabolites, *e.g.* antibiotics and toxins. Efforts to pinpoint the genes specific for toxin synthesis in dinoflagellates have been hampered by non-operonic gene arrangement and gene expansion. In this way the phosphopantetheinyl transferase is an appealing candidate for study because it is in low copy number (two to three per species), is assumed to not be regulated by downstream processes, performs a rate limiting step, and phylogenetics can be used to predict specificity for lipids or metabolites. Three families of PPtases have been identified in model species: AcpS-type, Sfp-type and Type I integrated PPtases, but the PPtases in dinoflagellates do not match any of the previous PPtase families. Thus, the transcriptomes from forty-two dinoflagellate species were mined for PPTases using three putative sequences from *Amphidinium carterae,* a basal dinoflagellate that produces the toxin amphidinol, to produce a phylogeny within the dinoflagellates. This analysis revealed three clades with PPTases II and III present in all species and PPTase I absent in several species implicating this gene as the non-essential toxin producer while II and III would be required for essential processes such as lipid synthesis. However, functional analyses of the three genes from *A. carterae* using a NRPS based reporter gives the opposite result with PPTase III showing strong activation, II producing mild activation, and PPTase I unable to activate the reporter. Successful phosphopantetheination of the reporter was confirmed using a gel shift assay and efforts are currently underway to use a semi-quantitative mass spectrophotometric approach. The current interpretation of these results is that PPTase III initiates metabolite synthesis through PKS and NRPS pathways, PPTase II initiates lipid synthesis but is promiscuous enough to allow for low level phosphopantetheination of other sites, and that PPTase I performs a specialized function or is non-functional. This hypothesis is currently being confirmed using the same methods to test for phosphopantetheination of the endogenous acyl carrier protein, which is the substrate for lipid synthesis. If correct, this would mean that the ability to initiate polyketide and non-ribosomal peptide based synthesis is present in all examined dinoflagellate species, irrespective of their designation as toxic or non-toxic.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-179

Transcriptomic responses of the cyanobacteria *Planktothrix* under high light and high temperature

S. Kim Tiam [1,*], K. Comte [1], C. Dalle [2], M. Delagrange [3], B. Ducos [3], C. Duval [1], M. Gugger [2], D. Kirilovsky [4], B. Marie [1], C. Bernard [1]

[1]UMR 7245 Molécules de Communication et Adaptations des Microorganismes, Muséum National d'Histoire Naturelle, [2]Collection des Cyanobactéries, Institut Pasteur, [3]Laboratoire de Physique Statistique, École Normale Supérieure, [4]Institute for Integrative Biology of the Cell (I2BC), CNRS, CEA, Paris, France

Abstract: Cyanobacteria are photosynthetic prokaryotes having a key role in ecosystem functioning but are also a source of concern for managers since they can form toxic blooms.

Environmental risk assessment related to cyanotoxin exposure is particularly complex since the toxin concentrations cannot be predicted based on the cyanobacterial species forming the bloom or on the bloom intensity. The characterization of the biologic role of toxins could be a key factor for concentration prediction and a better management of environmental risks.

The main objective of this study was to understand the link between toxin synthesis, photosynthesis and photoprotection. We hypothesized that cyanobacteria synthetizing toxins in stress conditions will be more resistant.

In order to test this hypothesis, five *Planktothrix* strains were selected: green (*Planktothrix agardhii*) or red (*Planktothrix rubescens*) ecotypes, non-microcystin or microcystin producers, and a wild-type green strain and its mutant for toxin production. Ecological advantages offered or not by toxins in stress condition (high light and high temperature), through their role in photosynthesis and photoprotection, were studied.

A 24-hour experiment composed with a 4-hour stress exposure followed by a recovery phase was performed. The cultures in triplicate exposed to high light intensity (150 µmol/m^2/s) or high temperature (33°C) were compared with controls (20°C, 6 µmol/m^2/s).

The effects of stress were evaluated on genes involved in photosynthesis (*psaA, psbA, acpA, cpcA, fnr*), photoprotection (*ocp, frp, flv1, flv3, hlip*), temperature tolerance (*dnaK, groEL, groES, hslO, hspA, hspG*) and oxidative stress regulation (*sodB, gor*). The upregulation of gene clusters involved in the biosynthesis of toxins (*mcy*) and other secondary metabolites (*mdn, aer, oci, apt, mic*) under stress conditions showed their role in stress response. This approach allowed to identify the secondary metabolites transcriptomic signatures of cyanobacteria exposed to high light intensity or temperature.

The new fundamental knowledge obtained in this study will help to identify the role of cyanotoxin and other secondary metabolites in stress conditions and could be generalized to other toxin-producing cyanobacteria.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-180

Transcriptome analysis reveals the molecular mechanism involved in phosphorus acclimation in a dinoflagellate *Prorocentrum donghaiense*

S.-F. Zhang [1,*], C.-J. Yuan [1], L. Lin [1], D.-Z. Wang [1]
[1]Xiamen University, Xiamen, China

Abstract: Dinoflagellates are one of the major contributors to primary production in the ocean and major causative agents of harmful algal blooms in the coastal waters. Phosphorus (P) is an essential macronutrient limiting marine dinoflagellate growth and productivity. However, the molecular mechanisms involved in P acclimation are poorly understood in marine dinoflagellates. Here, we compared the transcriptomes of a marine dinoflagellate *Prorocentrum donghaiense* grown in inorganic P-replete, P-deplete, and inorganic- and organic P-resupplied conditions using RNA-Seq and characterized differentially expressed genes. Transcripts of 27,434 genes altered significantly in P-deplete cells. Genes encoding low- and high-affinity phosphate transporters were down-regulated while genes participating in organic P utilization, nucleotide metabolism, photosynthesis, glycolysis and cell cycle were up-regulated. Remarkably, several genes involved in regulating photoperiod and circadian rhythm, such as flavin-binding kelch repeat F-box protein 1, were firstly identified in marine dinoflagellates and were down-regulated in P-deplete cells. Our results indicated that, in contrast with other algal species, *P. donghaiense* possessed a specific ability to utilize organic P, and ambient P depletion disturbed the circadian rhythm of *P. donghaiense* which subsequently triggered the response mechanisms to ambient P change.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-181

Deciphering cell cycle progression of the in situ blooming dinoflagellate cells with quantitative proteomics

H. Zhang [1,*], J.-L. Liu [1], Y.-B. He [2], Z.-X. Xie [1], S.-F. Zhang [1], Y. Zhang [1], L. Lin [1], S.-Q. Liu [2], D.-Z. Wang [1]
[1]State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, [2]BGI-Shenzhen, Shenzhen, China

Abstract: Over many years, a consensus has emerged that dianoflagellate blooms are caused by a short burst of cell proliferation. However, little is known about cell cycle progression of the *in situ* blooming cells. Here, we compared protein expression profiles of the *in situ* blooming cells of a dinoflagellate *Prorocentrum donghaiense* collected at different cell cycle phases using an iTRAQ-based quantitative proteomic approach. The blooming cells completed a cell cycle within 24 hrs with distinct cell cycle phases and cell cycle proteins presented similar periodic expression patterns to other eukaryotic cells. Proteins associated with photosynthesis, porphyrin and chlorophyll synthesis, carbon, nitrogen and amino acid metabolisms exhibited high expressions at the G_1 phase; proteins participating in DNA replication and mismatch repair were more abundant at the S phase; while protein synthesis and oxidative phosphorylation were highly enriched at the G_2/M phase. Interestingly, three core histones and one histone variant were first identified in *P. donghaiense* and highly expressed at the G_2/M phase. Our results indicated that the *in situ* blooming cells maintained the normal cell cycle progression, and the occurrence of the *P. donghaiense* bloom was more likely an accumulation result of cell growth rather than a short burst of cell proliferation.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-182

Metaproteomics reveals major molecular events occurring in the blooming cells during the succession of phytoplankton blooms

H. Zhang [1,*], P.-F. Wu [1], S.-F. Zhang [1], Z.-X. Xie [1], D.-X. Li [1], L. Lin [1], D.-Z. Wang [1]
[1]State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, China

Abstract: Phytoplankton blooms are a natural phenomenon in the ocean. Understanding of oceanographic and ecological mechanisms regulating bloom formation and succession has been improved. However, molecular events occurring in the blooming cells are lack. Here, we applied metaproteomic approach to compare global protein expression profiles of the blooming cells during bloom succession of *Heterosigma akashiwo* and *Prorocentrum donghainese*. Compared with the other species, high expressions of carbon assimilation and fixation related proteins as well as light harvesting proteins in the *H. akashiwo* cells might facilitate its earlier bloom formation. In the blooming cells of *H. akashiwo,* proteins involved in nitrite and ammonia uptake, SLCA10, SPX3, and high affinity nitrate and phosphate transporters were abundant, benefiting cells to acquire extracellular bicarbonate and inorganic nutrients. In addition, phoD-like phosphatase, 3'(2'), 5'-bisphosphate nucleotidase and phospholipase were also highly expressed to activate cells to utilize extra- and intracellular organic phosphorus. In the blooming cells of *P. donghaienese,* high abundances of proteins were associated with nitrate/nitrite uptake, polar amino acid ABC, low affinity phosphate transporters and hydrolytic enzymes acquire extra- and intracellular inorganic and organic nutrients. This study highlighted different utilization strategies of two phytoplankton species regarding uptake and assimilation of carbon, nitrogen and phosphorus, and nutritional niche divergence might regulate bloom formation and succession of different phytoplankton species.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-183

C4 photosynthesis strengthen carbon assimilation during the bloom period of a marine dinoflagellate

H. Zhang [1,*], T.-Q. Liu [1], Y.-P. Zhou [2], L. Lin [1], D.-Z. Wang [1]

[1]State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, [2]School of Chemistry & Chemical Engineering, Shanxi University of Science & Technology, Xi`an, China

Abstract: Dinoflagellate blooms have drawn the worldwide concerns as its huge economic and ecological impacts. Although considerable efforts have devoted to nutrients regulation on dinoflagellate blooms, knowledge of CO_2 assimilation during its bloom period still lacks. Here, we used transcriptomics, metaproteomics and isotope ratio MS techniques to prove the existence of C_4 cycle of *Prorocentrum donghaiense*, and the C_4 cycle was induced to highly express within the blooming cells in marine environment. Higher cell density and Fv/Fm at stationary phase were observed in CO_2 sufficient when compare to CO_2 exhaustion condition. The proportion of ^{13}C of particle organic carbon were increased as the CO_2 exhaustion aggravated in laboratory, and same results was observed in blooming cells of *P. donghaiense* in marine environments. Moreover, nearly all genes involved in C_4 cycle were cloned in pure *P. donghaiense* cells and higher abundances were monitored in CO_2 exhaustion conditions. Abundance of C_4 cycle proteins also increased as bloom proceeded in marine environment. In summary, we firstly proved the existence of C_4 cycle in *P. donghaiense* and its expression showed negative correlation with dissolved inorganic carbon concentration. This study highlighted the viral adaptive mechanisms of CO_2 assimilation during blooming conditions, and also deepened our knowledge on maintenance mechanisms of dinoflagellate blooms in marine environment.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-184

Life without Chargaff's Rules: Mapping 5-hydroxymethyluracil in genomic DNA

A. R. Place [1,*], E. Williams [1], T. Bachvaroff [1]

[1]2Center for Environmental Science, Institute of Marine and Environmental Technology, University of Maryland, Baltimore, USA

Abstract: One of the tenets for life on earth is that it obeys **Chargaff's rules** which states that DNA from any cell of all organisms should have a 1:1 ratio (base Pair Rule) of pyrimidine and purine bases and, more specifically, that the amount of guanine should be equal to cytosine and the amount of adenine should be equal to thymine. We have known since the 70's that a major group of primitive eurkaryotes, the dinoflagellates, uniformly break this rule. On average for all core dinoflagellate genera, 40.4±13.1 % (18) of thymine bases are modified to 5-hydroxymethyluracil in genomic DNA. This has resulted in single cell large genomes (~5.9 pg/cell and greater) without nucleosomes, permanently condensed chromosomes with 10 fold lower protein than all other eurkaryotes, lack of gene specific transcription factors, and trans splicing of all mRNAs. We will present our attempts at determining why this modification occurs and mapping its location in the genomes.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-185

USE OF HIGH THROUGHPUT SEQUENCING TO ASSESS DIFFERENTIAL GRAZING ON HARMFUL CYANOBACTERIAL BLOOMS BY ZOOPLANKTON IN LAKE ERIE, NEW YORK

M. Ladds [1,*], J. Jankowiak [1], C. Gobler [1]

[1]School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, USA

Abstract: Harmful algal blooms (HABs) have increased globally within both marine and freshwater habitats. Cyanobacteria, which can be extremely toxic, dominate HABs within freshwater systems and are linked to reduced zooplankton grazing pressure, but the relative grazing pressure on differing genera of cyanobacteria is poorly understood. The use of 16S and 18S barcoding and high throughput sequence analysis offers a tool to assess the genera of cyanobacteria grazed upon by zooplankton during HABs. To assess its utility, a set of grazer addition experiments with *Daphnia pulex* and *Daphnia magna*, as well as zooplankton dilution experiments were performed at the periphery and closer to the epicenter of the cyanobacterial HABs in Lake Erie at the Maumee River and Sandusky Bay. Pigment-based phytoplankton community analysis showed that there was active grazing cyanobacteria at all sites but more rapid grazing rates on diatoms when they were abundant. 16S high throughput sequencing revealed multiple genera including *Prochlorococcus, Synechococcus, Planktothrix, and Anabaena* increased in relative abundance, or were actively grazed, by both indigenous zooplankton and *Daphnia* spp. In contrast *Microcystis* was not grazed or became more abundant in the presence of enhanced grazing pressure. Assessment of 18S high throughput sequencing indicated that multiple diatom genera including *Skeletonema, Thalassiosira, Actinocyclus*, and *Aulacoseira* decreased in relative abundance during experiments, suggesting they were actively grazed, while other genera such as *Navicula* did not. Collectively, this study provides evidence that some bloom-forming cyanobacteria such as *Microcystis* are poorly grazed relative to other phytoplankton during freshwater HAB and thus is a likely reason for their dominance. This study also highlights general utility of using high throughput sequencing to assess grazing on HABs and other plankton.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-186

HAB species of the northern Adriatic (Mediterranean Sea) recorded using metabarcoding

A. Baricevic [1,*], C. Chardon [2], D. Maric - Pfannkuchen [1], N. Kuzat [1], V. Vasselon [2], M. Smodlaka - Tankovic [1], F. Rimet [2], A. Bouchez [2], M. Pfannkuchen [1]

[1]Center for Marine Research, Ruder Boskovic Institute, Rovinj, Croatia, [2]UMR-CARRTEL, INRA, Thonon-les-Bains, France

Abstract: The northern Adriatic Sea has a long-term monitoring programme and the well-known diversity of phytoplankton. Still, species identification is routinely based only on morphological analysis using light microscopy (LM) what may be limiting for the taxonomic determination of many phytoplankton species. To complement LM for species identification, diversity of four long-term monitoring northern Adriatic stations was for the first time assessed using metabarcoding together with LM. Special focus of the study was on HAB species detection since several genera containing harmful algae are virtually indeterminable during routine LM and other genera are presumed to be present in only marginal abundances.

In the 18S-V4 and rbcL dataset obtained from all the stations, ten microphytoplankton genera (*Alexandrium*, *Amphidinium*, *Azadinium*, *Gymnodinium*, *Gyrodinium*, *Heterocapsa*, *Karlodinium*, *Protoceratium*, *Pseudo-nitzschia* and *Symbiodinium*) with known HAB and toxic species representatives were identified. Some genera (*Azadinium*, *Heterocapsa*, *Karlodinium* and *Symbiodinium*) represent first records for the region studied. Genera *Gyrodinium* and *Alexandrium* had the highest OTU counts associated to them. Very good database (Silva, GenBank, DinoREF) representation of the genus *Alexandrium* enabled species level identification of *A. minutum* and *A. pseudogonyaulax*. Shared HAB genera detected by both metabarcoding and LM were of 50%. Abundance and incidence patterns of the genera previously included in long-term microscopy monitoring matched the metabarcoding data.

With the more profound approach in the HAB species identification then microscopy alone, metabarcoding managed to add significant knowledge to harmful algae diversity of the northern Adriatic Sea. Our study therefore confirms that metabarcoding represents a promising tool for HAB monitoring.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-187

Identification of core paralytic shellfish toxin biosynthesis genes in two strains of the dinoflagellate *Alexandrium ostenfeldii*

H. Savela [1 2,*], A. Kremp [3], S. Murray [2]

[1]Department of Biochemistry/Biotechnology, University of Turku, Turku, Finland, [2]C3 Climate Change Cluster, University of Technology Sydney, Sydney, Australia, [3]Leibnitz Institute for Baltic Sea Research Warnemuende, Roshtock, Germany

Abstract: *Alexandrium ostenfeldii* is a dinoflagellate which thrives in the shallow coastal waters of the Baltic Sea, where it produces the neurotoxins saxitoxins, also called paralytic shellfish toxins (PST). While one of the PST biosynthesis genes, *sxtA*, has been previously identified in *A. ostenfeldii*, the rest remain unidentified. To further study *sxt* genes in the species, we sequenced the transcriptomes of saxitoxin (STX) and gonyautoxin (GTX2 and GTX3) producing strains AOF0927 and AOVA30, originating in Åland, Finland and Gotland, Sweden, respectively. Three biological replicates of both strains were grown at their native salinity of 6 psu, at 16 °C, light–dark cycle 14:10 in F/2 medium. Total RNA was extracted from cells harvested at the exponential growth phase. After library generation, 150 bp paired-end reads were sequenced from all samples (n=6), resulting in an average of 135 million reads per sample. Read data from replicates were pooled and transcriptomes assembled with Trinity 2.6.5, with both final assemblies consisting of ~350 000 contigs. Dataset completeness was assessed using BUSCO v3, and homologs of 82% of the conserved genes included in the BUSCO core eukaryote dataset were identified in the transcriptomes of both strains.

Identification of core *sxt* genes and putative homologs was carried out using HMMER v.3.1b2 and InterProScan v.5.27-66.0. Transcripts of the *sxtA* gene which encodes the enzyme responsible for the first step of PST biosynthesis were readily identified in both transcriptomes (E=0), and several transcripts with high levels of similarity ($E<1.3\times10^{-145}$) to known dinoflagellate *sxtG* sequences both at the nucleotide and amino acid level were likewise recovered. Several potential homologs of cyanobacterial dehydrogenases *sxtH*/*T* and *sxtU* genes, as well as the sterole desaturase *sxtD* were identified in both transcriptomes, whereas only one putative *sxtI* transcript was recovered for both strains. No significant similarity between cyanobacterial SxtS and SxtB and *A. ostenfeldii* transcripts was found on the basis of HMMER profiles. However, annotation against the InterPro database led to the identification of predicted cytidine deaminase (*sxtB*) polyA-containing transcripts in both transcriptomes, as well as multiple transcripts belonging to the same protein family as SxtS, the oxoglutarate/iron-dependent dioxygenases. Overall, the candidate *sxt* genes were characterized by a high GC%, typical for dinoflagellate genomes, and they shared little sequence similarity with the corresponding cyanobacterial *sxt* genes. Similarly to other studies on dinoflagellate *sxt* genes, the results suggest that several crucial steps in the PST biosynthesis pathway in *A. ostenfeldii* are performed with enzymes which may not share a common origin with the corresponding cyanobacterial genes.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-188

Gene expression in Pseudo-nitzschia reveals the methylerythriol phosphate metabolic pathway in the synthesis of domoic acid

S. Hardardottir [1,*], S. Wohlrab [2], D. Marie Hjort [3], B. Krock [4], T. Nielsen [5], U. John [4], N. Lundholm [1]

[1]Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark, [2]Helmholtz Centre for Polar and Marine Research, Alfred Wegener Institute, Bremenhafen, Germany, [3]The Geological Survey of Denmark and Greenland, Copenhagen, Denmark, [4]Alfred Wegener Institute, Bremenhafen, Germany, [5]National Institute of Aquatic Resources, Copenhagen, Denmark

Abstract: Even though it has been decades since the discovery of *Pseudo-nitzschia* as a source of the marine biotoxin domoic acid (DA), the biosynthetic pathway has only partly been revealed. Using functional genomic approaches we acquired information on genes involved in biosynthesis and regulatory functions for DA production in *P. seriata*. In our study, we used predator cues to induce DA production in P.seriata and compared the response with a control not exposed to predator cues, and a parallel study with a non-toxic diatom, *Fragilariopsis* sp. Over 1000 genes were differently expressed in P.seriata cells exposed to predator cues for two days compared to the control. The majority (>70%) of these genes were up regulated including several genes involved in major cellular processes and metabolism such as amino acid and carbohydrate metabolism as well as genetic information processing and environmental signal transduction mechanisms. Previous studies suggested that the cellular route for DA production is a fusion of two unknown precursors, one deriving from the isoprenoid pathway and the other from the citric acid cycle. We found a clear signal for the methylerythriol phosphate metabolic pathway to be the isoprenoid pathway for precursor synthesis of geranyl pyrophosphate to form DA. Six out of eight genes involved in this pathway were upregulated but none in the conventional mevalonate pathway. Fragilariopsis surprisingly did not change gene expression compared to control conditions. These findings indicate that the two species have evolutionary distinct strategies for coping with grazer threats and that only *Pseudo-nitzschia* cells receive and respond to the predator cues.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-189

Comparative transcriptomic analysis of different *Prymnesium* strains

K. Anestis [1,*], G. S. Kohli [1], S. Wohlrab [1], P. J. Hansen [2], E. Varga [3], T. Ostenfeld Larsen [3], U. John [1]

[1]Ecological Chemistry, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, [2]Department of Biology, Copenhagen University, Helsingør, [3]Department of Biotechnology and Biomedicine, Technical University of Denmark, Lyngby, Denmark

Abstract: Blooms of the haptophyte *Prymnesium parvum* have provoked massive fish-kills in the past with devastating economic consequences. *Prymnesium* species produce a group of supersized ladder-frame polyether compounds collectively called prymnesins. Prymnesins are bioactive compounds with hemolytic, ichthyotoxic and cytotoxic ability and might play a role in fish-kills caused by *Prymnesium* blooms. The chemical profiling of different *P. parvum* strains has revealed the presence of various compounds differing in their structure and biological activity. Even though, there is a vast literature on the abiotic factors inducing toxicity in *P. parvum*, little is known about the molecular mechanisms underlying toxin production in *Prymnesium*. The diversity of the produced compounds reveals complexity with regards to the molecular mechanisms involved in the synthesis of the correspondent toxins, although the carbon backbone is most likely synthesized via polyketide synthases (PKS). Given the polyketide structure of prymnesins, previous studies have mainly focused on the role of polyketide synthase (PKS) genes. In the present study, we aim to explore the genetic toolkits used by *P. parvum* for the synthesis of toxic compounds. The comparative transcriptomic analysis of *Prymnesium parvum* strains and their corresponding toxin profiles revealed PKS genes that might be involved in toxin biosynthesis. The present study is going to evaluate the biosynthesis and evolutionary history of prymnesin production in *Prymnesium* strains and lead to the design of efficient tools to monitor harmful algal blooms.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-190

Annual variation in bloom-forming *Microcystis* genotypes from an Australian wastewater lagoon

C. Romanis [1,*], T. Mills [1], B. Neilan [1]

[1]School of Environmental and Life Sciences, University of Newcastle, Callaghan, Australia

Abstract: Cyanobacteria from the *Microcystis* genus are commonly responsible for nuisance and potentially toxic algal blooms that disrupt the supply of water from the Western Treatment Plant, Victoria, to the surrounding agricultural districts. It is therefore crucial to develop an understanding of the biological parameters that favour non-toxic bloom formation to reduce disturbances to the water supply. To assess genetic variation within seasonal populations of bloom-forming *Microcystis spp.* we analysed the algal bloom bacterial populations from the Western Treatment Plant for three consecutive years (2015-2018). Weekly sampling of each lagoon was conducted to capture variation in algal biomass population using metagenomic 16S rRNA sequencing. Bacterial community composition and biodiversity showed a large variation within and throughout the sampling periods; including the emergence of *Microcystis* as the predominant algal biomass occurring at different time-points throughout the proliferation period each year. This variation may be attributed to environmental parameters. The relative genetic diversity of microcystin-producing *Microcystis spp.* was quantified using the phytoxigene^{TM} CyanoDTec real-time PCR kit to ascertain the ratio of cells with the potential to produce microcystin (*mcyE* genotypes) to the total population for each bloom. The ratio of *mcyE*-containing cells to the total *Microcystis* population was highest during periods of bloom formation for each sample year, indicating the potential ecological function of toxic taxa in bloom proliferation.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-191

VALIDATION OF A SANDWICH HYBRIDIZATION ASSAY FOR DETECTING THE HARMFUL CYANOBACTERIA MICROCYSTIS

D. Greenfield [1,*], N. Dearth [2], W. Jones [2], R. Mortensen [2]

[1]City University of New York, New York, [2]University of South Carolina, Columbia, USA

Abstract: Cyanobacteria are responsible for the largest number of harmful algal blooms (HABs) worldwide. The genus *Microcystis* is a major contributor to these HABs, and several species produce the hepatotoxin microcystin that affect humans, pets, and wildlife. *Microcystis* blooms frequently recur in lakes, rivers, and stormwater detention ponds, all of which are associated with residential and recreational areas, thereby posing public health risks. Several molecular techniques enable rapid and accurate identification and quantification of HABs, facilitating 'early warnings' of potentially toxic blooms. This presentation describes the adaptation of one such technique, sandwich hybridization assay (SHA), for *Microcystis* spp. SHA enables direct (e.g. no amplification of genetic material) cell identification and quantification using ribosomal RNA (rRNA) with two DNA probes, capture and signal, that bind to the targeted rRNA sequence creating a "sandwich" wherein results are measured by absorbance. Capture probes were designed using 16S rRNA sequences from GenBank®, and the assay was validated using cultures of *M. aeruginosa, M. botrys,* and *M. wesenbergii*. The effects of temperature and irradiance on SHA results were evaluated in a laboratory setting to determine if environmental variability affected SHA findings and presumably cellular RNA. Field collections involved environmental samples from a multi-specific (*Anabaena, Microcystis,* and *Anabaenopsis)* cyanobacteria bloom that occurred in a South Carolina stormwater detention pond during the summer of 2016 and was associated with several instances of moderate to high levels of microcystin. Once implemented within monitoring efforts, this technology will enhance *Microcystis* bloom tracking and prediction in a wide range of settings thereby helping to protect public health and safety.

Disclosure of Interest: None Declared

"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs

P-192

Transcriptomic signature of long-term CTX exposure in *Naso brevirostris*

G. Nuel [1], F. Letourneur [2], J.-P. Jais [3], F. Tores [4], M.-Y. Dechraoui Bottein [5], R. Clausing [5,*], K. Sdiri [5], A. Rau [6], F. Jaffrézic [6], M. Chinain [7]

[1]LPSM (CNRS 8001), Sorbonne University, Paris, France, [2]INSERM – Centre de Recherche Inserm, Cochin Hospital, Paris, , [3]Imagine Institute; bioinformatic platform, Paris Descartes Universit, [4]Imagine Institute; bioinformatic platform, Paris Descartes University, Paris, France, [5]Environment Laboratories (IAEA) , Monaco, Monaco, [6]INRA – Institut national de la recherche agronomique (INRA), Jouy-en-Josas, France, [7]Laboratoire des Micro-Algues Toxiques, Institut Louis Malardé-UMR, Papeete Tahiti, French Polynesia

Abstract: The transcriptomic response to ciguatoxin (CTX) exposure has already been studied in murine models and zebrafish embryos. Although the results obtained were quite interesting, these experiments have two important limitations: 1) the considered species do not naturally occur in ciguatera-endemic zones; 2) short-term exposure does not represent natural conditions of fish exposure on the reef. The main objective of our project is to overcome these two limitations by considering long-term feeding exposure in Naso brevirostris, an herbivorous fish species that is often found with CTX throughout the Indo-Pacific and is also often used as a food source by the local populations. In our experiment, 12 Naso brevirostris (6 cases and 6 controls) were maintained in 100 L tanks with controlled environmental conditions. The exposed fishes were fed a gel-food diet enriched with a highly toxic strain of Gambierdiscus polynesiensis for 16 weeks. The control fishes were fed with the same gel-food without algal cells. After 16 weeks fish were euthanized, and liver tissue collected and conserved in RNAlater (Qiagen). RNA was extracted and quality control assessed using standard methods. The RNA was sequenced using a Nextseq 500 resulting in 1.2 billion high quality 75bp. Transcriptome assembly and rough annotation were performed using the Trinity software suite. We obtained a total of 107,336 transcripts. Using a conservative non-parametric Wilcoxon-Mann-Whitney test, we found 812 transcripts with a false discovery rate (FDR) of 0.29 meaning that we expect to have at least 500-600 true positives among them. Using random forest, we used the expression of these 812 transcripts to predict the case/control status in order to assess the power to discriminate between exposed and non-exposed fishes using a RNAseq signature. The exposed prediction probability had a median of 0.066 for the control fishes and a median of 0.912 for the cases, resulting in a total of 40.4% of maximum entropy. In order to avoid any statistical artefact, we processed negative controls (dataset with random case/control) with the exact same analysis. Over 100 replications, we systematically obtained a FDR of 1.0 for all transcripts, and the predictions through random forest were always close to pure randomness with prediction probabilities close to 0.50 for all samples with almost 100% entropy. Conclusions: Toxin accumulation in the dosed fish was confirmed and found at levels above the threshold for human intoxication. We found a strong signal of differential expression in liver. It is also clear that a long-term exposure has a discriminative expression signature. It is quite remarkable that such a strong signal can be detected despite the low power of our experimental design, and this opens an exciting line of research for studying the adaptation of fishes to long-term CTX exposure through gene expression. This work is part of the CARISTO-Pf project that was funded by France and French Polynesia.

Disclosure of Interest: None Declared

HumanToxicology

P-193

Cyanobacterial toxins and the intestines – Are we missing the first site-of-action for specific organ toxicity?

B. Kubickova [1,*], P. Babica [1], L. Šviháľková Šindlerová [2], K. Hilscherová [1]

[1]Research Centre for Toxic Compounds in the environment (RECETOX), Masaryk University, Faculty of Science, [2]Institute of Biophysics of the Czech Academy of Sciences, Brno, Czech Republic

Abstract: Gastrointestinal disturbances (e.g. nausea, vomiting, abdominal pain, diarrhoea) are one of the most frequently reported symptoms upon exposure to cyanobacterial blooms. The most frequent exposure route to water-borne cyanobacteria is via accidental consumption of contaminated water, either intended (insufficiently treated drinking water) or accidentally (recreation). Subsequently, cells of GIT are the first to come into contact with cyanobacterial water bloom components, and the epithelial lining of the GIT is the first barrier to be overcome by cyanobacterial toxins and metabolites to reach and affect internal organs.

Despite the relatively high frequency of adverse GIT outcomes in response to cyanobacterial bloom exposures, the cyanobacterial toxins or other water bloom components responsible for these effects are not fully recognized, and also the underlying mechanisms remain to be identified and characterized.

To address this data gap, literature was systematically reviewed to combine information on freshwater cyanobacterial blooms, toxins and other secondary metabolites in connection to their effects on the human GIT, also taking into account relevant disturbances in the humoral immune system and potential links to immunomodulating (cyano-) bacterial agents such as lipopolysaccharides.

Microcystins, the most extensively studied and common toxins in cyanobacterial blooms, are taken up via the bile acid transport system present in the gut and inhibit ubiquitous cytoplasmic protein phosphatases. Despite disruptive effects are reported primarily in the metabolically highly active liver tissue, destruction of cells lining the (small) intestine has also been reported. Furthermore, protein synthesis-inhibiting cyanotoxin cylindrospermopsin caused ulcerations and bleeding in the stomach and small intestines of mice. These adverse effects on GIT tissues are likely to be magnified by co-action with other virulence factors inherent to the complex mixture of cyanobacterial blooms, for example, the inflammatory-mediator LPS, eventually leading to enterocolitis rather than liver failure upon exposure. In addition to acute GIT symptoms, there is evidence that the frequent and prolonged exposure of the GIT to low levels of potentially carcinogenic and tumour-promoting cyanobacterial toxins (e.g. microcystins) may contribute to colorectal cancer incidence.

In conclusion, the intestines as the first barrier and the entry of cyanotoxins into the organism need to be considered as recipients of cyanobacterial toxicity, also with respect to the complex composition of naturally occurring cyanobacterial blooms and interactive mixture effects of their components on the GIT and immune system.

This project has received funding from the H2020-MSCA-ITN-2016 project No. 722493 NaToxAq and the Czech Science Foundation grant No. GA16-24949S.

Disclosure of Interest: None Declared

HumanToxicology

P-194

Brevetoxin-modulation of hydrogen peroxide production and antioxidant enzyme activity on human monocytic cell line

A. Rivera [1], C. Melo [1 2], A. Astuya [1], A. Llanos [1], J. J. Gallardo [3], M. Martorell [2,*]

[1]Department of Oceanography and COPAS Sur-Austral, [2]Nutrition and Dietetics Department, Faculty of Pharmacy, [3]Department of Chemical Engineering, Faculty of Engineering, Universidad de Concepción, Concepción, Chile

Abstract:

Background: Harmful algal blooms (HABs) cause massive death of shellfish and fish, but lower concentration of the toxins could be bioaccumulates over time. Brevetoxins (PbTxs) present in HABs in human cause neurotoxic shellfish poisoning. The consumption of sea products exposed to sublethal doses of PbTxs for a long time might favor the appearance of chronic diseases through oxidative stress and inflammation mechanisms. The aim of this study was to evaluate the effect of PbTxs on the reactive oxygen production and the activity of antioxidant enzymes in a human monocytic cell line.

Methodology: THP-1 cells were maintained in RPMI 1640 medium (10% FBS, 0.025 M glucose, 0.05 mM betamercaptoetanol and 1 mM sodium pyruvate) at 37ºC and 5% CO_2. Five experimental groups were assigned and incubated for 2 hours: control, lipopolysaccharide (LPS, 1 µg/mL) and PbTx 2 at 0.25, 0.5 and 1.0 µg/mL doses. After the incubation, one aliquot was used for H_2O_2 production and the rest of cells were lysed with deionized water for enzyme activity analysis. H_2O_2 production was measured using 2,7-dichlorofluorescin-diacetate (DCFH-DA) as indicator and using LPS at 1.0 µg/mL as stimulator. Fluorescence (Ex, 485 nm; Em 538 nm) was recorded at 37 °C for 1 h in Synergy H1 reader (Bio-tek Instruments, Inc). Catalase activity was measured by the spectrophotometric method of Aebi based on the decomposition of H_2O_2. Superoxide dismutase (SOD) activity was measured by an adaptation of the method of McCord and Fridovich.

Results: H_2O_2 production was significantly increased 1.47 times in the LPS group, and changed 1.31, 1.18 and 0.95 times in the 0.25, 0.5 and 1.0 µg/mL PbTx 2 groups, respectively. Moreover, catalase and SOD activities were significantly higher at 0.5 µg/mL PbTx 2 dose.

Conclusion: PbTx 2 enhances H_2O_2 production, similar to LPS-stimulated, although at lower dose. Highest PbTx 2 tested doses dismissed H_2O_2 production and might be related to the activation of the antioxidant machinery. Although antioxidant enzymes activities were clearly affected, further studies will be needed for determine the effects of PbTx 2 on oxidative stress and the mechanisms involved.

Acknowledgement

This work was supported by the Vicerrectoría de Investigación y Desarrollo from University of Concepción, Chile (217.073.033-1.0).

Disclosure of Interest: None Declared

HumanToxicology

P-195

In vitro genotoxic potential of MC-LR, -RR and -LF revealed by the micronucleus assay and detection of key markers in the HepaRG cells

R. Merabet [1 2,*], K. Hogeveen [2], R. Lanceleur [2], D.-E. Benouareth [1], V. Fessard [2]

[1]Biology , 8 MAI 1945 Guelma University, Guelma, Algeria, [2]Contaminant Toxicology Unit, ANSES, Fougères, France

Abstract: Microcystins (MCs) are cyclic hepatotoxins produced by various species of cyanobacteria that inhibit serine/threonine protein phosphatases. Their structure includes two variable amino acids leading to more than 100 variants. While MC-LR was reported to be the most toxic variant and, as so, has been largely investigated, increasing evidence suggests that MC-LF might be even more toxic. The aim of the current study was to characterize and compare the genotoxicity of three MC variants MC-LR, -RR and -LF using high content analysis and the *in vitro* cytokinesis-block micronucleus assay in the human hepatoma cell line HepaRG. Undifferentiated HepaRG cells were processed for high content analysis after 24 hours of treatment with concentrations ranging from 0.39 to 50 µM for MC-LR and -RR and from 0.05 to 6.25 µM for MC-LF. Apart from viability (cell count) and apoptosis (active Caspase-3), 4 cellular markers were evaluated for detection of DNA damage (γH2AX, phospho H3, p53 phospho S15 and ATM phospho S1981). A significant increase in each of these markers was observed and was correlated with the onset of cytotoxicity. The responses with MC-LF were observed at lower concentrations compared to MC-LR and MC-RR. Following a 24 hour treatment of differentiated HepaRG cells with MC-LR and MC-RR to concentrations ranging from 1.56 – 25 µM, a significant concentration dependent increase in micronuclei was observed with the two variants. These findings provide key information in the mechanism of action involved in the genotoxicity of MCs and confirm that MC-LF is more potent than MC-LR.

Disclosure of Interest: None Declared

HumanToxicology

P-196

Metabolism, absorption, and effects of marine biotoxins using in vitro systems combined with MS based analysis

M. D. Klijnstra [1,*], L. Portier [1], T. Bovee [1], A. Gerssen [1]
[1]RIKILT Wageningen University and Research, Wageningen, Netherlands

Abstract: Some marine biotoxins are highly toxic when i.p. injected into mice. However, there are hardly any human intoxication cases known, e.g. for the marine biotoxins 13-desmethylspirolide C (SPX-1) and palytoxin (PlTX). Most probably because these toxins are not adsorbed from the human intestine and/or rapidly inactivated in the liver. In vitro systems were used to establish whether this is true, i.e. human digestion, human intestinal Caco-2 cells in a transwell model for absorption, and human liver S9 fractions for metabolism. Samples before and after the treatments were tested with the neuro-2a bioassay for toxicity testing and in case of SPX-1 the nicotinic acetylcholine receptor assay for receptor activation. Furthermore, samples were measured with liquid chromatography coupled to an orbitrap high resolution mass spectrometer (LC-hrMS) to quantify the conversion or uptake of the toxin and the formation of metabolites (with use of compound discoverer). SPX-1, PlTX, yessotoxin (YTX) and azaspiracid 1 (AZA-1) were used for experiments. A human digestion model was conducted only for SPX-1 and PlTX, showing that these toxins were not affected by digestion, i.e. not deactivated.

For SPX-1, only 7 and 14% passed from the apical compartment to the basolateral compartment after respectively 4 and 16 hours in the Caco-2 transwell model. Moreover, both the receptor assay and LC-hrMS analysis showed that 90 to 100% of SPX-1 was converted by the liver S9 fraction. These outcomes might explain why no human intoxications from this toxin are known, as it is hardly absorbed from the intestine, and the small amounts taken up are rapidly inactivated in the liver.

For YTX even no measureable quantity had passed the Caco-2 cells after 4 or 16 hours of exposure. Moreover, with the S9 mix approximately 60% of the YTX was converted into inactive metabolites. These outcomes might explain why YTX compared to AZA-1 is much less toxic in vivo, while it is equally potent to AZA-1 in many in vitro tests.

For results of AZA-1 and PlTX, measurements still have to be performed at the moment the abstract is submitted. However, the data above are in line with the expectations and show that SPX-1 and YTX hardly pass the intestinal Caco-2 cells, while preliminary results show that the known human toxin AZA-1 is rapidly absorbed.

Disclosure of Interest: None Declared

HumanToxicology

P-197

Determination of human transporters involved in cellular uptake and excretion of cylindrospermopsin

J. Ravat [1], V. Fessard [1,*], A. Huguet [1]

[1]Toxicology of contaminants, French Agency for Food, Environmental and Occupational Health & Safety, Fougères, France

Abstract: Cylindrospermopsin (CYN) is a cyanotoxin produced by several freshwater cyanobacterial species. Due to eutrophication, these organisms are able to massively proliferate in the environment. The particularity of CYN is that it can be released in water during blooms (up to 98% of the total produced). Therefore, humans can be exposed to this toxin through drinking water and consumption of contaminated food. The crossing of CYN through an in vitro intestinal barrier model was recently investigated to evaluate systemic exposure following oral intake. During these studies, excretion mechanisms of CYN have been suggested. Therefore, the objective of the present study was to investigate the efflux transporters involved in CYN excretion. We used differentiated Caco-2 and HepaRG cells as human intestinal and hepatic cell models respectively. CYN cytotoxicity was studied with and without different inhibitors of efflux transporters (verapamil for P-gp, MK-571 for MRP2, and Ko143 for BCRP). Our results indicated that, when MK-571 and Ko143 are added, the cytotoxicity of CYN was significantly increased. Indeed, EC_{50} obtained with HepaRG cells exposed to CYN were 50% and 15% lower with MK-571 and Ko143 respectively as compared to control. On the contrary, verapamil significantly decreased the cytotoxicity of CYN (+20% EC_{50}). With Caco-2 cells, a significant increased cytotoxicity of CYN was observed only with MK-571. Our results highlight that various transporters are involved in CYN cellular uptake and excretion. We suggested that MRP2 and BCRP are implied in CYN efflux, while OCT2 is involved in CYN influx. Nevertheless, further investigations will be conducted to confirm these results.

Disclosure of Interest: None Declared

HumanToxicology

P-198

Toxicity and genotoxicity of portimine on a human hepatic cell line

V. Fessard [1,*], S. Huet [1], C. Besnard [1], T. Harwood [2], K. Hogeveen [1]
[1]Toxicology of contaminants, French Agency for Food, Environmental and Occupational Health & Safety, Fougères, France, [2]Cawthron Institute, Nelson, New Zealand

Abstract: Portimine is a toxin produced by the dinoflagellate *Vulcanodinium rugosum*. Although it belongs to the cyclic imine group, its effects differ from the other toxins, showing less toxicity after intraperitoneal injection in mice. Moreover, compared to the pinnatoxins that are produced by the same dinoflagellate, it is a weaker antagonist of nicotinic acetylcholine receptors, and is toxic *in vitro* in several cell types including macrophages, fibroblasts, lymphocytes and kidney epithelial cells. It has also been demonstrated to induce apoptosis using various endpoints.

In this study, we investigated the potential of portimine to induce genotoxicity (γH2AX, comet and micronucleus assays) and apoptosis through caspase-3 activation in human HepaRG cells capable of differentiating to hepatocytes and biliary-like cells. A High Content Analysis approach was used for γH2AX and active caspase-3 quantification. Human HepaRG cells were exposed for 24 hours to a concentration range of portimine up to 40 nM.

The toxin was cytotoxic (IC50 around 10 nM with the MTT assay) primarily affecting hepatocytes. However, portimine did not induce genotoxicity in HepaRG cells. No DNA damage was detected by the comet assay and no chromosomal damage was shown in the micronucleus assay. While no increase in active caspase-3 was observed, the induction of DNA double strand breaks detected by γH2AX was observed concomitantly with a decrease of cell count, although this is most likely due to cell death.

Disclosure of Interest: None Declared

HumanToxicology

P-199

In vitro toxicological assessment of lipophilic phycotoxins on *in vitro* enteric glial cells

O. Réale [1,*], A. Huguet [1], K. Hogeveen [1], V. Fessard [1]

[1]Toxicology of contaminants, French Agency for Food, Environmental and Occupational Health & Safety, Fougères, France

Abstract: Lipophilic phycotoxins produced by dinoflagellates can be accumulated in various edible filter-feeding bivalves. Human consumption of contaminated shellfish can induce gastro-intestinal and neurological symptoms. If studies have shown that phycotoxins induced toxic effects on intestinal epithelial cells, they also underlined their ability to cross the epithelial barrier to some extent. Therefore, phycotoxins can potentially impact the underlying cells such as enteric glial cells (EGCs) that are involved in gut homeostasis, motility and barrier integrity. However, no data are available regarding the susceptibility of EGCs and their possible role in the gastro-intestinal symptoms reported. Our study focused on investigating the effects of six phycotoxins (okadaic acid (OA), yessotoxin (YTX), pectenotoxin-2 (PTX2), azaspiracid-1 (AZA1), spirolide (SPX) and palytoxin (PlTX)) on the rat EGC cell line CRL2690. Cell viability, morphological cell changes, production of reactive oxygen species (ROS), inflammation, cell cycle and specific glial markers were evaluated using RT-PCRq and High Content Analysis (HCA) approaches. Our results showed that EGCs were more sensitive to AZA1 and PlTX while SPX had no effect on EGCs up to 130 nM. AZA1, OA, YTX and PTX2 induced neurites alterations at 24h, modification of the cell cycle and enteric glial reactivity since specific glial cell markers were increased: GFAP (intermediate filament described to be up regulated in glial cell stress), iNOS (nitric oxide synthase catalysing the production of NO leading to oxidative stress) and S100β (a calcium-binding protein implicated in apoptosis, oxidative stress and inflammation pathways). Inflammation was confirmed by early nuclear translocation of NFκB-p65 unit. Investigations on apoptosis (caspase-3 activation) and production of ROS have also confirmed glial cells damage. In conclusion, our results indicate that the toxic effects in EGCs induced by OA, YTX, PTX2 and AZA1 may also impact the intestinal barrier integrity.

Disclosure of Interest: None Declared

HumanToxicology

P-200

Metabolism of the lipophilic phycotoxin 13-desmethylspirolide

V. Fessard [1,*], J. Alarcan [1], E. Dubreil [2], A. Huguet [1], R. Araoz [3], F. Brée [4], B. Bouaita [5], D. Hurtaud-Pessel [2], S. Hessel-Pras [6], A. Lampen [6], L. Le Hégarat [1]

[1]Toxicology of contaminants, [2]French Agency for Food, Environmental and Occupational Health & Safety, Fougères, [3]Toxines, Récepteurs et Canaux ioniques , CNRSCEA/Université Paris Saclay, Gif/Yvette, [4]EUROSAFE, [5]Biopredic, Rennes, France, [6]Food Safety, German Federal Institute for Risk Assessment, Berlin, Germany

Abstract: 13-desmethylspirolide C (13-SPX-C) is a phycotoxin produced by dinoflagellates which can accumulate in shellfish. 13-SPX-C induces neurotoxic effects in rodents through blockade of nicotinic acetylcholine receptors. As no human intoxication following consumption of contaminated seafood has ever been clearly reported, this toxin is not regulated. Nevertheless shellfish consumers can be exposed to low levels of 13-SPX-C which are recurrently found in shellfish. In order to follow the behavior of the toxin after ingestion and to establish if it can be detoxified thus explaining the absence of human intoxication, we assessed the metabolism of 13-SPX-C with external metabolic activation systems. Using rat and human liver S9 fractions, both phase I and II reactions were screened. Then, the activity of the resulting metabolites towards nicotinic acetylcholine receptors was undertaken using a receptor-binding assay. Finally, the role of the cytochrome P450 1A2 in 13-SPX-C metabolism was determined using a new innovative *in vitro* tool: the CYP1A2-Silensomes™. Our results indicate that 13-SPX-C was almost completely metabolized with both rat and human liver S9. The metabolites showed less affinity towards nicotinic acetylcholine receptors than 13-SPX-C. Finally, we showed that CYP1A2 was involved in 13-SPX-C biotransformation. Thus, liver first-pass metabolism participates in the detoxification of 13-SPX-C.

Disclosure of Interest: None Declared

HumanToxicology

P-201

In vitro assessment of binary mixtures effects of phycotoxins in human intestinal cells

V. Fessard [1,*], J. Alarcan [1], S. Barbé [1], B. Kopp [1], S. Hessel-Pras [2], A. Lampen [2], L. Le Hégarat [1]

[1]Toxicology of contaminants, French Agency for Food, Environmental and Occupational Health & Safety, Fougères, France, [2]Food Safety, German Federal Institute for Risk Assessment, Berlin, Germany

Abstract: Lipophilic phycotoxins are secondary metabolites produced by phytoplanktonic species. They accumulate in filtering shellfish and can cause human intoxications. Humans could be exposed simultaneously to several phycotoxins but toxicological effects of phycotoxin mixtures on human health are largely unknown. Published data on phycotoxins co-exposure suggested that okadaic acid (OA) could be simultaneously found with pectenetoxin-2 (PTX-2), yessotoxin (YTX) or spirolide-1 (SPX-1). Therefore, the aim of this study was to examine the effects of three binary mixtures OA/PTX-2, OA/YTX and OA/SPX-1 on the human intestinal Caco-2 cells using high-content analysis approach. A multi-parametric approach was used: cell viability (cell count), oxidative stress (ROS formation), inflammation (Interleukine-8), and DNA damage (γ-H2AX) were simultaneously assessed. We first investigated the effects of each toxin in single condition and we observed that OA induced cytotoxicity, DNA breaks and IL-8 release. PTX-2 only induced slightly DNA strand-breaks, whereas SPX-1 and YTX showed a negative response for all endpoints. The addition of another toxin to OA resulted in antagonistic effects at low concentrations but additive or synergistic effects with increases concentrations. Due to noticeable combined effects on some key toxicity pathways, more attention should be paid on possible human health with phycotoxin found in mixtures.

Disclosure of Interest: None Declared

HumanToxicology

P-202

Are anatoxins responsible for a human intoxication associated with consumption of food supplements containing *Aphanizomenon flos-aquae*?

R. Bire [1,*], T. Bertin [1], M. Bourgeois [1], C. Duval [2], T. Guérin [1], V. Dubée [3], B. Marie [2]
[1]Université Paris-Est, ANSES, Laboratory for Food Safety, Maisons-Alfort, [2]Muséum National d'Histoire Naturelle - UMR CNRS 7245 MCAM, Equipe "Cyanobactéries, Cyanotoxines et Environnement", [3]Service de réanimation médicale, Hôpital Saint Antoine, Paris, France

Abstract: A 66-year old woman was admitted at Saint Antoine hospital for a motor deficit of the four limbs with an impairment of respiratory function and swallowing, progressively installed over one month. The clinical picture evoked myasthenia gravis (autoimmune disease of the neuro-muscular junction) that was ruled out after an electrophysiological study of neuro-muscular conduction that did not show the characteristic elements of myasthenia gravis. The patient was then seen in consultation by specialists in amyotrophic lateral sclerosis (ALS) because the symptoms would be compatible with this disease, especially since she has a family history. The conclusion of this consultation was that ALS may well be diagnosed. The atypical evolution observed might be related to the fact that the patient has indicated taking daily for three years food supplements containing *Aphanizomenon flos-aquae,* a cyanobacterium known to produce potent cyanotoxins among which anatoxins. Food supplement capsules belonging to the same lot as the one taken by the patient were analysed by enzyme-linked immunosorbent assay for the presence of anatoxin-a. The results came out as positive for this toxin. Chemical analyses by liquid chromatography coupled to mass spectrometry, both in low and high resolution, were performed on three different types of extracts (water, acidified water, acidified methanol) to confirm the presence of anatoxin-a and potentially identify some of its analogues.

Disclosure of Interest: None Declared

HumanToxicology

P-203

LPS from cyanobacterial water blooms induces inflammatory responses in intestinal epithelial cells

L. Švihálková Šindlerová [1 2,*], P. Babica [2], L. Bláha [2], L. Kubala [1]

[1]Department of Free Radical Pathophysiology, Institute of Biophysics of the CAS, v. v. i., [2]Research Centre for Toxic Compounds in the Environment , Faculty of Science, Masaryk University, Brno, Czech Republic

Abstract: Cyanobacterial water blooms in freshwater reservoirs represent a serious human health hazard. They might produce high amounts of cyanotoxins (microcystin, cylindrospermopsin, etc.). On the other hand, they are the source of lipopolysaccharides (LPS) which represent an important part of the cell wall of cyanobacteria and gram negative bacteria and are released into the water during cell division and lysis. It is well documented that even short recreational exposure to cyanobacterial water bloom during swimming, water-skiing or canoeing can result in gastroenteritis.

To study LPS ability to induce pro-inflammatory responses, LPS extracts from cyanobacterial water bloom biomasses dominated by different species were prepared. Namely, it was *Microcystis aeruginosa* (M), *Dolichospermum (ex. Anabaena) curvum* (D), *Aphanizomenon klebahnii* (A) and *Planktothrix aghardii* (P). First, pyrogenicity of these LPS was tested using LAL and Pyrogene assays. Water bloom LPS fractions were compared with commercially available LPS of pathogenic *Escherichia coli* (EC). There was a wide range of pyrogenic activity among cyanobacterial water blooms, from relatively low activities in case of D, A and P to a very high activity of sample M, comparable with the activity of EC.

Further, intestinal epithelial cell lines (IEC) Caco-2 cells and HT-29 were exposed to the LPS. Production of pro-inflammatory interleukin 8 (IL-8) was assessed by ELISA after 24 hours. Surprisingly, concentration of IL-8 released by these cells was comparable to EC treatment not only in the case of sample M, but also in D and P.

Based on the results, two most active LPS originating from water blooms abundant with the species most common in the Czech Republic (M and D) were chosen for the cytokine array experiments. Pools of media from Caco-2 cells exposed in 7 independent experiments were used. The LPS induced production of many different pro-inflammatory cytokines, with the predominance of IL-1β and IL-12.

Furthermore, all LPS preparations activated mouse macrophage RAW264.7 cell line. In this model, water bloom LPS were less active than model LPS of *E. coli*. On the other hand, all LPS from water blooms induced comparable effect on the expression of TNFα, despite their significantly different pyrogenic activities in LAL and Pyrogene tests. LPS fraction from M and D samples produced a comparable band pattern when analysed by SDS-PAGE.

Taken together, LPS from cyanobacterial water bloom may induce pro-inflammatory processes in intestinal epithelial cells as well as in macrophages and therefore they might contribute to the development of gastroenteritis. Moreover, we can speculate that potential risk to human health could be underestimated at least in some samples if it is based on the results of traditional pyrogenic tests, Pyrogene and LAL.

This study was supported by Czech Science Foundation (16-24949S).

Disclosure of Interest: None Declared

HumanToxicology

P-204

Cyanobacteria in the air: do the human lungs like it?

P. Laboha [1,*], O. Brozman [1], B. Kubickova [1], J.-P. Hildebrandt [2], P. Babica [1]

[1]Research Centre for Toxic Compounds in the Environment (RECETOX), Masaryk University, Brno, Czech Republic, [2]Zoological Institute and Museum, Animal Physiology and Biochemistry, University of Greifswald, Greifswald, Germany

Abstract: Toxic compounds produced by cyanobacteria pose hazards to wildlife and human health. Detection of cyanobacterial taxa and cyanotoxins in aerosols and dust particles raises the question of potential hazards associated with human exposures via inhalation. While most attention has been oriented towards harmful cyanobacterial blooms in aquatic ecosystems worldwide, a number of cyanobacterial taxa and cyanotoxins has been detected also in the air, including species originating from both water and terrestrial environments. Indeed, respiratory symptoms have been observed in humans and linked to cyanobacterial exposure. Our study focused on *Microcystis* sp., one of the most common and abundant cyanobacteria across Europe. The susceptibility and vulnerability of human bronchial epithelia to various cyanobacterial compounds, including (i) microcystin-LR, (ii) extracts of *Microcystis* PCC7806 culture and of complex algal bloom with the dominance of *Microcystis* sp., and (iii) lipopolysaccharide (LPS) isolated from PCC7806 and the complex algal bloom were investigated *in vitro*. To assess inhalation toxicity in the airway epithelia, monolayers of immortalized human bronchial epithelial cells 16HBE14o-, HBE1 and Beas-2B were used. The tested compounds/extracts varied in their cytotoxicity assessed as morphologic alterations, resazurin reduction capacity, esterase activity and membrane integrity. Non-cytotoxic concentrations were further tested to evaluate alterations of other cellular events, such as phosphorylation of regulatory enzymes, mitogen-activated protein kinases (MAPKs), and production of pro-inflammatory molecules (cytokines). Since even the water extract of cyanobacteria elicited cytotoxicity at the concentration of 7 mg/mL (dry weight cyanobacterial biomass/mL of cell medium), this study suggests possible hazards of inhalation of cyanobacterial compounds in the air. Further research of cyanotoxins-induced toxicity and underlying mechanisms is needed, as well as more data on environmental concentrations of cyanotoxins in aerosols and exposure assessment. The research was supported by the Czech Science Foundation Grant No. GJ17-25279Y.

Disclosure of Interest: None Declared

Risk assessment for algal and cyanobacterial toxins

P-205

Cylindrospermopsis raciborskii invasion in Portuguese freshwater systems

C. Moreira [1,*], C. Gomes [1], V. Vasconcelos [1 2], A. Antunes [1 2]
[1]CIIMAR, Matosinhos, [2]FCUP - Faculdade de Ciências da Universidade do Porto, Porto, Portugal

Abstract: *Cylindrospermopsis raciborskii* is an invader into temperate environments. In Portugal its occurrence was firstly recorded in the South reservoirs (2000) where the water temperatures are higher. In 2008, it was recorded in the Center Region (Vela Lake). Recently an ongoing project (CYANOTOX) has been responsible for the surveillance of freshwater systems in Portugal where north regions are included. The main aim targets early warning techniques (PCR) in the detection of cyanobacteria and cyanotoxins through the surveillance of relevant freshwater ecosystems located in the national territory. In this sense water samples were collected and processed for PCR detection along with strain isolation, microscopic observation and bloom occurrence from May to September. The molecular identification of *C. raciborskii* was achieved through the PCR amplification of primers cyl2/cyl4 in water samples. The molecular data showed for the first time the appearance of *C. raciborskii* in northern lakes while microscopic observation failed in identifying this cyanobacterium species in those lakes. The microscopic isolation also recovered *C. raciborskii* from the Vela Lake while phylogenetic analysis allowed assessing the genetic diversity and population structure of this species in Portugal. Overall the dispersion of *C. raciborskii* into northern regions identified adaptation mechanisms and supports its biogeographical invasiveness. Finally the obtained data using genomic tools proved to be valuable in determining the invasion of *C. raciborskii* into temperate environments.

Disclosure of Interest: None Declared

Risk assessment for algal and cyanobacterial toxins

P-206

First Detection of Tetrodotoxin in Italian Mussels. Is it an Emerging Threat to Humans in the Mediterranean Area?

C. Dell'aversano [1,*], L. Tartaglione [1], F. Varriale [2], A. Penna [3], M. Giacobbe [4], S. Pigozzi [5], A. Milandri [5], P. Bordin [6], L. Bille [6], A. Turner [7]

[1]Pharmacy, [2]University of Napoli Federico II, Napoli, [3]University of Urbino, Urbino, [4]IAMC - CNR, Messina, [5]Fondazione Centro Ricerche Marine, Cesenatico, [6]Istituto Zooprofilattico delle Venezie, Legnaro, Italy, [7]CEFAS, Weimouth, United Kingdom

Abstract: Tetrodotoxin (TTX) is one of the most potent neurotoxins, originally found in ovary and liver of pufferfish (*Tetraodontidae*) [1]. Successively, TTX was isolated from many other marine and terrestrial animals, as xanthid crab, trumpet shellfish, blue-ringed octopus, chaetognatha, gastropods, starfish, and frogs. The wide distribution of TTX in genetically unrelated organisms has made TTX origin for long time controversial, with different kind of bacteria being identified as TTX-producing organisms [2]. Even *Alexandrium tamarense* – one of the paralytic shellfish poisoning toxins (PST) producing organisms – was proposed as potential biogenetic source of TTX [3].

Although fatal human poisonings following consumption of TTX-contaminated seafood have been reported so far only in Japan, the accumulation of TTX in fish, oysters and mussels collected in Europe (Spain, Portugal, England, Greece) has been recently reported. So, in the frame of a collaborative study on evaluation of PST-related risk in the Mediterranean area, mussels collected in the Siracuse bay (Sicily, Italy) over a three year period (2015-2017), were analyzed by hydrophilic interaction liquid chromatography coupled with both high resolution and tandem mass spectrometry (HILIC-HRMS and HILIC-MS/MS). Both techniques highligted the presence of high PST contamination levels, with samples collected in 2016 containing up to 10851 mg STX eq/kg. Unexpectedly, together with PST, tetrodotoxin was detected in Sicilian mussels. Although this was the first report of TTX in Italy, contamination levels found in mussels (0.8-6.4 µg/kg) were well below the regulatory limit of 2 mg TTX eq/kg established for TTX in Japan. Interestingly, much higher contamination levels of TTX (413 mg/kg) have been detected in mussels collected in 2017 in the northeastern Adriatic coasts of Italy (Lagoon of Marano), in the frame of the monitoring programme for marine biotoxins regulated in the EU.

[1] Wu Z, Xie L, Xia G, Zhang J, Nie Y, Hu J, Wang S, Zhang R. 2005. A new tetrodotoxin-producing actinomycete, Norcardiopsis dassonvillei, isolated from the ovaries of puffer fish Fugu rubripes. Toxicon 45:851-859.

[2] Yasumoto T, Yasumura D, Yotsu M, Michishita T, Endo A, Kotak Y. 1986. Bacterial production of tetrodotoxin and anhydrotetrodotoxin. Agric. Biol. Chem. 50:793–795.

[3] Kodama M, Sato S, Sakamoto S, Ogata T. 1996. Occurrence of tetrodotoxin in Alexandrium tamarense, a causative dinoflagellate of paralytic shellfish poisoning. Toxicon. 34:1101-1105.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-207

Assessment of ciguatera benthic dinoflagellates from the Atlantic coast of Guatemala

E. Blanda [1,*], J. Garcia-Perez [1], M. Vargas-Montero [2], L. Carrillo-Ovalle [1]

[1]Centro de Estudios del Mar y Acuicultura, Universidad de San Carlos de Guatemala, Guatemala, Guatemala, [2]Centro de Investigación en Estructuras Microscopicas , Universidad de Costa Rica, San Jose, Costa Rica

Abstract: Ciguatera fish poisoning is the most common type of marine food poisoning worldwide, which can cause gastrointestinal, cardiovascular and both acute and chronic neurologic diseases in humans, even death. The illness is caused by the consumption of coral reef fishes, which accumulate, through the food chain, toxins produced by benthic dinoflagellates. Endemic areas are mainly the tropical and subtropical Pacific and Indian Ocean and the tropical Caribbean. Species of the genus *Gambierdiscus* are the primary responsible, followed by *Prorocentrum* spp., *Coolia* spp., and *Ostreopsis* spp.. Those genera are usually found over reef areas attached to corals and macroalgae available to fishes to the food chain.

To assess the presence of dinoflagellates responsible for Ciguatera poisoning, a monitoring throughout the years 2016 and 2017 was planned off the Atlantic coast of Guatemala. Three areas were selected: a shallow area (1-3m), where the vegetation is mostly seagrass on a sand bottom (Estero Lagarto) and two coral reef sites: King fish (7-10m) represented mostly by macroalgae and a low percentage of corals, and Cabo Tres Puntas (15-18m) where the percentages of corals is higher than macroalgae. Dinoflagellates were collected using two different techniques: an artificial substrate method, which collect benthic algae for 24h and the traditional macroalgae substrate method. Samples collected were filtered and analysed on light microscopy demonstrating the presence of *Gambierdiscus* spp., *Coolia spp., Prorocentrum spp.* and *Ostreopsis spp.* widely distributed throughout the collection sites. To verify the species of *Gambierdiscus,* a scanning electron microscope was used to take morphological pictures of the theca. The tabulation system was used to determine the number and position of plates but, so far, it was insufficient to reveal the exact species. This work provides new information on benthic dinoflagellates implicated in ciguatera poisoning in the Atlantic, and it is the first report for Guatemalan waters. We recommend a long-term monitoring of the area and endure with the identification of the species of the different genera (through scanning electron microscopy and PCR analyses), hence this information is imperative due to the variety of potentially toxic dinoflagellates living in this marine ecosystem.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-208

Sand-dwelling Dinoflagellates from different types of sediment in the North-Western Waters of the Arabian Gulf

W. A. Al-Rashed [1,*], F. Al-Yamani [1]

[1]Ecosystem-based marine management resources, Kuwait Institute for Scientific Research, Kuwait, Kuwait

Abstract: Benthic dinoflagellates inhabit different substrata such as coral, macroalgae and sediment. The benthic dinoflagellates in sediment lived in the interstitial sand grains (sand-dweller) in the interface between seawater and sand. A comprehensive ecological survey program research on benthic dinoflagellates was conducted to document species from different sediment types. Sediment samples were collected from four stations, including the inshore coral habitats (Qitat Funaitees), offshore coral coral islands (Kubbar), Doha beach, and north of Kuwai Bay at Bubiyan Island (Station A). Qualitative and quantitative studies were run on the sediment samples of the four stations in the period from September 2012 to August 2014. Prorocentrum fukuyoi was the representative benthic dinoflagellate species of Doha station in sediment (220 cells/ g ww of sediment) in winter 2014. It revealed that benthic dinoflagellates were highly diverse in species composition, but they were less in abundance per substrate. Future research is recommended to study the toxicity of the benthic dinoflagellates as they could have severe impact on the economy.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-209

Assessing the risks of Ciguatera in the Caribbean under regional integration. Project IAEA-RLA7020

C. Alonso-Hernandez [1,*]
[1]Centro de Estudios Ambientales de Cienfuegos, Ciudad Nuclear, Cuba

Abstract: With the support of the International Atomic Energy Agency, a regional network to evaluate HABs has been established in the Caribbean region. Training and infrastructure have been strengthened under the RLA7014 and RLA7020 projects.

The objective of this work is to present the results obtained under these projects and to show spatially the presence of *Gambiediscus* sp in the region.

Sampling of benthic dinoflagellates using natural and artificial substrates has been implemented in the coasts of Colombia, Costa Rica, Cuba, Dominican Republic, Guatemala, Nicaragua, Pámama and Venezuela. In all the sites studied, the presence of *Gambiediscus* sp.

A network of five laboratories (Costa Rica, Colombia, Cuba, El Salvador and Nicaragua) have implemented the RBA technique for the analysis of toxicity in marine organisms. Advances, limitations and challenges are also presented.

The results achieved allow to improve the evaluations of the Ciguatera in the Caribbean region with the support of the International Atomic Energy Agency, a regional network to evaluate HABs has been established in the Caribbean region. Training and infrastructure have been strengthened under the RLA7014 and RLA7020 projects.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-210

Cysts, germination and other findings in *Gambierdiscus* spp. laboratory cultures

P. Assuncao [1,*], E. Portillo [1]

[1]Biotechnology Department, Instituto Tecnológico de Canarias, Pozo Izquierdo, Spain

Abstract: Dinoflagellates belonging to the genus *Gambierdiscus* are the former causative agent of ciguatera fish poisoning (CFP), a human illness induced by the consumption of fish that have accumulated ciguatoxins through their diet. Cysts formation is a part of dinoflagellate life cycle and play an important role in the ecology of the species, since remain in the sediment layer during unfavorable conditions for vegetative growth, reinoculating the water column when favorable conditions are restore. These long-term resting cysts can remain dormant for years before germination. Under favorable conditions, temporary cysts can germinate within 3 days, resting cysts can take as long as 5 months to germinate. On the other hand, total toxin concentration of cysts can be at least six-fold than vegetative cells. In this work we describe several observations of cells germination, division and cysts of *Gambierdiscus* spp. under laboratory conditions. We also show the inhibitory effect of *Gambierdiscus australes* cysts and temperature over diatoms. Temperature can be used as a way of diminish diatoms contamination in *Gambierdiscus* cultures and ease the establishment of monoalgal cultures.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-211

CIGUATERA IN FLORIDA KEYS PATCH REEFS: BIOGEOGRAPHIC INDICATORS OF GAMBIERDISCUS DENSITY AND TEMPORAL ABUNDANCE (CFP: BIG DATA)

M. Hian [1,*], M. Parsons [1]
[1]Coastal Watershed Institute, Florida Gulf Coast University, Fort Myers, USA

Abstract: Ciguatera fish poisoning (CFP) is a global public health concern that is associated with *Gambierdiscus,* a genus of harmful algae found in coral reef environments that includes species known to produce toxins (ciguatoxins). Outbreaks of CFP have often been linked to elevated abundance of *Gambierdiscus* cells and disturbance-related degradation of coral reefs. However, the influence of human activities on CFP risk, both directly and indirectly within the broader context of reef health, has yet to be defined for highly exploited patch reefs in the Florida Keys. The objectives of this study were to define spatial and temporal patterns in reef health and *Gambierdiscus* abundance across the three regions (Upper, Middle, Lower), to determine whether the drivers of those patterns were natural or anthropogenic, and to identify biogeographic indicators of risk. To address these objectives, this study combined field sampling with a "big data" approach to spatial analysis. Six patch reefs (two per each of three regions) were selected as study sites from existing research stations. Datasets from long-term monitoring of benthic cover, fish species abundance, land use, and water quality were compiled and analyzed in ArcGIS to characterize the ecological context of each site. Analysis of samples of host macroalgae collected from all study sites biannually revealed that *Gambierdiscus* cell densities were consistently highest in the Upper Keys and lowest in the Middle Keys, regardless of season. Conversely, reef health was lowest in the Upper Keys and improved along a gradient to the Lower Keys. Multivariate analysis of site similarity indicated that this regional pattern was driven more strongly by grazing than substrate availability. Additionally, there is evidence that human activities have an indirect influence on CFP risk through reef health, as well as through overfishing, and the destruction of inshore habitats like seagrass and mangroves. Due to a strong positive correlation with cell densities, this study suggests that mangrove cover could be useful as a biogeographic indicator of potential CFP risk. Whereas surgeonfish, with a strong negative correlation with cell densities, could indicate the actual flow of toxins into higher trophic levels. The concordance of high regional risk and high population density necessitates continued monitoring of fish in those areas and the development of more comprehensive predictor of potential CFP outbreaks.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-212

Abiotic Influences and Community Dynamics of *Gambierdiscus* species in the Florida Keys.

J. Schroeder [1,*], M. Parsons [2]

[1]Graduate Assistant, [2]College of Arts and Sciences, Florida Gulf Coast University, Estero, USA

Abstract: *Gambierdiscus* is an epibenthic dinoflagellate genus containing some species that produces a toxin that causes Ciguatera Fish Poisoning (CFP). These dinoflagellates live on macrophytes that are consumed by herbivorous fish, thereby introducing the toxin into the reef food web. These small fish are later predated by larger fish, leading to toxin biomagnification in the larger fish tissues. Other dinoflagellates co-exist on the same macrophyte hosts that *Gambierdiscus* spp. reside on including potentially toxigenic *Prorocentrum* spp. and *Ostreopsis* spp. The research presented here is on the community ecology of *Gambierdiscus* spp. and these other dinoflagellates, and the abiotic factors that affect their abundance. The knowledge obtained from this project will provide valuable information on the community structure of the benthic dinoflagellate community dynamics and the influence of abiotic factors in relation to community composition.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-213

NUCLEAR TECHNIQUE FOR DETECTION OF SAXITOXIN IN SHELLFISH USING RECEPTOR BINDING ASSAY (RBA) METHOD AT LAMPUNG BAY

T. R. Dyah Larasati [1,*], U. Sugiharto [1], M. Tajri [2], A. Sumartono [1], W. Andayani [1]
[1]Centre for Isotopes and Radiation Application, National Nuclear Energy Agency, Jakarta, [2]Balai Besar Perikanan Budidaya Laut Lampung, Pesawaran, Indonesia

Abstract: The nuclear technique with RBA (Receptor Binding Assay) to detect saxitoxin, one of the PSP (Paralytic Shellfish Poisoning) toxins in shellfish, has been done in Lampung Bay. The study was conducted when the color of sea changed to brown and mass fish deaths occured in 2012, 2014 and 2015. This phenomenon was suspected to be a red tide. PSP toxicity analysis in Indonesia was done using a bioassay mouse with a minimum detection limit of 40 ug STX / 100 g of wet shell. An alternative method of quickly determining specific and sensitive toxicity of PSP level in green shells is indispensable. One of the alternative methods is the nuclear technique with RBA. In this method, 3H-saxitoxin was used as a tracer and capable of detecting PSP level up to 1 µg STX / 100 g of wet shell. Environmental parameters, nutritional content, and plankton species were monitored during the sampling.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-214

Human sickness and toxicity relating to imported Red Snapper implicated with a case of ciguatera fish poisoning in the United Kingdom

A. D. Turner [1,*], B. Maskrey [1], A. Robertson [2]
[1]Food Safety, Cefas, Weymouth, United Kingdom, [2]Dauphin Island Sea Lab, Dauphin Island, USA

Abstract: In June 2017, a suspected CFP incident occurred in Stoke-on-Trent, central England, in the UK associated with the consumption of Red Snapper fillets. Three people were affected, all from the same family, all of whom consumed fillets from the same product package and batch of fish. The fish were caught in the Indian Ocean, processed in India and imported to the UK. Two of the three family members were admitted to hospital with symptoms of diarrhoea and vomiting, followed by parasthesia and shortness of breath. The reporting doctor provided a presumptive diagnosis in the absence of analytical confirmation that CFP was the likely cause of illness, based upon the characteristic signs and symptoms, together with the history of eating species of fish that are known to carry ciguatoxins.

One fillet of snapper remained uncooked in frozen storage from the family, which was subsequently submitted for toxicity screening and toxin analysis. Cytotoxicity testing confirmed the presence of sodium channel blocking activity in the fish tissue. The results revealed levels of ciguatoxicity above guidance limits, meaning that human illness would be expected following consumption of this fish. Chemical detection using LC-MS/MS was also used to confirm the presence of chromtaographic peaks indicative of Caribbean ciguatoxins.

Taking the shared medical symptoms, and the known food consumption patters, together with the known potential for CFP to occur in this species of fish, the subsequent evidence for ciguatoxin-like activity and ciguatoxins in the same fish samples provides strong evidence for this fish to be contaminated. Furthermore, given the levels screened, such toxicity would be expected to cause intoxication in humans. To our knowledge, this represents the first confirmed occurrence of CFP in the UK from imported fishery products.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-215

Report of a major outbreak of ciguatera fish poisoning in Mangalore, India

I. Karunasagar [1], A. D. Turner [2,*], B. Maskrey [2], A. Robertson [3], S. Shivanagouda Hosagoudar [1], P. Rai [1], S. Adappa [1], S. Hiremath [4], S. Kogaluru Shivakumaraswamy [1], M.-Y. Dechraoui Bottein [5], R. Clausing [5], L. Turner [6], A. Godhe [7], I. Karunasagar [1]

[1]University of Nitte, Nitte, India, [2]Food Safety, Cefas, Weymouth, United Kingdom, [3]Dauphin Island Sea Lab, Dauphin Island, USA, [4]Justice K.S. Hegde Hospital, Nitte, India, [5]International Atomic Energy Agency , Monaco, France, [6]University of Plymouth, Plymouth, United Kingdom, [7]University of Gothenburg, Gothenburg, Sweden

Abstract: On 30th September 2016, over 200 people, most of them working in a seafood processing unit complained of food poisoning symptoms including abdominal pain, vomiting, weakness and tingling sensations around the mouth and limbs. 1-6 pieces of cooked heads of large red snapper were consumed per person. Gastrointestinal symptoms started appearing in about 4-5 hours and gradually, the number of affected individuals began reporting to 5 different tertiary care hospitals around Mangalore the same evening. By midnight, over 200 people were sick. The majority of those affected reported neurological symptoms including circumoral paraesthesia, pain and numbness of limbs. Around 25% of the affected individuals were treated as outpatients, as the symptoms were mild and could be managed without admission as there was shortage of emergency beds. 75% of individuals were hospitalized due to severity of symptoms. About 10% experienced cardiac symptoms like sinus bradycardia, low pulse and hypotension. Conservative management was resorted to and several of them discharged in 48h on becoming asymptomatic. 10% of the patients continued to remain in the ICU for a few more days due to persistence of cardiac symptoms.

Since the outbreak occurred among staff of a fish processing unit, fish heads and whole fish from the incriminated batch of fish consumed was obtained for further studies. Based on clinical symptoms and the involved fish species, ciguatera fish poisoning (CFP) was suspected. Fish heads, muscle, liver, gonads were extracted using two solvent/solvent extraction procedures leading to both dichloromethane CTX (ciguatoxin) and methanol MTX (maitotoxin) crude extracts. The CTX extract was used for mouse bioassay (MBA), receptor binding assay (RBA), neuroblastoma cell toxicity assay (N2A) and LC MS/MS analysis. The RBA indicated the presence of ciguatoxin-like activity equivalent to 1.10, 1.36 and 2.61 ng PCTX3C/g muscle, intestine and liver tissues respectively. Ciguatoxicity was not found in the gonads. LC MS/MS analysis showed chromatographic peaks indicative of Indian Ocean and Caribbean ciguatoxins in the same tissue samples. The ciguatoxins from the Indian ocean have not yet been fully identified; however four molecules (I-CTX-1 through -4,molecular weight of 1140, 1140, 1156 and 1156 respectively) have been isolated, inducing similar symptoms on mice (LD50 ~0.5 mg/kg), and capable of competing with brevetoxins as for ciguatoxin from the Pacific and the Caribbean. Yet to our knowledge the toxic equivalent factor for human consumption between the I-CTX and the P_CTX has not yet been determined.

Fish identification was done based on PCR amplification of cytochrome oxidase subunit-1 and 16S rDNA from mitochondrial DNA as Lutjanus bohar (red snapper).

Overall, these results confirm the presence of ciguatoxin activity in the fish involved in the intoxication.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-216

Characterization of bioactive metabolites from benthic dinoflagellate *Gambierdiscus balechii*

W. H. Lee [1 2,*], C.-K. Lu [3 4], K. C. Yip [1 2], Y. L. Mak [1], J. Wu [1], L. L. Chan [1 2]

[1]State Key Laboratory in Marine Pollution, [2]Department of Biomedical Sciences, City University of Hong Kong, Kowloon Tong, Hong Kong, [3]National Research Institute of Chinese Medicine, Ministry of Health and Welfare, [4]Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, -

Abstract: Dinoflagellate is a group of unicellular algae that are often described as chemically diverse. Studies have been carried out to determine the relevant knowledge about marine biotoxin produced by dinoflagellates. For instance, ciguatoxin is one of the most famous biotoxin produced by *Gambierdiscus* due to the highly potent nature to human that known as ciguatera fish poisoning. The major transmission route is made by consuming intoxicated coral reef fish that accumulated ciguatoxin in their diet. In this study, toxigenic strains of *Gambierdiscus balechii* was cultured in ES-DK enriched medium from artificial seawater. Harvested dinoflagellates was extracted by organic solvent, mainly MeOH. The crude extract was further fractionated by liquid-liquid partitioning, solid phase extraction clean-up and high-performance liquid chromatography. The isolated fractions were used to study the bioactivity of the metabolites produced by the *Gambierdiscus* strain, including anti-bacteria activity, algicidal function and toxicological study. The results showed that bioactive metabolites other than commonly known maitotoxin and ciguatoxin were also produced by the *Gambierdiscus* strain. These data suggest the feasibility of novel metabolites or new analogues of ciguatoxin from *Gambierdiscus* strain. The isolated metabolites will be useful for further biomedical and ecotoxicology research.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-217

The first report of occurrence of *Gambierdiscus silvae* in the coastal areas of Japan.

H. Funaki [1,*], S. Yoshii [1], T. Yoshioka [1], Y. Tanii [1], K. Hashimoto [1], T. Nishimura [1], K. Tanaka [1], I. Yanagida [2], H. Yamaguchi [1], M. Adachi [1]
[1]Kochi university, Nankoku, [2]INO NPO, Okinawa, Japan

Abstract: The marine benthic dinoflagellates genus *Gambierdiscus* are known to produce potent marine toxins such as ciguatoxins (CTXs) and maitotoxins (MTXs), which can have a negative impact on human health. Unveiling the mechanism of CFP outbreaks, it is important to clarify the species compositions of the genus in coastal areas. In the Japanese coastal areas, five species/phylotypes of dinoflagellate *Gambierdiscus* (*G. australes, G. scabrosus, Gambierdiscus* sp. type 2, *Gambierdiscus* sp. type 3, and *Gambierdiscus* cf. *silvae*) have been reported. Among them, *Gambierdiscus* cf. *silvae* is the species that is dominantly found at the deep sites (30 m depth) of Okinawa Prefecture (Adachi et al. 2016). We examined the morphological and phylogenetic position of *Gambierdiscus* cf. *silvae* dOHHG1 isolated from the coastal area of Okinawa Prefecture, and its toxicity was assessed using a mouse assay. The cells of *Gambierdiscus* cf. *silvae* dOHHG1 were 59.0 ± 8.7 µm deep, 54.2 ± 8.4 µm wide, and 36.3 ± 4.6 µm long. The plate formula of the strain was Po, 4′, 0a, 6′′′, 6c?, 6s?, 5′′′, 0p, and 2′′′′. The morphological features of these cells resembled those of *G. silvae* S. Fraga & F. Rodríguez, in the following aspects: anteroposteriorly compressed cell shape; broad 2′′′′ plate; hatchet-shaped 2′ plate; oblique dorsal end of 2′′′′ plate; absence of distinct fold formed by 1′, 4′, and 1′′ plate, and elongated 1′ plate. Phylogenetic analysis of *Gambierdiscus* cf. *silvae* dOHHG1 based on LSU rDNA D8–D10 revealed that this strain was closely related to *G. silvae*. Thus, we identified *Gambierdiscus* cf. *silvae* dOHHG1 as *G. silvae*. The mouse toxicity (dichloromethane soluble fraction) of *G. silvae* dOHHG1 was observed to be the second-highest among all Japanese *Gambierdiscus* species/phylotypes. This is the first report about the occurrence of *G. silvae* in the Pacific Ocean. The toxicity analysis results emphasize the importance of monitoring *G. silvae* in the coastal areas of Japan.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-218

Prevalence of Caribbean ciguatoxins and benthic algal toxins in fishes from the Florida Keys Marine Sanctuary

A. B. Catasus [1,*], M. Parsons [1], A. Robertson [2]

[1]Coastal Watershed Institute, Florida Gulf Coast University, Fort Myers, [2]Department of Marine Sciences, University of South Alabama, Mobile, USA

Abstract: Ciguatera fish poisoning (CFP) is a common syndrome affecting coastal communities in the Western Atlantic and Indo-Pacific regions. This severe illness is caused by the ingestion of reef fish contaminated with ciguatoxins (CTXs). The source of CTXs has been associated with epiphytic dinoflagellates of the genus *Gambierdiscus* (among others) which reside on macroalgae that are grazed by herbivores. The entry of algal CTX metabolites (often referred to as gambiertoxins) into the food web initiates a cascade of trophic transfer events and biotransformations that have not been fully elucidated in the Greater Caribbean region. A critical knowledge gap addressed in the present study was to gain better predictive capacity of CTX presence in fishes across trophic levels in a sub-tropical hotspot for CFP. A variety of fish including herbivores (e.g. representatives of Acanthuridae, Pomacathidae) up to apex predators (e.g., *Sphyrena barracuda*, *Mycteroperca bonaci*) were collected from a long term monitoring site in the Florida Keys. Extracts of fish were assessed for neurotoxicity and CTX activity using the sensitive *in vitro* neuroblastoma (N2a) assay. Twenty seven of the 66 fish (40%) examined during this study expressed sodium channel dependent toxicity in both sensitized and non-sensitized cell treatments highlighting the likely presence of multiple toxin classes in these reef fish. Monospecific sodium channel activity was observed in 7% of fish represented by one species: *S. barracuda.* Given the complexity of potential toxin classes, further efforts are now focused on the identification of toxin congeners, rather than toxicity alone. The presence of toxic fish indicates a need for risk assessment and management consideration in the Florida Keys.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-219

A French initiative for harmonizing the Neuro2A cell based assay for ciguatoxin detection: preliminary results

V. Fessard [1,*], R. Lanceleur [1], C. Canepa [1], F. Maillot [2], J. Turquet [2], K. Lhaute [3], P. Hess [3], J. Viallon [4], T. Darius [4], M. Chinain [4], M.-Y. Dechraoui Bottein [5], R. Biré [6]

[1]Toxicology of contaminants, French Agency for Food, Environmental and Occupational Health & Safety, Fougères, [2]Hydro Réunion, Ste-Clotilde, [3]Laboratoire Phycotoxines, IFREMER, Nantes, France, [4]Laboratory of Toxic Microalgae, Institut Louis Malardé, Papeete, French Polynesia, [5]Environment Laboratories, International Atomic Energy Agency, Monaco, Monaco, [6]French Agency for Food, Environmental and Occupational Health & Safety, Maisons-Alfort, France

Abstract: Ciguatera Fish Poisoning (CFP) is an intoxication due to consumption of fish contaminated with ciguatoxins (CTXs). Symptoms are mainly gastro-intestinal, neurological and, to a lesser degree, cardiovascular. If CFP frequently occurs in tropical and subtropical areas, an emerging risk for European countries is not excluded since *Gambierdiscus* dinoflagellates were detected in the Mediterranean Sea and in the Eastern Atlantic Ocean and ciguatera cases were reported in the Canary Islands and suspected in Madeira. A maximum permitted level of 0.01 ng g−1 P-CTX1 equivalent toxicity has been recommended but, until now, European regulations have not given any indication for a reference method nor for regulatory safety CTX limits in fishery products. In 2017, a French consortium supported by the PHYCOTOX group of research (https://www.phycotox.fr/) launched an interlaboratory comparison on the Neuro2A cell based assay that, among the few methods available for CTX detection, can detect very low concentrations of CTXs. The project aims to set up the cell-based assay in several laboratories, assess its performance in comparison with other methods such as the receptor binding assay, and develop a harmonized protocol. Five laboratories are involved in this round robin testing.

The results of the laboratories will be presented i) with the pure toxins P-CTX3C and brevetoxin 3 and ii) with the same dinoflagellate cultures and fish samples distributed to the different laboratories. We will also outline the difficulties encountered when performing the assay and the implementations which can be proposed.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-220

Intercalibration of counting methods for *Ostreopsis* spp. blooms in the Mediterranean Sea

A. Penna[1 2,*], S. Casabianca[1], F. Sbrana[3], N. Gjeci[4], S. Capellacci[1], V. Asnaghi[5], E. Ottaviani[6], V. Giussani[7], L. Pugliese[1], C. Jauzein[8], R. Lemée[9], M. A. Hachani[10], S. Turki[10], L. Acaf[11], M. Abboud-Abi Saab [11], A. Fricke[12], L. Mangialajo[12], R. Bertolotto[13], C. Totti[14], S. Accoroni[14], E. Berdalet[15], M. Vila[15], M. C. Chiantore[5], M. Vassalli[3]

[1]DISB, University of Urbino, Pesaro, [2]CONISMA, Roma, [3]Institute of Biophysics, CNR, [4]OnAir srl, [5]University of Genova, [6]On Air srl, Genova, [7]ARPAL, Genova, Italy, [8]IFREMER, Centre de Brest, Plouzané, [9]INSU-CNRS, Laboratoire d'Océanographie , Villefranche-sur-Mer, France, [10]Laboratoire Milieu Marin, INSTM, Tunis, Tunisia, [11]National Council for Scientific Research, Beirut, Lebanon, [12]Université Côte d'Azur, CNRS, Nice, France, [13]ARPAL, Genova, [14]DISVA, Università Politecnica delle Marche, Ancona, Italy, [15]Institut de Cìences del Mar, CSIC, Barcelona, Spain

Abstract: This study describes the adoption and validation of two innovative methods for the automated count of *Ostreopsis* spp. concentration in sea water: a molecular assay based on qPCR and an opto-electronic device allowing to automatically acquire images from the whole sample and recognize the target algae on the basis of a machine learning algorithm. The two approaches were tested on samples coming from different locations in 5 countries along the Mediterranean Sea, collected in the framework of the cross-border EU ENPI CBCMED Project M3-HABs, and compared with the standard visual counting method. The results demonstrate the effectiveness of both automatic approaches which provide a valuable tool, mostly cost and time effective, for the establishment of large-scale and effective monitoring of *Ostreopsis* spp. blooms. Moreover, the two automatic methods demonstrated the ability to discriminate for the presence of a different but similar species, *O. fattorussoi*, for which new species-specific qPCR primers were developed.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-221

Clean-up of Fish Extracts for Ciguatoxin Analysis Using Receptor Binding Assay

B. Porntepkasemsan [1,*], W. Kulsawat [1], P. Nochit [1]
[1]Thailand Institute of Nuclear Technology (Public Organization), Nakhon-Nayok 26120, Thailand

Abstract: In general, a receptor binding assay (RBA) provided a robust method for the detection of ciguatoxin (CTX) and could easily be a CTX screening tool. However, when testing fish extract for CTX using RBA, the matrix effect has to be taken into consideration. It was observed that fish accumulated more lipid prior to reproductive stage which interfered with RBA by increasing non-specific [^{3}H]Pb-Tx-3 binding. In this study, the matrix effect was conducted in pelagic fish obtained from a HACCP fish farm that was assured not exposed to CTX and referred herein as nontoxic fish extract. Optimization experiments were carried out to maximize recoveries and the effectiveness of the clean-up. A modification in the purification step with respect to the IAEA Tecdoc1729 was carried out. Adequate clean-up of fish extracts using two times of hexane extraction allowed for the elimination of unnecessary lipid matrix; in which no CTX toxicity were found. This resulted in a substantial reduction of matrix effects which 72% of non-target lipids were removed. While in applying the Florisil solid phase extraction (SPE), it performed less satisfactory with respect to the low recovery. The obtained clean-up procedure was assessed in herbivorous and carnivorous fish extracts of Thai waters. It revealed the most effective for both species.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-222

Application of a Receptor Binding Assay to the Analyses of Ciguatera toxin in Reef fish, Thailand

W. Kulsawat [1,*], B. Porntepkasemsan [1], P. Nochit [1]

[1]Thailand Institute of Nuclear Technology (Public Organization), Nakhon-Nayok 26120, Thailand

Abstract: Ciguatera is the worldwide marine toxins which accumulates in many types of reef fish. However, it has been largely neglected in many countries due to lack of standard protocol. This study examined 72 carnivorous reef fish belonging to 4 distinct species i.e. grouper, snapper, streak spinefoot, and trevally. Fish samples were wild caught from the coastal waters of Thailand. Extracts from 20 gram fish flesh of 27 grouper, 15 snapper, 15 streak spinefoot and 15 trevally were purified and determined for the presence of ciguatera toxin (CTX). A receptor binding assay in microplate format has been applied to this study. It was found that of seventy-two fish samples indicated negative results. The negative control test was conducted by analyzing random samples under differing method. Those samples were subjected to chemical analysis via liquid chromatography tandem-mass spectrometry (LC/MS/MS) which revealed negative results as the previous tests. It indicated that the receptor binding assay technique was shown to be an effective tool for CTX monitoring programme.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-223

Effects of temperature and nutrients availability on the growth and toxicity of *Gambierdiscus* spp.

S. Longo [1,*], K. Henry [1], A. Ung [1], J. Viallon [1], H. T. Darius [1], P. Hess [2], M. Chinain [1]
[1]Institut Louis Malardé UMR 241 EIO, Papeete, French Polynesia, [2]Phycotoxins Laboratory, Ifremer, Nantes, France

Abstract: Ciguatera is a global seafood poisoning resulting from the consumption of fish and marine invertebrates contaminated by potent neurotoxins known as ciguatoxins (CTXs). These compounds are produced by a benthic dinoflagellate, *Gambierdiscus* spp., which proliferates episodically in the environment. Adverse climatic events (cyclone, tsunami, coral bleaching, …) and anthropogenic disturbances are arguably some of the factors likely to promote ciguatera outbreaks in highly damaged coral reef ecosystems.

Considering the high reliance of local populations on lagoon resources for their subsistence, and the high incidence rates consistently reported over the past decades in most of its islands, French Polynesia is currently regarded as one of the most affected areas worldwide. In a context of global ocean warming (+ 0.1°C / century), acidification (- 0.4 pH units / century) and increased eutrophication in coral reef ecosystems, there is therefore reason to fear an increased risk of ciguatera.

The objective of the present study is to investigate how the variations in some key-environmental parameters such as temperature, pH and nitrogen availability may affect the growth and toxicity of *Gambierdiscus* in *in vitro* conditions. To this end, cultures of the six *Gambierdiscus* species currently reported in French Polynesia will be carried out and their growth rates and toxin production tested under different eco-physiological conditions.

Here, we present our preliminary results on the effects of highly contrasted temperatures on the growth and toxicity of *G. polynesiensis* cultures (as assessed by the neuroblastoma cell based assay). The impacts of nitrogen depletion *versus* repletion on the toxicity of different strains of *G. polynesiensis* originating from various island groups of French Polynesia will also be evaluated, with reference to standard culturing conditions (used in routine for strain acclimation and culture maintenance).

Implications of these preliminary findings in terms of increased ciguatera risk will be discussed.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-224

Coolia spp. (Dinophyceae) in the Canary Islands (NE Atlantic Ocean): the highest species richness observed worldwide

H. David [1] [2], A. Laza-Martínez [2,*], F. Rodríguez [3], S. Fraga [3] [4], E. Orive [2]

[1]MARE, University of Lisbon, Lisbon, Portugal, [2]Plant Biology and Ecology, University of The Basque Country (UPV/EHU), Leioa, [3]Centro Oceanográfico de Vigo; Instituto Español de Oceanografía, Vigo, [4]Praza Mestra Manuela 1, Nigran, Spain

Abstract: Benthic dinoflagellate studies have increased worldwide during the last decade, improving our knowledge on their diversity and biogeography. Nevertheless, tropical and subtropical areas in the Eastern Atlantic Ocean have been poorly studied. The Canary Islands are located close to NW African coast, in a subtropical region. Studies from these islands during the last years reported an unexpected species richness of the genus *Gambierdiscus*. In the present communication we address the genus *Coolia* from this area. We sampled macroalgae from five sites in Tenerife Island in September of 2013, when water temperature ranged 23-24 °C. Samples were collected in the intertidal and subtidal zones by snorkeling, and screened for epiphytic dinoflagellates. Thirty two clonal *Coolia* strains were isolated. Light and scanning electron microscopy analyses were performed for morphological characterization and LSU rDNA sequencing (D1-D2 region) for molecular identification. From general cell morphology two groups of strains could be distinguished: one with cells showing some degree of antero-posterior flattening, and another with cells more robust and rounded. Phylogenetic analyses showed that the former contained 3 ribotypes that corresponded to *C. monotis*, *C. malayensis* and *C. palmirensis*. The latter group consisted of *C. tropicalis*, the two ribotypes contained in the original description of *C. canariensis* and a new ribotype closely related to these. Thus, all known *Coolia* species, except *C. santacroce* and *C. areolata*, plus a new ribotype are present in the Canary Islands, the highest *Coolia* species richness reported in the world for a single location. Species with different biogeographic patterns converge in this archipelago. The ribotype containing the type of *C. canariensis* and the new ribotype reported here have only been observed in the Canary Islands, whereas the clade sister of *C. canariensis* has been reported also from the west Pacific. *Coolia malayensis*, *C. tropicalis* and *C. palmirensis* show a pantropical distribution. Finally, *C. monotis* shows a temperate North Atlantic (including Mediterranean Sea) distribution.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-225

Biodiversity, distribution and toxicity of benthic dinoflagellates in a subtropical reef: the first comprehensive study in Hong Kong

V. T. Lam [1,*], S. K. Yiu [1], P. T. Leung [1 2], M. Yan [1 2], T.-C. Wai [1 2], P. K. Lam [1 2 3]

[1]State Key Laboratory in Marine Pollution, City University of Hong Kong, Hong Kong, [2]Research Centre for the Oceans and Human Health, City University of Hong Kong Shenzhen Research Institute, Shenzhen, [3]Department of Chemistry, City University of Hong Kong, Hong Kong, China

Abstract: Marine dinoflagellates are well known causative agents for harmful algal blooms (HABs) and seafood poisoning. Their records are worldwide, and their occurrences are more frequently reported in tropical and subtropical regions. Marine benthic dinoflagellates, unlike the planktonic form, generally inhabit substrate surface such as macroalgae, coral rubbles or/ and sediments in reef ecosystems. The outbreaks of benthic species are generally subtle and have no clear visible environmental phenomena, such as blooms/ red tides as shown by planktonic dinoflagellates, and therefore are often overlooked before the occurrence of food poisoning cases. Hong Kong has subtropical climate, is regularly affected by the HABs of planktonic dinoflagellates. The occurrence of algal blooms and biodiversity of the benthic dinoflagellates, as well as their potential risks to the local marine environments, are however virtually unknown.

This study, for the first time, successfully isolated >200 strains of benthic dinoflagellate from local coral communities and rocky reefs. A total of 20 benthic dinoflagellate species of genera *Amphidinium, Coolia, Fukuyoa, Ostreopsis* and *Prorocentrum* were comprehensively revealed using morphological and molecular techniques; and a benthic algal culture collection has been systematically established. These species were eventually examined by bioassays, and potentially toxic benthic dinoflagellates have been discovered. This study has updated the current biodiversity and distribution of the toxic benthic dinoflagellates, and contributes to the understanding of their emerging threats to the sub-tropical reef systems locally and regionally. In addition, the established algal culture collection is an important resource for supporting further studies of HABs

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-226

Is the eastern Mediterranean a "*Gambierdiscus* biodiversity hotspot"? New data from Greece and Cyprus

K. ALIGIZAKI [1,*], M. ILIADOU [2], I. KAPPAS [2], M. ARSENAKIS [1]

[1]LABORATORY UNIT ON HARMFUL MARINE MICROALGAE, BIOLOGY DEPT, [2]BIOLOGY DEPT, Aristotle University of Thessaloniki, THESSALONIKI, Greece

Abstract: The first record of *Gambierdiscus* in the Mediterranean Sea (Crete) in 2003 raised significant concern regarding a possible onset of Ciguatera in the area. In order to investigate this risk, research on *Gambierdiscus* distribution has been intensified especially in Crete the last decade, while recently, in the frame of EuroCigua project, the samplings have expanded in Cyprus as well. Morphological characterization (epifluorescence and scanning electron microscopy) and molecular identification based on the SSU and D1-D3/D8-D10 LSU rRNA regions indicate the presence of at least three different taxa in western Crete other than *G. carolinianus* isolated from the same area previously: *Gambierdiscus silvae*, which is recorded for the first time in the Mediterranean Sea, a second taxon, morphologically and genetically identical to *Gambierdiscus* sp., the putative new species initially identified in Crete, and a third taxon, closely related to *G. belizeanus*. In Cyprus, *Fukuyoa* sp. has also been identified, while this genus was detected in 2010 in the central Aegean continental coasts. Strains of the above *Gambierdiscus* taxa from both Crete and Cyprus are propagated under culture conditions for further toxicity testing using the neuroblastoma N2A cytotoxicity assay. Preliminary results indicate high toxicity activity for *G. silvae* and low for *Gambierdiscus* sp., in line with earlier studies. After the Canary Islands in the NE Atlantic, the eastern Mediterranean Sea and especially Crete seems to represent a “biodiversity hotspot” of *Gambierdiscus/Fukuyoa*, with at least five species being identified so far.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-227

Assessing the potential for temperate range extension of dinoflagellates associated with ciguatera fish poisoning in Australia

M. E. Larsson [1,*], S. Murray [1], M. Doblin [1]

[1]Climate Change Cluster (C3), University of Technology Sydney, Sydney, Australia

Abstract: Some species from the benthic dinoflagellate genus *Gambierdiscus* produce potent neurotoxins, such as ciguatoxins (CTXs) and maitotoxins (MTXs), which can accumulate in the marine food web and cause the human illness Ciguatera Fish Poisoning (CFP). The genus is typically distributed throughout tropical environments, although in recent years, populations have been documented in more temperate locations. In this study, we used an ecological approach to assess the potential of *Gambierdiscus* to extend its range in eastern Australia and a toxicological approach to evaluate what this means for managing the risk of human exposure to CFP in temperate waters. Four species of *Gambierdiscus* (*G.* sp. 1, *G.* sp. 2, *G. carpenteri* and *G. lapillus*) were identified from the tropical Great Barrier Reef region of Australia but only *G. carpenteri* was found at a temperate location. Using a novel Ca^{2+} influx bioassay, *G.* sp. 1, *G*. sp. 2 and *G. lapillus* where found to produce compounds with CTX-like activity and all species produced compounds with MTX-like activity. Fitness curves across environmental gradients of temperature, salinity and irradiance suggest the distribution of *Gambierdiscus* is not limited by these abiotic factors, so the current and future species distribution is likely to be determined by other factors. Rafting on detached macrophyte fragments, transported south with the East Australian Current, was identified as the likely natural long-distance dispersal mechanism aiding introduction of *Gambierdiscus* into temperate locations, and successful colonisation in novel habitats was found to only require a single pulse of very few cells. This study advances knowledge about the ecology and toxicology of *Gambierdiscus* species from eastern Australia and provides experimental evidence of the mechanisms that could facilitate temperate range extension of the genus.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-228

Screening of ciguatoxin-like toxicity in fish from Madeira

À. Tudó [1,*], N. Sagristà [1], P. Aguayo [1], L. Reverté [1], M. Campàs [1], M. Rambla-Alegre [1], C. Flores [2], J. Caixach [2], N. Gouveia [3], C. Santos [4], A. Marques [5], J. Diogène [1]

[1]Institut de Recerca de Tecnologies Agroalimentàries (IRTA), Sant Carles de la Ràpita, [2]Mass Spectrometry Laboratory/Organic Pollutants, IDAEA-CSIC, Barcelona, Spain, [3]Regional Fisheries Management - Madeira Government, DSI-DRP, [4]Instituto das Florestas e Conservação da Natureza, IP-RAM, Secretaria Regional do Ambiente e Recursos Naturais, Regional Government of Madeira, IFCN IP-RAM, Madeira, [5]Portuguese Institute of Sea and Atmosphere (IPMA), Lisbon, Portugal

Abstract:

Outbreaks of ciguatera have been reported in the last years in the Eastern North Atlantic Ocean, mainly in the Canary Islands and in Madeira. Ciguatoxins (CTXs) and derivates have been detected in more than 400 fish species. These species are spatially and temporally unpredictable and, therefore, more effort is required to determine which are the species susceptible to accumulate these biotoxins and how accumulation works.

In the frame of the ECsafeSEAFOOD project, 40 fish of 16 different species from seawaters of Madeira were collected between October 2013 and December 2014. Fish were dissected and muscle and liver were extracted with acetone followed by dyethyl ether/water and hexane/aqueous methanol (80%) liquid/liquid partitions. Subsequently, samples were tested by the neuroblastoma cell-based assay (N2A) to screen for the presence of CTX-like toxins.

Twelve fish of 6 different species were CTX-like positive by N2A. All of moray eel samples (n=9) of 3 species of moray eel (*Muraena helena*, *M. augusti* and *Gymnothorax unicolor*) showed CTX-like positivity in liver and 4 of them showed positive CTX-activity also in muscle. Three other fish *Balistes capriscus*, *Mycteroperca fusca* and *Bodianus scrofa* showed CTX-like toxicity only in liver. In all cases, values of CTX-1B equivalents detected in liver were higher than the levels detected in muscles. Quantification values in both tissues were above the value of 0.01 ppb of CTX-1B eq., which is considered as the level that may cause ciguatera fish poisoning.

These results suggest liver is a better indicator for bioaccumulation of ciguatoxins than muscles. However, muscle may be a better tissue to conduct food safety assessment considering that liver is not consumed in Macaronesia. In addition, these CTX-like positive samples are presently being analysed via liquid chromatography coupled to high resolution mass spectrometry (LC-ESI-HRMS) in order to confirm the presence of CTXs.

Acknowledgments: The research leading to these results has received funding from the European Union’s Seventh Framework Programme (FP7/2007-2013) under grant agreement no 311820 (ECsafeSEAFOOD). The authors acknowledge Direçao Regional das Pescas and Parque Natural da Madeira. A.Tudó acknowledge IRTA-URV-Banco Santander for the respective PhD grant (2016 PMF-PIPF-74).

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-229

EFSA FPA for the characterization of ciguatera fish poisoning in the EU (EuroCigua): Role and activities of the University of Vigo

A. Gago-Martinez [1,*], J. M. Leao [2], P. Estevez [1], D. Castro [3], A. Pequeño [4], O. Vilariño [2]

[1]Analytical and Food Chemistry, [2]Analytical and Food Chemistry, University of Vigo and EU Reference Laboratory for marine biotoxins , [3]Analytical and Food Chemistry, [4]Analytical and Food Chemistry, University of Vigo , Vigo , Spain

Abstract: In the last years the increasing presence of emerging toxins along the European coast have been evidenced, being climate change and globalization the main reason to justify this emergence. Ciguatera Fish Poisoning (CFP) toxins are a group of emerging toxins of particular interest because of their incidence in subtropical European waters such as Canary Islands (Spain) and Madeira (Portugal) associated to the contamination of several fish species. The risk evaluation and characterization of ciguatera fish poisoning in European fish is the overall objective of the EuroCigua project, in which the University of Vigo is involved coordinating an Specific Grant (SG4), specially focused on the method development for the confirmation of the CFP toxins (CTXs) involved in the fish contamination, as well as in the development of reference materials for CTxs. The project is co-funded by the European Food Safety Authority (EFSA), and it is organised in four specific agreements: management and scientific coordination, epidemiology, evaluation of CTXs in seafood and environment and characterization of CTXs. The activities of the research team of the University of Vigo are focused on the characterization of CTXs by developing an efficient analytical method based on the use of Liquid Chromatography coupled with tandem Mass Spectrometry (LC-MS/MS) which allow to identify CTXs in contaminated fish. The method developed is also applied to the confirmation of the toxicity of samples previously screened as positive using Neuroblastoma (N2a) assay with CTX-like toxicity cell assay. Samples where the presence of CTXs has been confirmed are selected to be used for the preparation of reference materials to be further used for method development and implementation for the characterization of the CFP risk along the EU. A summary on the progress of the work carried out so far is presented and future aims and perspectives will be also discussed in this work.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-230

Gambierdiscus from Seychelles: morphology, molecular identification and toxicity

L. Lavenu[1,*], N. Chomerat[2], L. Diaz Asencio[3], C. Gerry[4], C. Belmont[4], S. Hollanda[4], A. Tunin-Ley[5], J. Turquet[5], M.-Y. Dechraoui Bottein[1]

[1]International Atomic Energy Agency, Environment Laboratories (IAEA-EL), 4a Quai Antoine Ier, MC-98000, Monaco, Monaco, [2]Ifremer, ODE/LER BO, Station de biologie marine de Concarneau, Place de la Croix, F-29900 , CONCARNEAU , France, [3]Centro de Estudios Ambientales de Cienfuegos, Cienfuegos, Cuba, [4]SFA, Mahe, Seychelles, [5]Hydrô Réunion, Sainte Clotilde, Réunion

Abstract: Benthic dinoflagellates associated with human ciguatera poisoning are present in the Indian Ocean and species such as *Fukuyoa (*formerly *Gambierdiscus) yasumotoi*, G. toxicus and *G. belizeanus* have been reported since the mid 90's. Nevertheless, very few information is available concerning the occurrence of *Gambierdiscus* species and toxicity in the Seychelles waters. Water samples were collected in March 2017 in Bay Ternay (West Mahe) and around Saint Anne island. Four strains were successfully isolated and maintained in culture. *Culture a*liquots were fixed for species identification using microscopy and molecular tools (sequencing of D1-D3 and D8-D10 of LSU rDNA) and toxin production analyzed in 21 days culture using the N2A bioassay and the receptor binding assay (RBA). All four culture were identified as *Gambierdiscus belizeanus*. No relevant toxicity was observed in three cell cultures using the N2A, and 1 strain showed low CTX activity equivalent to 4 fmol PCTX3C euiv/cell. Under microscopic examination, we noticed the great mobility of these cells which grew relatively fast in comparison with other Gambierdiscus cells. Culture growth curves are being fully characterized. Ciguatoxins in the Indian Ocean have not yet been chemically characterized although ciguatera remains in the region the most common fish poisoning. Expanding this project to areas with high ciguatera occurrence in the Indian Ocean could help in characterizing the responsible toxins.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-231

SOME PRACTICAL ASPECTS ON THE OPTIMIZATION OF N2a ASSAY FOR DETERMINATION OF CIGUATOXINS IN FISH

A. Gago-Martinez [1], D. Castro [2], A. Pequeño [2], O. Vilariño [3,*]

[1]Analytical and Food Chemistry , University of Vigo and EU Reference Laboratory for marine biotoxins , [2]Analytical and Food Chemistry, University of Vigo , [3]Analytical and Food Chemistry, University of Vigo and EU Reference Laboratory for marine biotoxins , Vigo , Spain

Abstract: Ciguatoxins (CTXs) are a group of neurotoxins responsible for the syndrome Ciguatera Fish Poisoning (CFP) endemic of tropical and subtropical regions, as a result of the consumption of contaminated fish, these toxins are well known in different areas worldwide, mostly around Pacific Caribbean and Indian Ocean. CTXs typically occur in fish as a result of biotransformation of the gambierol, as the main precursor produced by the benthic dinoflagellate of *Gambierdiscus* genus. Recent reports indicate the emergence of CFP in other geographic areas worldwide and in particular in different locations in Europe, where some outbreaks and several intoxications have been identified. A cell-based assay conducted on neuroblastoma cell line of murine origin (N2a) has been developed as a cell assay, based on the evaluation of the cellular toxicological effects affecting the cell viability, caused by toxins. N2a has been applied to different groups of marine biotoxins, specifically neurotoxins, acting on voltage-gated sodium channel (VGSC) of excitable cells. The neurotoxin character of CTXs makes them good candidates to be evaluated by N2a, nevertheless the detection of CTX-group toxins poses a challenge, due to their high toxicity at very low levels but N2a assay is a very sensitive assay which allows the detection of these toxins at levels far below the ones recommended as security levels (1 ppb). The great potential of N2a as a very sensitive tool for the screening of CTXs is clearly recognized, nevertheless this work is focused on the application of N2a with other purposes, in particular on its application as supplementary tool to advance in the development and optimization of LC-MS/MS, as a confirmatory tool to evaluate the toxicity of unidentified MS fragments for which no standards are available, and as a tool to be used on the evaluation of the efficiency of the sample pre-treatment, by measuring the toxicity of the eluates from the different extraction steps, which would justify the lack of recovery on the different steps of the sample pre-treatment. Some critical points on the application of N2a in particular those typically associated to the use of cell lines as well as those related to the complexity of the matrix are presented and discussed in this work with the aim of providing recommendations for an implemented method set up.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-232

Characterization of a set of six CTXs standards purified from toxic *Gambierdiscus* cultures and fish

M. Chinain [1], P. CRUCHET [1], A. UNG [1], J. VIALLON [1], H. T. DARIUS [1], M. RAMBLA-ALEGRE [2], J. DIOGENE [2], C. FLORES [3], J. CAIXACH [3], L. CHAN [4 5], J. WU [4 5], C.-K. LU [6 7], S. ENGUEHARD [8,*]

[1]Laboratory of Toxic Micro-algae, Institut Louis Malardé - UMR 241 EIO, PAPEETE-TAHITI, French Polynesia, [2]IRTA, 43540 Sant Carles de la Ràpita, [3]Mass Spectrometry Laboratory/Organic Pollutants, IDAEA-CSIC, 08034 Barcelona, Spain, [4]State Key Laboratory in Marine Pollution, [5]Department of Biomedical Sciences, City University of Hong Kong, Kowloon Tong, Hong Kong, [6]National Research Institute of Chinese Medicine, Ministry of Health and Welfare, [7]Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, -, [8]Novakits, 44100 Nantes, France

Abstract: Ciguatera Fish Poisoning (CFP) is a circumtropical disease resulting from the consumption of fish and marine products naturally contaminated with a suite of toxins named ciguatoxins. This illness is recognized as a major threat to food sustainability and food security not only in the Pacific but also at a global scale. The reliable detection and quantification of CTXs in contaminated seafood products is one of the key-component of most CFP risk assessment and management programs.

Over the past 10 years, significant progress has been achieved in the development of a variety of analytical tools. However, the multiplicity of CTXs, the difficulties in synthesizing these complex molecules and the notorious lack of commercially available reference materials (standards) necessary for the calibration of detection tests, still constitute a major impediment towards the implementation of effective mitigation and prevention programs. In this context, many laboratories have dedicated extensive effort in obtaining CTXs standards purified from either toxic cultures of *Gambierdiscus* spp. or from toxic fish material.

Here, we report the characterization of a set of six CTX standards purified from mass-cultures of a French Polynesian strain of *Gambierdiscus polynesiensis* (TB-92) as well as from toxic samples of the moray-eel *Gymnothorax javanicus.* Toxic fractions were obtained by bio-guided fractionation following standard extraction and purification protocols developed at Institut Louis Malardé. The identification and degree of purity of these standards has been further evaluated via liquid chromatography coupled to high resolution mass spectrometry (LC-ESI-HRMS and HR-LC-MS/MS) analysis performed in two distinct laboratories for comparison purposes. The identity of each CTX was confirmed by its exact masses, isotopic patterns and fragment ions obtained from AIF and PRM mode analysis in a Thermo Exactive-Orbitrap and Q Exactive hybrid quadrupole-Orbitrap mass spectrometer. Quantification of these fractions was also approached by LC-MS/MS calibration curve established with standards widely used in research laboratories and by qNMR experiments. Additional data relative to the biological activity of these 6 standards as assessed by the neuroblastoma cell-based assay (CBA-N2a) and the receptor binding assay (RBA) are also presented.

Plans to make these new CTXs standards commercially available to laboratories are currently under consideration, an outcome that is particularly promising in the context of the multiple international initiatives recently implemented for a better control of ciguatera and its adverse effects on populations.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-233

Diversity of benthic dinoflagellates along Kuwait’s coast (NW Arabian Gulf) with emphasis on potentially toxic species

M. Saburova [1,*], I. Polikarpov [1], F. Al-Yamani [1]

[1]Ecosystem-Based Management of Marine Resources Program, Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research, Al-Kuwait, Kuwait

Abstract: Benthic dinoflagellates in sub-tropical and tropical areas have attracted considerable research attention due to their morphological and taxonomic diversity, ecological significance in marine benthic ecosystems, and capability to produce toxins and cause toxic blooms in the coastal habitats. A taxonomic survey of the benthic dinoflagellates along Kuwait’s coast revealed that the bottom sediments and the surface of brown and red seaweeds were inhabited by taxonomically diverse and abundant assemblages of sand-dwelling and epiphytic dinoflagellates. Dinoflagellate species belonging to five orders were recorded in this study including Dinophysiales, Gymnodiniales, Gonyaulacales, Peridiniales, and Prorocentrales. High diversity of the sand-dwelling dinoflagellates was supported mainly by gymnodinioid and peridinioid taxa. In contrast, members of *Coolia*, *Ostreopsis*, and *Prorocentrum* were among the most abundant and diverse epiphytic dinoflagellates. A number of known elsewhere toxin producers were recorded including the ichthyotoxic dinoflagellates of the genus *Amphidinium*, the okadaic acid producing *Prorocentrum* species, the cooliatoxin producing *Coolia*, the palytoxin producing *Ostreopsis*, and the ciguatera-related *Fukuyoa*. The presence of known toxigenic taxa of benthic dinoflagellates indicates a potential hazard for marine environment and underscores the need for further detailed studies on benthic dinoflagellates and their toxicity in Kuwait’s coastal area.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-234

Marine phytoplankton species producing B'HABs on the Caribbean coast of Costa Rica

M. Vargas [1,*]

[1]Research Centre for Microscopic Structures, University of Costa Rica, San José, Costa Rica

Abstract: Recent studies in Costa Rica have reported the existence of some phytoplankton species that produce Ciguatera at both side coasts. Although in our country an episode of ciguatera poisoning has never been reported, various species of dinoflagellates with the capacity of producing the Ciguatera toxin have been found in Costa Rica. This study was conducted from November 2015 to August 2017 at the Caribbean coast of Costa Rica. Sample collection sites were chosen, between the Uvita Island located in front of the downtown of Limón and the reef zone in Puerto Vargas National Park, at the southern Caribbean. Collected samples of different macroalgae species in these areas were subject to alternative calibration method with artificial substrate, suggested by Tester et al. 2014. The samples were processed in the laboratory to perform both quantitative and qualitative studies, using the established methodologies to calibrate the collection methods. Phytoplankton samples were analyzed using scanning electron microscopy showing five species of potentially producing B'HABs benthic dinoflagellates.

Within the species of found dinoflagellates, there were large quantity of *Gambierdiscus* spp, *Ostreopsis* spp, *Prorocentrum Lima* and *Coolia tropicalis* and C. monotis, among others. In our country, this surveillance program is of a great importance to determining the presence of ciguatoxins in fish, because of the potential risk for public health. Moreover, this study reveals the importance of the possible geographic expansion of these dinoflagellate species at the Central American Caribbean coast and the potential B'HABs events in the area

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-235

Global Distribution of the Genera *Gambierdiscus* and *Fukuyoa*

P. Tester [1,*], L. Wickliffe [2], H. Enevoldsen [3]

[1]Ocean Tester, LLC, [2]JHT, Inc. for National Ocean Service, NOAA, Beaufort, USA, [3]IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Intergovernmental Oceanographic Commission of UNESCO, Copenhagen, Denmark

Abstract:

Historically, species in the genus *Gambierdiscus* Adachi & Fukuyo were viewed as pantropical organisms distributed between 35°N and 34°S, throughout the Caribbean, the Hawaiian Islands, French Polynesia, Australia and the Indian Ocean. Recently, however, three new species have been described in areas where *Gambierdiscus* was previously unknown, two from the Canary Islands and one from Japan. Between 2016-2017 four new species were added to the genus from the South Pacific Ocean. In total, there are now fifteen described *Gambierdiscus* species and three species of *Fukuyoa* (formerly *Gambierdiscus*), a closely related genus. Among these eighteen species and some, yet, unsubscribed ones (ribotypes), there is a wide toxicity range. While some species do not appear to produce phycotoxins in amounts above the level of concern, others produce extremely toxic ciguatoxin compounds.

Data from recently published literature (ca. 2009 – 2018) with molecular validation of the species identifications, were gathered and subsequently archived in HABMAP, the ISSHA-IOC project contributing to a first Global HAB Status Report by mapping the known global distribution of all toxin producing microalgae in the IOC-UNESCO Ocean Biogeographic Information System, OBIS. Mapped distributions were developed in ArcGIS using HABMAP data to provide visualizations of the global distribution for *Gambierdiscus* and *Fukuyoa* species. Furthermore, we merged published toxicity information on *Gambierdiscus* and *Fukuyoa* species, to the distributional data, to provide greater insight on where toxic events may develop and food webs could become compromised. These resultant data sets on species distribution and toxicity can inform regional monitoring programs, health officials and coastal managers, providing vital insights for sustained ecosystem-level management and reduce the risk of human illness.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-236

Biological activities of benthic dinoflagellate secondary metabolites

A. Leynse [1 2,*], A. Robertson [1 2]

[1]Marine Sciences, University of South Alabama, Mobile, [2]Dauphin Island Sea Lab, Dauphin Island, USA

Abstract: Secondary metabolites of plants, fungi, bacteria, and algae have long been known to provide an ecological service to their producers. Additionally, many of these compounds have proven to be commercially useful in biotechnology and pharmaceuticals. Epibenthic dinoflagellates are well known for their production of bioactive secondary metabolites. However, most of the known metabolites produced by these organisms have been identified through investigations targeting causative agents of seafood borne illness and/or events of mass mortality of marine organisms. Consequently, the ecological relevance of these compounds is largely unknown. Meanwhile metabolites unaffiliated with toxicity, remain poorly characterized.

Using a marine natural products approach, the metabolomes of species belonging to the genre, *Gambierdiscus*, *Ostreopsis*, and *Coolia* isolated from the Long Key, Florida and St. Thomas, U.S. Virgin Islands were investigated. Extracts were initially separated based on solubility, via sequential liquid-liquid partitioning, then through a series of chromatography and analytical methods. Unique activities indicative of both known and novel bioactive compounds were identified with the *in vitro* mouse neuroblastoma (N2A) assay. Additionally, anti-microbial activities of multiple extracts were identified via the disk diffusion assay. These findings reveal the epiphytic dinoflagellates as a promising source for both ecologically and commercially relevant natural products. Further study is aimed at the identification and ecological function of these compounds.

Disclosure of Interest: None Declared

Ciguatera and related benthic HAB organisms and toxins

P-237

Determination of Ciguatoxins in the Moray Eel *Gymnothorax javanicus* from Okinawa and Amami Islands, Japan.

N. Oshiro [1,*], H. Okubo [1 2], M. Ito [3], K. Kuniyoshi [1], T. Kojima [2], K. Tachihara [4], H. Asakura [1], T. Yasumoto [5]

[1]Division of Biomedical Food Research, National Institute of Health Sciences, Kawasaki, [2]Faculty of Life & Environmental Sciences, Teikyo University of Science, Tokyo, [3]Graduate School of Engineering and Science, [4]Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Nishihara, [5]Tama Laboratory, Japan Food Research Laboratories, Tama, Japan

Abstract: The moray eel *Gymnothorax javanicus* is well known for the ciguatera fish poisoning (CFP) risk. Since the first isolation of ciguatoxin-1B (CTX1B) from moray eels collected in the French Polynesia, the high toxicity of moray eel viscera and CTX1B being the major toxin is the common assumption throughout the Pacific. To testify the assumption, we collected moray eels from a distant corner of the Pacific, Okinawa and Amami Islands, Japan, and analyzed the toxins by LC-MS using calibrated toxins. Of the 66 flesh specimen analyzed, 21 specimens (32%) contained CTXs. Notably, 11 specimens (17%) exceeded the action level (0.01 μg/kg) recommended by US FDA. The highest value recorded was 0.071 μg/kg. CTXs were present in all the liver samples: 21 samples from fish with toxic flesh and 2 from those with no detectable CTXs (<0.002 μg/kg) in the flesh. The CTX levels in the liver were 20 to 1,300 times higher than those in the flesh. The possible correlation on the CTXs levels to total length, weight, age of the specimens was discussed. In conclusion, the principal toxin (CTX1B) and distribution of CTXs in the body did not differ between the two distant places, French Polynesia and Okinawa-Amami area.

Disclosure of Interest: None Declared

HABs causing aerosol and/or direct contact toxicity

P-238

Summer bloom of *Vulcanodinium rugosum* in Cienfuegos Bay (Cuba) associated to dermatitis in swimmers

A. R. Moreira Gonzalez [1 2,*], A. Comas-González [2], A. Valle-Pombrol [2], M. Seisdedo-Losa [2], C. Alonso-Hernández [2], O. Hernández-Leyva [3], L. F. Fernandes [4], N. Chomérat [5], G. Bilien [5], F. Hervé [6], G. A. Rovillon [6], P. Hess [6], L. L. Mafra Jr. [1]

[1]Centro de Estudos do Mar, Universidade Federal do Paraná, Pontal do Paraná, Brazil, [2]Centro de Estudios Ambientales de Cienfuegos, Ciudad Nuclear, [3]Centro Provincial de Higiene, Epidemiología y Microbiología de Cienfuegos, Cienfuegos, Cuba, [4]Departamento de Botânica, Universidade Federal do Paraná, Curitiba, Brazil, [5]IFREMER, Concarneau, [6]IFREMER, Nantes, France

Abstract: During summer of 2015 (from July 23rd to 26th), around 60 cases of acute skin irritation were recorded among swimmers in popular beaches of Cienfuegos Bay, central-southern Cuba, leading to beach closure enforced by public authorities. Sixty-one percent of the victims were infants (10 to 14 years old) and 82% were male. Patients were treated at local hospitals and one severe case demanding artificial ventilation was reported. Overall, the convalescence period lasted for 7-10 days. Beaches affected by this dermatitis outbreak experienced the formation of large, discontinuous reddish-brown patches in the seawater. Microscopic observations of seawater samples revealed the dominance of the recently described peridinoid dinoflagellate *Vulcanodinium rugosum* Nézanet Chomérat among the phytoplankton assemblage. Motile cells exhibited a distinctive apical pore complex, with remnants of mucilaginous material. Spherical, cyst-like cells encased in a gelatinous matrix were also abundant. As observed under scanning electron microscopy, all thecal plates were ornamented with numerous circular trichocyst pores and prominent longitudinal ridges, including a narrow and invaginated 1′ plate. The morphological identification of *V. rugosum* was confirmed genetically by sequencing the internal transcribed spacers ITS1 and ITS2 and the 5.8S rDNA region. *V. rugosum* formed a massive (max. 9.6×10^7 cells.L^{-1}), nearly monospecific bloom during the first 20 days of the event, when diatoms and other dinoflagellates, including several red tide-forming species such as *Dinophysis caudata*, *Peridinium quinquecorne*, *Prorocentrum micans*, *Tripos furca* and *T. trichoceros*, started to appear at lower cell density. The bloom persisted for ~2 months, until September 20th, and beaches remained closed until September 30th. Toxic compounds were identified from a bloom extract by high resolution mass spectrometry and their concentrations estimated using low resolution tandem mass spectrometry. Cells contained mostly portimine (357 fg.cell^{-1}), pinnatoxin (PnTX)-E (94 fg.cell^{-1}) and PnTX-F (442 fg.cell^{-1}), as well as trace amounts of PnTX-D and possibly of PnTX-G, and isomers of PnTX-E and -F. The toxin profile differed markedly from those described for Mediterranean isolates, but it was very similar to the profiles exhibited by Pacific strains, particularly strains from Australia. Considering the lack of previous reports of *V. rugosum* in Cienfuegos Bay during the regular plankton monitoring carried out by public authorities, this suggests a recent introduction of the species into Cuban waters via ballast water from ships coming from the Pacific through Panama channel. Moreover, the occurrence of *V. rugosum* in Cienfuegos Bay represents the first report of this species in the Western Atlantic, and the first description of a natural bloom of this species associated with dermatitis cases worldwide.

Disclosure of Interest: None Declared

HABs causing aerosol and/or direct contact toxicity

P-239

A Citizen Science Platform to explore *Ostreopsis* blooms and their impacts

M. Vila [1,*], L. Viure [1], M. Estrada [1], R. Abós-Herràndiz [2], J. Àlvarez [3], E. Berdalet [1]

[1]Biologia Marina i Oceanografia, Institut de Ciències del Mar (CSIC), [2]Department of Primary Health Care, Catalan Health Institute (Generalitat de Catalunya), [3]Department of Health, Public Health Agency of Catalonia (Generalitat de Catalunya), Barcelona, Spain

Abstract: Since the late 90s, blooms of the tropical genus *Ostreopsis* in certain temperate beaches have been related to respiratory symptoms, skin and mucosa irritation in humans. People exposed to marine aerosol for several hours reported mild respiratory irritations (rhinorrhea, pharyngeal pain, cough), general malaise, headache, fever (≥38ºC) and eye irritation. Some of these symptoms and/or dermatitis were also noted after swimming.

There are many uncertainties concerning the human health impacts associated to *Ostreopsis* blooms. This is due, in part, to the fact that samplings for *Ostreopsis* cells in the benthos are not usually included in monitoring programs for water quality in beaches, and when done, they rarely coincide (time, space) with human health problems recorded by epidemiologists. Furthermore, affectations are underreported due to the lack of specificity of the health symptoms, which normally disappear within a few hours without particular treatment when people move away from the affected *Ostreopsis* bloom area. Recently, parallel epidemiology and ecology studies in a hot spot area in the NW Mediterranean (2013-2017) revealed that symptoms occurred within a particular physiological phase of the bloom (mainly in the transition from exponential to the stationary phase) and were not noticed during long periods of high cell abundances.

With the aim to explore the presence of *Ostreopsis* blooms in the Mediterranean coast and to detect its potential health impacts, we launched a pilot platform of Citizen Science in August 2017 through two outreach webpages. Technical cards with basic information on *Ostreopsis* blooms were displayed in the two websites. Images of water discolorations, floating aggregates and foams, and macroalgae communities covered by the mucilaginous biofilms produced by *Ostreopsis* helped to identify the massive presence of the microalgae by beach users when swimming or snorkelling. People were invited to take pictures, send them to the website and provide basic environmental conditions (i.e. water temperature, meteorological and sea conditions, habitat description, location, etc.). Particular information was requested concerning the submarine mucilage (covering the macroalgae, rocks or sand) and simultaneous surface foams (colour, size, presence of macroalgal fragments). Finally, citizens were asked whether they had noticed any irritant symptom on eyes, nose and skin. In this case, they were invited to contact us through email and further private communication (to guarantee personal data preservation) with us was established to get more insight into the health affectation. In this way we identified the presence of *Ostreopsis* in a new area where beach goers noticed irritant symptoms. With this Citizen Science Platform, people are empowered and share key information with sea researchers at local scale. This approach can be useful for other HAB events worldwide.

Disclosure of Interest: None Declared

Medical applications of algae, cyanobacteria and their toxins

P-240

Gymnodimine-A as blueprint for developing novel molecules with pharmacological activity towards human nicotinic acetylcholine receptors

R. Araoz[1 2,*], L. Chabaud[3], D. Servent[2], J. Molgo[1 2], C. Guillou[3]

[1]Neuro-PSI, UMR9197, CNRS, [2]CEA/ DRF/ JOLIOT/ SIMOPRO/ Toxines Récepteur et Canaux Ioniques, CEA-Saclay, [3]Institut de Chimie des Substances Naturelles, UPR 2301, CNRS, Gif sur Yvette, France

Abstract: Gymnodimine-A, a fast-acting cyclic imine neurotoxin produced by *Karenia selliformis*, is a potent antagonist of muscle and neuronal nicotinic acetylcholine receptors. The six-membered cyclic imine was determined as the pharmacophore of this neurotoxin based on: *i*) the observation that chemical reduction of the imine function abolished the toxicity of this molecule, *ii*) the co-crystallization of gymnodimine with Acetylcholine Binding Protein (AChBP), a surrogate of the extracellular domain of α7 nicotinic acetylcholine receptor (nAChR), showed the positioning of the cyclic imine in the orthosteric site of AChBP, and *iii*) the 6,6-cyclic imine core of gymnodimine obtained by chemical synthesis retained the antagonistic activity towards *Torpedo marmorata* muscle $\alpha1_2\beta1\gamma\delta$ nAChR and human neuronal α4β2 nAChR. Here we extend such approaches and chemically synthetized a group of 11 cyclic imine analogues to assess their antagonistic effects on muscle-type nAChR from *T. marmorata*, and on human neuronal α4β2 and α7 nAChRs expressed in *Xenopus laevis* oocytes. As expected, the cyclic imine analogues exert an antagonist action on nAChRs, confirming the six-membered cyclic imine group as the pharmacophore of gymnodimine-A. The structure-activity relationship studies performed highlights the relative importance of the chemical substitutions at the R1, R2 and R3 structural motifs of the cyclic imine core needed for antagonistic activity. The potency rank of LC191 molecule is *Torpedo* $\alpha1_2\beta1\gamma\delta$ nAChR (IC_{50} = 0.163 µM) > human α7 nAChR (IC_{50} = 2.206 µM) > α4β2 (IC_{50}= 7.881 µM). These results show the potential of the cyclic imine moiety of gymnodimine-A as a scaffold for developing pharmacological tools selective for a given nAChR sub-type.

Acknowledgements. RA, JM and JM acknowledge the funding support from Interreg/ Atlantic Area for ALERTOX-NET EAPA_317/2016.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

P-241

Effect of salinity on interspecific competition between *Alexandrium pacificum* (Group IV) and *Heterosigma akashiwo*

Y. Yamasaki [1,*], M. Fujita [1], S. Kawano [2], T. Baba [3]

[1]National Fisheries University, Shimonoseki, [2]The University of Electro-Communications, Tokyo, [3]Yamaguchi Prefectural Fisheries Research Center, Yamaguchi, Japan

Abstract: We investigated the effect of salinity on interspecific competition between the dinoflagellate *Alexandrium pacificum* (Group IV) (formerly *A. catenella*) and the raphidophyte *Heterosigma akashiwo* using bi-algal cultures under several culture conditions. When initial cell densities of *A. pacificum* and *H. akashiwo* were both 10^2 cells ml^{-1} at salinity 30, the growth of *H. akashiwo* was strongly suppressed by *A. pacificum*, and no intact *H. akashiwo* cells were observed. In contrast, the growth of *A. pacificum* was suppressed when initial cell densities of *A. pacificum* and *H. akashiwo* were 10^2 and 10^4 cells ml^{-1}, respectively. Interestingly, the growth of *A. pacificum* was notably suppressed in bi-algal cultures at salinity 20 when initial cell densities of *A. pacificum* and *H. akashiwo* were both 10^2 cells ml^{-1}. In addition, growth of *H. akashiwo* in bi-algal cultures with *A. pacificum* under cell-contact conditions was significantly inhibited, whereas growth of *H. akashiwo* was not inhibited by *A. pacificum* in non-contact culture conditions. Furthermore, live-cell-mediated hemolytic activity of *A. pacificum* was detected, but no hemolytic activity was detected in *A. pacificum* culture filtrate. Therefore, synergetic effects of stressors may play a strong role in the growth dynamics of *A. pacificum* and *H. akashiwo* in the field.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

P-242

Symbiotic interaction between *Microcystis aeruginosa* and *Rhizobium* species by quorum sensing and oxidative stress defense

B. Shin [1], Y. Lee [2], M. Kim [1], C. O. Jeon [2], W. Park [1,*]

[1]Korea Univesity, [2]Chung-Ang University, Seoul, Republic of Korea

Abstract: Roles of epiphytic bacteria on a freshwater microcystin-producing *Microcystis aeruginosa* remain unclear. Strong attachment of bacteria on the surface of *M. aeruginosa* KW cells was observed using confocal and scanning electron microscopies. Amplified ribosomal DNA restriction (ADRA) analysis revealed that *Pseudomonas mendocina, Rhizobium rosettiformans* and *R. radiobacter* account for 96% of culturable bacterial community. By measuring chlorophyll *a* concentration of axenic *M. aeruginosa* NIES-298 cells and xenic KW cells using spectrophotometry and flow cytometry, our observation suggested that epiphytic bacteria promote the growth of *M. aeruginosa*. The most dominant *R. rosettiformans* isolated from KW coculture cells accelerated the growth of axenic *M. aeruginosa* NIES-298. Our *Agrobacterium*-reporter based study revealed that all three dominant epiphytic bacteria produce N-acyl-homoserine lactone (AHL). Addition of various concentrations of C4~C10-AHLs to the culture of axenic *M. aeruginosa* NIES-298 suggested that only low concentration (5nM) of C10-AHL enhances the growth of axenic culture. Our bioinformatics suggested the absence of any catalase gene and presence of multiple peroxiredoxin genes in the genomes of many *M. aeruginosa* strains. This led us prove that catalase activity of axenic culture could be ignored compared to xenic and bacterial cells. Our data presumably suggested that epiphytic bacteria of *M. aeruginosa* produce specific AHL signals for growth promotion and provide oxidative stress defense to *M. aeruginosa* cells by sharing catalase activity.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

P-243

Recurrence-based network analysis revealed that low-abundant free-living bacteria lie in the center of bacterial interaction

C.-Y. Ahn [1,*], Y. Cui [1], S.-J. Chun [1], S. H. Baek [2], M. Lee [2], S.-R. Ko [1], S. Hwang [1], H.-M. Oh [1]

[1]Korea Research Institute of Bioscience & Biotechnology, Daejeon, [2]Korea Institute of Ocean Science and Technology, Geoje, Republic of Korea

Abstract: To investigate the potential interactions among bacterial species, phytoplankton, and environmental parameters, free-living (FL), nanoparticle-associated (NP), and microparticle-associated (MP) bacterial community compositions (BCCs) were analyzed. A total of 267 samples were collected from July to December 2016 in the bloom-frequent southern coastal water of Korea. The variations of BCC were mostly dependent on planktonic size fractions (i.e., lifestyles). In general, Alphaproteobacteria, SAR406, and Actinobacteria were more adapted to the FL lifestyle, while Gammaproteobacteria, Bacteroidetes, and Planctomycetes were better adapted to the particle-associated (PA) lifestyle. In comparison to PA bacteria, FL bacteria responded more sensitively to the ambient environmental conditions, mostly abiotic bacteria. The influence of phytoplankton community fluctuations was lower in reshaping BCC. Most of the interspecies interactions were related to FL bacteria. The recurrence-based association network revealed the importance of Rhodospirillaceae, SAR406, SAR11, and Oceanospirillales in community stabilization and functions. The taxonomic positions of hub bacteria were retained on a monthly basis, while they varied considerably at the OTU level. This finding supported the resilience of the ecosystem under changing environments. In conclusion, the bacterial lifestyles of BCC and FL bacteria were more sensitive to surrounding environmental conditions, which play pivotal roles in ecological processes and biogeochemical cycles.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

P-244

Species specific interactions among microalgae and parasitic marine alveolates

I. Sassenhagen [1,*], L. Jardillier [2], U. Christaki [1]

[1]Laboratoire d'Océanologie et des Geosciences, Université du Littoral, Wimereux, [2]Institut Diversité, Ecologie et Evolution du Vivant, Université Paris-Saclay / Université Paris-Sud, Paris, France

Abstract: Marine alveolates (MALV), a highly diverse group of dinoflagellates, are among the most common microorganisms in all aquatic ecosystems based on meta-barcoding data. Previous studies have suggested that most MALV are parasites and might be able to terminate harmful algal blooms and control grazer communities. The hosts of most MALVs, however, are unknown and only few MALV species have been studied in detail in the laboratory. Thus, information are lacking about e.g. their morphology, life cycle, virulence, host range and specificity. Their impact on community composition and ecosystem functioning is, therefore, poorly understood.

To get new insights into host range and specificity of MALVs, we isolated and cultured different MALV species and tested their ability to infect various potential hosts. Furthermore, ribosomal markers from a large number of microalgal cells with parasites, individually isolated from several plankton communities, were cloned and sequenced allowing the combined identification of host and infecting parasite. The molecular data provide the first thorough identification of MALV hosts in different plankton communities, while the cultivation experiments improve our understanding of life cycles and host specificity in MALV.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

P-245

Allelopathic effects of several PUAs-producer micro and macroalgae on the toxic benthic dinoflagellate *Ostreopsis* cf. *ovata*

C. Totti [1,*], S. Accoroni [1], S. Pichierri [1], L. Pezzolesi [2], F. Guerrini [2], T. Romagnoli [1], R. Pistocchi [2]

[1]Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona, [2]Department of Biological, Geological and Environmental Sciences, Università di Bologna, Bologna, Italy

Abstract: *Ostreopsis* blooms regularly occur in many Mediterranean coastal areas in late summer-autumn affecting diatom-dominated microphytobenthic communities. Diatoms have been shown to produce and release a wide range of secondary metabolites that mediate interactions between individuals of different species. Moreover, studies carried out on the Conero Riviera (northern Adriatic Sea, Italy) highlighted that *Ostreopsis* abundances recorded on rocks were significantly higher than on the surface of seaweeds, suggesting that some allelopathic interactions might occur between *Ostreopsis* and macroalgal substrates as well.

Among the possible allelochemicals influencing the growth of *O.* cf. *ovata*, the role of polyunsaturated aldehydes (PUAs) has been hypothesized as their production has been reported by several diatoms and, more recently, by macroalgae.

In this study we report the effects of several diatoms, both benthic (i.e. *Tabularia affinis*, *Proschkinia complanatoides* and *Navicula* sp.) and planktonic (i.e. *Thalassiosira* sp. and *Skeletonema marinoi*), and macroalgae (*Ulva rigida*), all known to produce PUAs, on cell morphology, cytological features and growth of *O*. cf. *ovata.*

Our results showed a marked decrease of *O*. cf. *ovata* growth rate when cells were exposed to filtrates of all tested diatoms. The highest inhibition rate were observed for exposures to *P. complanatoides* and *Navicula* sp. filtrates and increased with the age of diatom culture. Moreover, a clear DNA degradation and abnormal forms of *O*. cf. *ovata* cells were found using *Navicula* sp. filtrate after 10 days of the inoculum. *U. rigida* had inhibitory effect both in form of fresh thalli and dry powder but not as algal filtrate, suggesting either that *U. rigida* does not release any allelopathic compound in the medium in absence of *O*. cf. *ovata* or that alleged released allelochemicals are rapidly degradable.

A complete algicidal effect of either diatoms or macroalgae was never observed, but exposed *O.* cf. *ovata* cells produced a large amount of resting stages.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

P-246

Effect of the endoparasite Amoebophrya sp. on toxin content and composition in the dinoflagellate *Alexandrium catenella* (Dinophyceae)

S. Kim [1,*], M. G. Park [2]

[1]Department of Oceanography, Pukyong National University, Busan, [2]Department of Oceanography, Chonnam National University, Gwangju, Republic of Korea

Abstract: Members of the *Amoebophrya ceratii* complex are endoparasitic dinoflagellates that parasitize a number of their dinoflagellate relatives, including toxic and/or harmful algal bloom-forming species. Despite many studies on the occurrence, prevalence, biology and molecular phylogeny of *Amoebophrya* spp., little attention has been given to toxin dynamics of host population following parasitism. Using Amoebophrya sp. infecting the paralytic shellfish toxin (PSP)-producing dinoflagellate *Alexandrium catenella*, we addressed the following questions: (1) does parasitism by *Amoebophrya* sp. alter toxin content and toxin profiles of the dinoflagellate *A. catenella* over the infection cycle? and (2) do parasite dinospores produced at the end of the infection cycle retain host toxins and thus potentially act as a vector to convey PSP toxin through the marine microbial food-web? Toxin time-course experiments showed that the PSP toxin contents did not vary significantly over the infection cycle, but mean toxin content for infected cultures was significantly higher than that for uninfected cultures. Host toxins were not detected in the free-living, dinospore stage of the parasite. Therefore, our results indicate that *Amoebophrya* sp. does not function as a vector for transferring PSP toxins to higher trophic levels. Rather, *Amoebophrya* infections appear to play an important role in maintaining healthy ecosystems by transforming potent toxins-producing dinoflagellates into non-toxic dinospores, representing ‘‘edible food’’ for consumers of the marine microbial food-web during toxic algal bloom event.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

P-247

Potential influence of cyanobacterial blooms on the bacterial community assemblages in two Algerian freshwater ecosystems

F. Z. GUELLATI [1,*], S. ARIF [2], K. TAMBOSCO [3], C. QUIBLIER [4], M. BENSOUILAH [1], J.-F. HUMBERT [5]
[1]Ecobiologie des milieux marins et littoraux, Badji Mokhtar University of Annaba, [2]Ecobiologie des milieux marins et littoraux, University Badji Mokhtar, Annaba, Algeria, [3]Ecobiologie des milieux marins et littoraux, UMR 7618 UPMC-CNRS-INRA-IRD-Paris 7-UPEC, [4]Muséum National d'Histoire Naturelle, UMR 7245 MNHN-CNRS, [5]Institut d'Ecologie et des Sciences de l'Environnement de Paris (iEES), UMR 7618 UPMC-CNRS-INRA-IRD-Paris 7-UPEC, Paris, France

Abstract: The potential interactions occurring between heterotrophic bacteria and cyanobacteria in freshwater ecosystems have received a growing attention in the recent years in the goal to better understand the dynamics of cyanobacterial blooms. Most of these studies have been performed in North countries where environmental conditions are very different from those encountered in the South ones, which might have a great influence on these bacterial communities (BCs).

In the present study, we have compared the seasonnal variations in the composition and structure of BCs in two northeast Algerian lakes displaying different trophic statues, Lake Oubeira (El-Tarf) being eutrophic to hypereutrophic while the Hammam Debagh reservoir (Guelma) is mesotrophic. If these two lakes face *Microcystis wesenbergii* blooms, the biomasses of these blooms are very different in each of them, which offers a good opportunity to compare the influence of these blooms on their associated BC. In this goal, we performed a one-year monitoring in 2014, including the measurements of several environmental parameters and of chlorophyll-a biomasses and the sampling of BCs. An Illumina sequencing approach performed on a 16S rRNA fragment has permitted to characterize these bacterial communities.

The BCs of the two lakes were dominated by Proteobacteria (around 65% of the reads), as it is frequently found in freshwater ecosystems from North countries. Interestingly, in the eutrophic Lake Oubeira, the second dominant phylum was that of Fusobacteria (10% of the reads), which is not known to be abundant in freshwater ecosystems but is frequently found in the gut microbiome of fishes. These bacteria were very abundant during the *M. wesenbergii* bloom, from August to November 2014. Monthly changes were found during all the year in the structure of BCs in the Hamman-Debagh reservoir. On the contrary, in Lake Oubeira, the BC community displayed a remarkable stability during all the *M. wesenbergii* bloom, suggesting that the bloom had a direct impact on the BC of the whole lake. Finally, our data have also evidenced that the heavy rains occurring on the two lakes and their watershed from April to May 2014 have also a direct influence on the BCs of these lakes.

To conclude, our study has shown that the occurrence of heavy bloom in one of the two lakes, Lake Oubeira, has a great influence on the structure and the dynamics of the BC of this lake, which suggests the occurrence of direct interactions between bacteria and the cyanobacteria. Moreover, the high temperature in this lake during the cyanobacterial bloom season have lead to the selection of a group of Bacteria which are not been frequently found in lakes of North countries, suggesting that the combination of climate changes and cyanobacterial blooms can lead to great changes in BCs from the lakes located in North latitudes.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

P-248

Unexpected infection of *Alexandrium catenella* by the parasite Parvilucifera sp in Patagonian fjords, southern Chile

J. E. Paredes [1,*], M. Seguel [2], D. Varela [1]

[1]centro i~mar, Universidad de Los Lagos, [2]Centro Regional de Análisis de Recursos y Medio Ambiente (CERAM), Universidad Austral de Chile, Puerto Montt, Chile

Abstract: Due to its relevance as HABs species, an extensive monitoring program and many studies in natural population and in the laboratory have been performed on *Alexandrium catenella* in Chile. In this context, in summer of 2018, we sampled water of several localities from south of Chiloe to the north of Aysén fjords with the aim to collect cells of this species for experiments. Most of the localities did not show vegetative cells, except at Seno Canaled (44° 35' 26'' S; 73° 18' 51''W) where the cell density did vary between 2.4 and 7.9 cells/mL. There, 10 liters of seawater was filtrates (through 85, 65 and 20 µm sieves) to separate plankton and concentrate *A. catenella* cells. Then the filtrated was inoculated in flasks with L1 medium. Flasks (n=30) were transported to the laboratory (Universidad de Los Lagos, Puerto Montt, 340 Km north) where species identification was performed, cells density of *A. catenella* was periodically determinate, and also its morphological characteristics were recorded through photography. As results, at the third day of culture we found normal and encyst cells of *A. catenella*, and also in some flask containing *Dinophysis acuta, Protoceratium reticulatum, Polykrikos sp., Protoperidinium sp.* and *Thalassiosira sp* species in lower density. Surprisingly from the fifth day, an apparent diminution in the cell density of *A. catenella* was observed, reaching zero value on the eleventh day. To explain its phenomena we conjectured that the allelopathic, predation or competitive exclusion could have affected to *A. catenella* cells viability. However, cells morphology comparison revealed that *A. catenella* and also *Protoceratium reticulatum* were infected by the parasite *Parvilucifera sp*. Thus, in order to confirm the infection, sporocytes coming from the infected *A. catenella* cultures were inoculated in new flasks (n=3) containing 6700 cell/mL of normal and non-infested *A. catenella* cells. Morphological analyses revealed the presence of zoospores surrounding cells and the generation of early trophocyte after ca. nine days. Fourteen days later the generation of early sporocyte was observed. Despite several decades of monitoring and studies of *A. catenella* from Patagonian fjords, this is the first record of parasite *Parvilucifera sp.* infecting vegetative cells of this species. Considering the role of parasite-host interaction, futures studies need to quantify the effect on population and community.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

P-249

New oomycete parasites of toxic *Pseudo-nitzschia* spp. and other diatoms

R. Siano [1,*], A. Garvetto [2], C. Gachon [2], N. Elisabeth [3], G. Bilien [3], Y. Badis [2], P. Arce [2], E. Bresnan [4]

[1]IFREMER, Brest, France, [2]Scottish Association for Marine Science, Oban, United Kingdom, [3]IFREMER, Concarneau, France, [4]Marine Scotland, Aberdeen, United Kingdom

Abstract: Holocarpic endoparasitoids of diatoms have been recorded and morphologically described since the late 19th century. Most of those parasites have been reported to infect both marine and freshwater diatoms and regrouped within the genus *Ectrogella*, which was believed to be an ancestral lineage within the oomycetes (Stramenopiles) on the basis of morphological characters. The first identified parasitoid of the marine toxigenic diatom *Pseudo-nitzschia pungens* has been also tentatively affiliated to the genus *Ectrogella*, but no genetic data were provided, opening the debate about its real systematic position. Recently, the first sequence of a parasitoid of *P. pungens* appears as the earliest diverging long-branch within the oomycete phylogeny.

In this study we collected single infected *Pseudo-nitzschia* cells from the Atlantic coast of Brittany (France) and Northwest Scotland (United Kingdom) belonging to four different diatom species, over a period of 6 years (2010 - 2016). Using nested single cell PCR or single cell whole genome amplification (SGA) we obtained the nearly complete 18SrDNA sequence for the isolated infected cells. Where possible sequenced draft genomes were queried *in silico* for the gene encoding the of cytochrome *c* oxidase subunit II (COII). Using the same methods, we obtained molecular data from holocarpic endoparasitoids infecting the diatom genus *Melosira*.

Robust phylogenetic analyses unambiguously demonstrated that our sequences are separated into two clusters within the phylum Oomycota, being related to the seaweed parasite genera *Anisolpidium* and *Olpidiopsis*. Yet, morphological features were not sufficient to unambiguously attribute our parasites to any *Ectrogella* species. In order to avoid a potentially unstable taxonomy, we named our two Oomycota clades OOM-1 and OOM-2, awaiting additional morphological and genetic information. A screening of global databases of the regions V4 and V9 of the 18SrDNA, demonstrated the presence of our parasites beyond the North Atlantic coastal regions. In a biweekly metabarcoding survey of the diatom communities in the Concarneau Bay (France, Brittany), high abundances of a subclade of OOM_1 occurred within a time lag of few days and coincided once with the decline of *Pseudo-nitzschia* spp. and then with that of *Cerataulina pelagica*. These findings, together with the genetic identification of the same oomycete infecting both *P. australis* and *Melosira* sp., support the hypothesis of a short-lasting infection cycle *in situ* and of a lack of host specificity of the studied parasites.

Our finding highlights a complex and still unexplored genetic diversity within oomycete parasitoids of diatoms and calls for new biological evidences to unveil the evolutionary history and the ecological role of these marine protists.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

P-250

Phylogenetic diversity of microbiomes from single cells of *Alexandrium catenella*

E. Becker [1], A. Bridle [1], C. Bolch [1,*]
[1]Institute for Marine and Antarctic Studies, University of Tasmania, Launceston, Australia

Abstract: Phytoplankton cells interact with closely-associated complex marine bacterial communities (microbiomes) that have dramatics effects on the growth, physiology and toxicity of the algal host. A substantial proportion of microbiomes inhabit the stable boundary layer around cells (the phycosphere), however, the difficulties of sampling this micro-environment in the presence of abundant background bacteria and plantkon mean we know little about microbiomes associated with single cells in nature. To address this problem, we developed an approach to enable phylogenetic analysis of microbiomes from single cells using the toxic dinoflagellate *Alexandrium catenella* as a test-case. Individual cultured *A. catenella* cells were isolated using sterile manual micromanipulation along with a series of plasticware and extraction reagent contamination controls. Total DNA was purified from cells using low-loss extraction methods and near full-length bacterial 16S rDNA amplified with primers 127F and 1492R, followed by a nested indexing PCR to enable paired-end sequencing on an Illumina MySeq platform. After subtraction of sequences associated with plasticware and extraction/reagent blanks, a wide diversity of bacterial taxa were detected with considerable variation in taxa associated with each cell. The total microbiome recovered from multiple cells was consistent with microbiomes obtained from *A. catenella* cultures using bulk cell extraction, indicating the approach is feasible and imparts minimal PCR-bias. Increased cell-washing steps (up to 5x) prior to extraction increased the relative abundance of gamma-Proteobacteria and Actinobacteria, suggesting these phyla have a tighter association with the dinoflagellate cell.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

P-251

Assessment of toxin induction in Namibian *Pseudo-nitzschia* species (incl. a novel species)

C. Lara [1,*], F. Gai [1 2], D. Louw [3], K. Grobler [4], B. Krock [5], Ø. Moestrup [6], N. Lundholm [1]

[1]Natural History Museum of Denmark, University of Copenhagen, Copenhagen, [2]Environment and Nature, Orbicon A/S, Taastrup, Denmark, [3]NatMIRC, Ministry of Fisheries and Marine Resources, Swakopmund, [4]NatMIRC, Ministry of Fisheries and Marine Resources, Lüderitz, Namibia, [5]Helmholtz Centre for Polar and Marine Research, Alfred Wegener Institute, Bremerhaven, Germany, [6]Marine Biological Section, University of Copenhagen, Copenhagen, Denmark

Abstract: Several species of the diatom genus *Pseudo-nitzschia* produce the neurotoxin domoic acid (DA) responsible for amnesic shellfish poisoning and toxic effects as well as death of e.g. marine mammals and seabirds. The present study has explored the toxicity of Namibian *Pseudo-nitzschia* strains as well as effects of grazing copepods on DA production. A broad diversity of Namibian *Pseudo-nitzschia*, incl. a novel species, collected in the northern Benguela Upwelling System, was used in the experiments. Located off the southwest coast of Africa, the Benguela is a highly productive system where large and frequent blooms of *Pseudo-nitzschia* occur, but knowledge of the conditions facilitating the blooms is limited. In general, triggers of DA production include biotic and abiotic factors, but general conclusions on what controls toxin production are complicated by interactions between factors, and differences at both species and strain level. Results on potential induction of toxin production with regard to intra and interspecific differences will be presented. Findings will contribute to the knowledge of *Pseudo-nitzschia* in a chiefly unexplored part of the world.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

P-252

HOST RANGE AND VIABILITY UNDER ANOXIC CONDITIONS OF PARVILUCIFERA COROLLA (ALVEOLATA, PERKINSOZOA)

F. R. Rodríguez [1,*], R. Figueroa [1], C. Arias [2], P. Domínguez [2]

[1]Red tides and harmful algae, Spanish Oceanographic Institute, [2]C.P.R. Vivas, Vigo, Spain

Abstract: Marine parasitoids of the genus *Parvilucifera* are considered specific endoparasites of dinoflagellates (Garcés et al. 2013). *P. corolla* was recently described (Reñé et al. 2017) from the Catalan coast (NW Mediterranean) and Canary Islands (NE Atlantic), although its host range was not included in that study. In the present work both the host selectivity and viability under anoxic conditions of parasitoids of *P. corolla* were examined. P. corolla was isolated from tidal ponds in Lanzarote (Canary Islands) and host range screened for 106 strains of dinoflagellates belonging to 23 genera. The parasitoid infected some or every *Akashiwo*, *Alexandrium*, *Amphidinium*, *Barrufeta*, *Coolia*, *Dinophysis*, *Gymnodinium, Heterocapsa, Lepidodinium, Levanderina, Ostreopsis, Prorocentrum, Protodinium, Scrippsiella, Takayama* and *Tripos* strains tested. On the contrary, species of the genera *Fragilidium, Fukuyoa, Gambierdiscus, Karenia, Karlodinium, Lingulodinium* and most *Prorocentrum* strains were resistant. Encystment was stimulated in the dinoflagellate *Symbiodinium natans* above a certain threshold of parasite:host ratio. *P. corolla* did not infect other algal groups (chlorophytes, cryptophytes, chrysophytes, diatoms, haptophytes and raphidophytes) but one strain of the prasinophycean *Pyramimonas,* although infections and sporangia were not viable. Mature sporangia of P. corolla survived in anoxic dark conditions at least during one month, as demonstrated by their capability to produce zoospores that developed new infections.

Acknowledgments: the authors acknowledge funding from CICAN, CCVIEO and CIGUATROP, and assistance with cultures to P. Rial and A. Fdez.-Villamarín.

References:

Garcés, E., Alacid, E., Bravo, I., Fraga, S., Figueroa, R.I. (2013). *Parvilucifera sinerae* (Alveolata, Myzozoa) is a generalist parasitoid of dinoflagellates. Protist 164, 245–260.

Reñé, A., Alacid, E., Figueroa, R.I. and Rodríguez, F. (2017). Life-cycle,ultrastructure, and phylogeny of *Parvilucifera corolla* sp. *nov*. (Alveolata, Perkinsozoa), a parasitoid of dinoflagellates. European Journal of Protistology 58, 9–25.

Disclosure of Interest: None Declared

Microbial interactions (allelopathy, parasites…)

P-253

Recent study of algal viruses infectious on *Karenia mikimotoi*

N. Nakayama [1,*], M. Hamaguchi [1]

[1]Japan Fisheries Research and Education Agency (FRA), National Research Institute of Fisheries and Environment of Inland Sea (FEIS) Harmful Algae Blooms Group, Hiroshima, Japan

Abstract: At least two viruses infecting the dinoflagellate *Karenia mikimotoi* have been isolated from the coastal waters of Japan so far. KmDNAV5 was the first identified and isolated from the Ago Bay in July 2013. The second one identified came from Uwajima Bay in July 2014. Both viruses have an icosahedral head, lack a flagellum, are of a similar size (about 80 nm) and have double-stranded DNA. In the growth test of *K.mikimotoi* KmH6 with virus inocculation, the KmDNAV5 caused lysis for 10 days in their host strains at 20˚C under a 12-h light-dark cycle; the light (130 to 150 µmol of photons $m^{-2}s^{-1}$) was provided by cool white fluorescent illumination. The host ranges of the KmDNAV5 examined among the 20 *K. mikimotoi* strains were wide i.e., each virus infected 7-8 strains, including the host used for isolation. Differently, the culture of *K. mikimotoi* KmUW3 by a new virus KmDNAV3 caused lysis from 1 day after inoculating the virus. The host range test demonstrated that it is more specific. Recently, the virus is expected to be a promising tool for the prevention of the occurrence of some harmful microalgae. In fact, *Heterocapsa circularisquama* and its ssRNA virus (HcRNAV) had displayed a synchronous fluctuation pattern in abundance in natural environments, and the virus infection has been considered to have a significant impact on the host's bloom dynamics. In this report, we will introduce the effects of the viruses on *K. mikimotoi* blooming and its potential as a preventative tool in the natural environment as well as the characteristics of the new virus.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

P-254

CONTRIBUTION OF THE HABf INDEX FOR FISH FARMS RISK ANALYSIS

A. Clément [1,*], T. Husak [2], S. Clément [1], F. Muñoz [1], M. Saldivia [3], C. Brito [4], R. Crescini [3], N. Correa [1], K. Teiguel [1], S. Saez [1]
[1]Plancton Andino, Puerto Varas, [2]Oxzo, Puerto Montt, [3]Plancton Andino, Castro, [4]Plancton Andino, Coyhaique, Chile

Abstract: In recent years in southern Chile we have observed numerous HAB event - particularly with flagellate species - which have been causing problem in fisheries and aquaculture. We have been monitoring and studying intensively the Chilean fjord ecosystem with an emphasis on key phytoplankton species, its photo-chemical photosynthesis, phytoplankton and particle characterization and distribution with FlowCam, bio-optical water properties, and remote sensing water color.

All the above results are valuable information as we develop an understanding of the oceanographic and ecological significance of the HAB events. However, we need in addition an on-line near real time indicator such as a **$\mathbf{HAB_f}$ Index** for the fish farmers, authorities and general users.

The **$\mathbf{HAB_f}$ Index** is based upon a relatively simple algorithm that takes into account different weighting factors and risk coefficients. Specifically, the summation of the water column weighted average of each harmful algae abundance divided by its critical or threshold fish kill value is the key part. In this analysis we include the different harmful algae synergist and specific effects on the impact on a salmon farm. Additionally, we evaluate several weight factors for each harmful species depending on the degree of fish kill damage.

We have tested the **$\mathbf{HAB_f}$ Index** retrospectively, examinating a large data set connected directly to the server and a business intelligence software (BIME).

While the results of the **$\mathbf{HAB_f}$ Index** show a close correlation with harmful algae bloom impacts on salmon farms, there are several challenges to solve. Among them are that FlowCam data should be normalized and included in the **$\mathbf{HAB_f}$ Index** calculation, synergistic effects, weighting factors, and threshold fish kill values.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

P-255

Following clues about whale mortality in the Gulf de Penas-Chile: are the toxic microalgae responsible?

G. Fuenzalida [1,*], L. Norambuena [1], E. Cascales [1], C. Alarcon [2], C. Toro [2], H. Pacheco [2], G. Pizarro [2]
[1]Centro de Estudios de Algas Nocivas (CREAN), Instituto de Fomento Pesquero, Puerto Montt, [2]Centro de Estudios de Algas Nocivas (CREAN), Instituto de Fomento Pesquero, Punta Arenas, Chile

Abstract: During march 2015, the largest mass whales mortality was detected in the Gulf of Penas, south of Chile. The species correspond to *Balaenoptera borealis* (Sei whales). One of the hypothesis for explain this event was the harmful algal blooms (HABs) originated by bio-oceanographic condition triggered by “El Niño” event, however, the description of the phytoplankton community or the associated physicochemical characteristics were not described for this area. In this context, during February 2018 we were part of a scientific expedition in which 13 sites were sampled between the Gulf of Corcovado to Gulf of Penas (44 - 47° S). The goal of the sampling was to describe the phytoplankton community including the distribution and abundance of microalgae using optical microscopy and molecular markers (real time PCR); detect different toxins by liquid chromatography (LC-MS/MS); measure nutrients concentrations (nitrite, nitrates, phosphate and silicates) to get a global picture of the spatial structure of the phytoplankton community in the area. Our results show that the distribution and abundance of microalgae species and the associated oceanographic conditions varied between both gulfs, channels and between sampling sites. Different toxins were also detected (domoic acid, pectenotoxins, dinophysistoxins and yessotoxins) in concentrations ranging from 2.8 to 87.55 ng/ml. In parallel with the optical identification of the principal species we developed a set of molecular markers that can be used for the identification of different toxic species by real time PCR to be used in the monitoring activities. Our result suggests the possibility that while toxins may be responsible (*e.g.* domoic acid) in the whale mortality by toxin accumulation coming from the HABs, but periodic monitoring study is necessary to achieve a greater understanding about these events.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

P-256

Cyanobacteria bloom impacts on fish: Insights from an ongoing study at a shallow, hypertrophic lake in Vermont (USA)

N. Flores [1,*], T. Miller [2], J. Kraft [3], J. Stockwell [1]

[1]Rubenstein Ecosystem Science Laboratory, University of Vermont, Burlington, [2]Joseph J. Zilber School of Public Health, University of Wisconsin-Milwaukee, Milwaukee, [3]Department of Animal and Veterinary Sciences, University of Vermont, Burlington, USA

Abstract: The negative effects of cyanobacteria blooms on aquatic habitat quality are known (*e.g.*, hypoxia, toxins), but the seasonal and long-term consequences of blooms on ecosystems are not well understood. For example, cyanobacteria can produce hundreds of bioactive metabolites, but studies that measure toxins in tissues of wild fish are mostly limited to microcystins, and many do not use methods that can distinguish congeners. Consequently, our knowledge of cyanotoxin dynamics in wild fish is limited. Understanding if cyanotoxins accumulate differentially in fish depending on environmental conditions, such as water toxin concentration, or ecological role, such as fish trophic level, will better inform how management agencies can respond to current and emerging problems related to harmful algal blooms. Moreover, fish from eutrophic lakes may be of poor nutritional quality. Cyanobacteria do not produce long-chain essential fatty acids (LC-EFA), which are required in the diet of higher organisms for essential functions such as immune system responses and cell membrane fluidity. To investigate the effects of cyanobacteria blooms on fish, we conducted a field study on a hypertrophic lake in Shelburne, Vermont (USA). Sampling was conducted in 2016 and 2017 from May (pre-bloom) through October (post-bloom). We collected water and adult fish (≥200 mm), including carnivorous yellow perch (*Perca flavescens*) and phytoplanktivorous golden shiners (*Notemigonus crysoleucas*). Water and fish tissues (brain, liver, & muscle) were tested for a suite of cyanotoxins using LC-MS/MS and analysis of LC-EFA in tissues (liver & muscle) using GC-FID is underway. We hypothesized that fish will have (1) increased cyanotoxin levels and (2) decreased LC-EFA tissue levels in response to blooms. We expect that cyanotoxin concentration will increase in both species with increased water toxin concentration, but golden shiners will contain higher average toxin concentrations than yellow perch because of direct ingestion of cyanobacteria. In contrast, we expect to see a decrease in LC-EFA in both fish species with bloom development. Due to their foraging habits and high concentrations of cyanobacteria during blooms, golden shiners are expected to have lower concentrations of total LC-EFA than adult yellow perch, which generally consume invertebrates and small fish, and thus, may be able obtain higher amounts of LC-EFA in their diet. So far, our analyses indicate cyanobacteria peptides in the water fluctuated across the two years, including up to seven congeners of microcystin, three anabaenopeptins, and two cyanopeptolins (2017 only). We also found the simultaneous occurrence of many of the peptides in fish tissues, with highest concentrations in the liver. Our study is one of the first to demonstrate the presence of different cyanobacteria peptides in wild fish tissues.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

P-257

Bioactivity assessment of the cyanobacterium *Woronichinia naegeliana* towards crustaceans *Thamnocephalus platyurus* and *Daphnia pulex*

B. Bober [1,*], J. Bialczyk [1], M. Adamski [1], E. Chrapusta [1], K. Duchnik [1]
[1]Plant Physiology and Development, Jagiellonian University, Krakow, Poland

Abstract: Cyanobacteria are known for the production a wide range of secondary metabolites of various chemical structure and biological activity that are released into aquatic environment. Although cyanobacterium *Woronichinia naegelian*a (Unger) Elenkin is appearing frequently in the phytoplankton of freshwater worldwide, information about it is scarce. The assessment of its bioactivity towards planktonic invertebrates such as *Thamnocephalus platyurus* and *Daphnia pulex* was performed. The greatest biological effect to the aqueous extract obtained from *W. naegeliana* cells was displayed by *T. platyurus*. The denoted semi-lethal concentration after 24 hours of exposure was 0.99 mg of dry weight mL^{-1}. A lower sensitivity to cyanobacterial extract was observed for *D. pulex*, although its toxicity grew with time. After 24 hours the effective concentration of 5.21 mg of dry weight mL^{-1} caused death or immobilization of *D. pulex*, while two times longer exposure to cyanobacterial extract caused its reduction by 1.8 times. One of the 18 fractions isolated from *W. naegeliana* cell extract containing microginin FR3 exhibited biological activity towards *T. platyurus.* The negative results of more detailed study on microginin FR3 using enzyme inhibition assays indicate a mode of action other than serine protease inhibition. The observed toxicity of *W. naegeliana* towards crustaceans might be a result of the synergic effect of the cyanopeptides produced by this species.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

P-258

Metabolic and physiological responses of the marine mussel *Mytilus galloprovincialis* fed with freshwater toxic cyanobacteria

F. L. de Oliveira [1], J. C. Martins [1], L. D-Q. Jiménez [2], A. Caméan [2], M. V. Turkina [3], V. Vasconcelos [1], A. Campos [1,*]
[1]Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal, [2]Area of Toxicology, Faculty of Pharmacy, University of Seville, Seville, Spain, [3]Department of Clinical and Experimental Medicine, Linkoping University, Linkoping, Sweden

Abstract: Many freshwater ecosystems worldwide are affected by the proliferation of toxic cyanobacteria. Toxic cyanobacteria can be extremely adverse to freshwater ecosystems leading to the contamination of the water and the aquatic food chains, and the loss of aquatic biodiversity. This study aimed to disclose the less known downstream impacts of freshwater toxic cyanobacteria and their toxins, carried by the river streams to the sea, on estuarine and coastal organisms. For this investigation 744 marine mussels (*Mytilus galloprovincialis*) were fed with three different microalgae species (10^5 cells/ml) twice a day for 15 days: the toxic cyanobacteria *Microcystis aeruginosa* and *Chrisosporum ovalisporum*, and the non-toxic *Parachlorella kesseleri* (experimental control), in controlled laboratory conditions. The toxicity of *M. aeruginosa* and *C. ovalisporum* strains was evaluated regarding cell content in microcystin-LR (0,023 pg/cell) and cylinsdrospermopsin (7,854 pg/cell) respectively. Mussel survival was not affected in this study. Moreover, no significant changes were observed in the capacity of mussels to produce byssus nor in mussel's growth according to the measurements in whole body dry weight. Nevertheless, the filtration rates recorded in this experiment reveal that marine mussels were able to filter and ingest the toxic microalgae the same way as the non-toxic green microalga *P. kesseleri*, meaning that mussels can feed on toxic cyanobacteria and thereby can be regarded as potential vectors of cyanobacteria toxins in the marine/estuarine food chain. Total levels of protein and glycogen in mussels changed in the course of the experiment but were not related with mussel's diet. Furthermore, potential sub-toxic effects of cyanobacteria on mussels was investigated with shotgun proteomics and multivariate statistics. 394 proteins were identified in this analysis, among them 17 significantly changed abundances in the digestive glands of mussels fed with toxic microalgae. The alterations in proteins with putative functions in cytoskeleton organization (ACT, TMP, PLS, TLN), immune response (LGALS, C1q) or in ion transmembrane transport and respiratory electron transport chain (ATP6V1A, ETFA) suggest possible sub-toxic effects induced by toxic microalgae in mussels. Moreover alterations in the treifol factor homologous protein also point to an impairment of gastrointestinal epithelium functions in mussels.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

P-259

Cylindrospermopsin impairs Zebrafish (*Danio rerio*) Development and gene expression

A. Moraes [1 2,*], V. Magalhães [1], S. Shah [2], H. Habibi [2]

[1]Institute of Biophysics Carlos Chagas Filho - Federal University of RIo de Janeiro, Rio de Janeiro, Brazil, [2]Department of Biological Science, University of Calgary, Calgary, Canada

Abstract: Cyanobacteria are known to produce a variety of toxic compounds known as cyanotoxins which threaten both human and environmental health by contaminating drinking water as well as aquatic ecosystems. Growing evidence indicates cyanotoxins act as developmental toxins, inhibiting or impairing several pathways of vertebrate development. Among them, cylindrospermopsin (CYN) is a 415 Da alkaloid produced by a range of cyanobacteria. Previous studies have described CYN as a protein synthesis inhibitor and capable of damaging several organs in mammals. However, little is known about its toxicity mechanisms in others vertebrates. This study aims to characterize the adverse developmental effects of CYN using zebrafish (*Danio rerio*) larvae as an aquatic model organism. A wide range of CYN concentrations (0 - 2000 µg/L) were assessed using a morphometric approach for various growth (length, eye volume) and developmental abnormalities (yolk and pericardial edemas, hemorrhaging, spinal malformations, touch reflex, and swim bladder malformation), alongside survival and hatching. In addition, we also investigated the expression of various genes. The results demonstrate that CYN exposure causes changes in development such as decreased growth and, increased developmental abnormalities in zebrafish larvae. Furthermore, the results provide evidence that CYN exposureresults in changes in transcript abundances of certain geneswith threshold of 10 µg/L. These results provide novel evidence in a vertebrate model that exposure to CYN results in multiple adverse effects and impairs the development of zebrafish.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

P-260

Chemical ecology of Benthic HABs: the impact of NW Mediterranean *Ostreopsis* cf. *ovata* on copepods

A.-S. Pavaux [1,*], J. Rostan [1], L. Guidi-Guilvard [1], S. Marro [1], S. Gasparini [1], R. Lemée [1]
[1]Sorbonne Université, Laboratoire d'Océanographie de Villefranche, CNRS, Villefranche-sur-mer, France

Abstract: Blooms of benthic toxic dinoflagellates genus *Ostreopsis* have increased in frequency and intensity, notably in the Mediterranean Sea. Adverse effects on Human health of those microalgae have been recorded, with skin/eye irritations or breath difficulties among main symptoms. The toxicity of those dinoflagellates was attributed to the presence of palytoxin and analogs. Deleterious effects of *Ostreopsis* blooms on marine organisms have already been reported, without knowing if they were due to anoxic/hypoxic conditions or to the presence of toxic secondary metabolites.

In this context, the aim of our work was to assess the effect of *Ostreopsis* cf. *ovata* on two copepods: *Sarsamphiascus* cf. *propinquus* (a phytal meiobenthic species) and *Acartia clausi* (a planktonic species). These two models were exposed, *in vitro*, to ecologically realistic concentrations of *O.* cf. *ovata* and *Licmophora paradoxa*, a non-toxic competitive benthic microalga. The potential toxic effect of *O.* cf. *ovata* was tested on survival rates, fecal pellets production (as a proxy of feeding) and reproduction (using fertility/fecundity ratios). Results indicated that *S.* cf. *propinquus* was the most tolerant organism to *O.* cf. *ovata* ever tested (LC_{50} (48h) > 20 000 cells.mL^{-1}), even if hatching success and egg production were affected by the presence of the toxic dinoflagellate, suggesting a reprotoxicity. *Acartia clausi* was 4 000 times more sensitive (LC_{50} (48h) < 5 cells.mL^{-1}) than the meiobenthic copepod. These results highlight the necessity to study species-specific responses to better understand the effects of *O.* cf. *ovata* on marine organisms.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

P-261

Embryotoxicity of microalgae Heterosigma akashiwo and Karlodinium veneficum in zebrafish (*Danio rerio*).

A. Llanos-Rivera [1,*], F. Novoa [1], K. Álvarez [1], M. Ruano [2], A. Astuya [2], J. J. Gallardo [3], L. López-Rosales [4], A. Sánchez-Mirón [4], F. García-Camacho [4]

[1]Oceanografia, [2]Oceanografia - COPAS, [3]Ingeniería Química, Universidad de Concepcion, Concepción, Chile, [4]Chemical Engineering, University of Almería, Almeria, Spain

Abstract: Bloom-forming ichthyotoxic microalgae commonly cause important losses to the Seafood Industry and impact negatively on marine ecosytems. Examples of this kind of microalgae are *Heterosigma akashiwo* (Raphidophyceae) and *Karlodinium veneficum* (Dinophyceae). Currrent understanding of the toxicity of *H. akashiwo* relates it to the production of neurotoxins (e.g. brevetoxins), reactive oxygen species (ROS) or polyunsaturated fatty acids (PUFAS). In the case of *K. veneficum*, the background indicates the presence of ichthyotoxic toxins (e.g. karlotoxins) with hemolytic, and cytotoxic properties.

In this work, the embryotoxic effects of methanolic extracts prepared from biomass pellets of both species on early development stages of zebrafish were studied. Biomass of both species were obtained from cultures carried out in pH-controlled photobioreactors (12L). Cells were harvested in stationary phase. Zebrafish embryos were exposure to different concentrations of methanolic extract ranging from 50 to 200 mg/L. The embryotoxicity assay was initiated with eggs in the gastrulation phase and it were mounted in 96 well plates with 3 replicates for each concentration. The embryos were observed daily recording the percentage of embryonic mortality (48 hours post fertilization), the percentage of hatching and the presence of larval malformations. For *K. veneficum*, the average toxicity percentage increased with the concentration of methanolic extract in the well. A maximum of embryonic mortality (>50%) was found at the maximum extract concentration assayed (200mg/L). Although at lower concentrations, the embryonic mortality decreased, sublethal effects on the hatched larvae were observed such as high frequency of malformations. In the case of the *Heterosigma* extracts, the tendency was similar. However, the magnitude of the effect was lower. Among the most frequent larval malformations observed were caudal torsion, pericardial and yolk sac edema. These results indicate the greatest harmfulness was in *Karlodinium veneficum* extracts.

Acknowledgments

Financial support: FONDEF-IdeA ID17I 10100 and INNBIO MECESUP UCO1401.

This research was also partially funded by the Spanish Ministry of Economy and Competitiveness (Project CTQ2014-55888-C3-02).

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

P-262

Immune and histopathological effects on the mussel *Perna perna* during short-term exposure to the toxic dinoflagellate *Prorocentrum lima*

R. Neves [1], T. Santiago [1], W. Carvalho [1], E. Silva [2], P. Silva [2], S. M. Nascimento [1,*]
[1]Departamento de Ecologia e Recursos Marinhos, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro,
[2]Departamento de Biologia Molecular, Universidade Federal da Paraíba, João Pessoa, Brazil

Abstract: Cosmopolitan *Prorocentrum lima* is widely distributed from temperate to tropical latitudes and found all year round growing on macroalgae in Brazilian waters. Every *P. lima* culture tested for toxins showed to produce okadaic acid (OA) and analogues. Evidences for molluscan contamination by *P. lima* toxins have been reported. This study assessed immune and histopathological effects of *P. lima* exposure on *Perna perna* mussel. The *P. lima* strain used (UNR-9) was isolated from Rio de Janeiro, Brazil and its toxicity was assessed using *Artemia salina*. Another *P. lima* strain isolated from the same site produces OA and small amounts of DTX-1. Assays consisted in 5-days exposure of 14 mussels to *P. lima* or non-toxic control with the green algae *Tetraselmis* sp. Cellular abundance employed (900 cells mL^{-1}) considered *P. lima* abundances in Rio de Janeiro coastline. Mussels were acclimated to experimental conditions 48-h before assay. Total hemocyte count (THC), relative size and internal complexity, phagocytic activity and reactive oxygen species production (ROS) were evaluated after 24, 48 and 96-h of initial exposure. Histopathological modifications were measure at 96-h. Pooled hemolymph (n=3) was analysed in flow-cytometer tubes with the fluorophores: SYBR Green I for THC and morphology, 2-μm latex-beads for phagocytosis or DCFH-DA for ROS production. Analyses were performed in a FACScalibur flow cytometer. Phagocytic activity and hemocyte relative size significantly decreased with time-course *P. lima* exposure. ROS production and phagocytic activity were significantly affected by toxic exposure and incubation time interaction. ROS production increased 6-times after 96-h exposure to *P. lima* simultaneously to a significant decrease in phagocytic activity. Parasites and unspecific histopathological conditions were detected with variable prevalence among all mussels. Mussels exposed to *P. lima* presented exacerbated hemocytic infiltration in the connective tissue of stomach, intestine and digestive gland (47.1%), while control mussels did not. Moderate to severe atrophy of digestive tubules was detected in 60% of *P. lima* exposed mussels while only 20% of mussels from control were affected. Granulocytomas and edema on branchial filaments from the inners lamellae were also detected in all mussels. Among parasites and commensals, intracytoplasmic colonies of Rickettsia-*like* organisms, protozoan *Nematopsis* sp., sporocysts of bucephalid trematodes and unknown copepods occurred in all mussels with similar prevalence (<25%), and cestode *Tylocephalum* sp. was the highest prevalent parasite (>90%). Immune and tissue responses suggest that mussels were subjected to increased stress after 96-h exposure to *P. lima*. The survival of mussels to *P. lima* short-term exposure and its toxic effects indicates that *P. perna* is a potential OA-vector.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

P-263

Cyanobacteria as common producers of teratogenic retinoid-like compounds into aquatic ecosystems

K. Hilscherová [1,*], E. Sychrová [1], M. Pípal [1], J. Priebojová [1], L. Sehnal [1], T. Procházková [1], M. Smutná [1]
[1]RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic

Abstract: Formation of cyanobacterial water blooms and related production of diverse bioactive and toxic compounds have been often linked with adverse effects on exposed organisms and potential risk to human health. Newly detected group of compounds produced by cyanobacteria are retinoid-like compounds, but there is little information on their production, levels in the environment or potential adverse effects and risks associated with their occurrence. Our studies employed bioanalytical approaches using reporter cell lines for the characterization of the retinoid-like potencies of extracts and exudates from laboratory cultured cyanobacteria from different orders and samples of environmental water blooms and their surrounding surface water collected from field studies. Sensitive analytical methods (LC-MS-MS) were used to characterize compounds contributing to the detected retinoid-like activities. The results document production of compounds with this bioactivity into surface waters by various cyanobacterial species and environmental water blooms. In some cases, the level of retinoid-like activity reached values that can cause adverse developmental effects in exposed organisms. The in vivo relevance of the detected bioactivities was analyzed using embryos of aquatic model species, zebrafish (*Danio rerio*) and frog (*Xenopus laevis*), aiming particularly at the identification of developmental and teratogenic effects. Exposure studies documented correspondence of retinoid-like activity in cyanobacterial samples with developmental effects in embryos. Observed effect phenotypes in both fish and frog embryos and effective concentrations of cyanobacterial samples corresponded to all-trans retinoic acid (ATRA) equivalents, which supports the hypothesis that the teratogenic effects of cyanobacterial samples are probably associated with retinoid-like activity. We have identified a set of compounds contributing to the detected retinoid-like activity in both laboratory and field samples. These include ATRA, 9/13cis retinoic acid (RA), as well as several novel cyanobacterial metabolites, such as 5,6epoxy-RA or 4keto-ATRA with high retinoid-like potency. Our studies document that the production of retinoids by cyanobacteria into the aquatic environment is a common phenomenon, since retinoid-like activity and presence of individual retinoids have been shown to be associated with cyanobacterial water blooms dominated by many different species. This study was supported by the Czech Science Foundation project No.18-15199S.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

P-264

Quantitative pathology and anti-oxidant enzyme activities in three bivalve species caused by *Dinophysis caudata*

L. Basti [1,*], R. Matsushima [2], T. Suzuki [2], S. Nagai [2]

[1]Deaprtment of Ocean Sciences, Tokyo University of Marine Science and Technology, Tokyo, [2]Fisheries Research Agency, Yokohama, Japan

Abstract: Several species of the genus *Dinophysis* are responsible for the widespread contamination of shellfish with diarrhetic shellfish toxins (DST), oakdaic acid (OA) and dinophysistoxins (DTX) responsible for the diarrhetic shellfish poisoning (DSP) in humans. Mollusc bivalves are known to accumulate DST at differential rates and are not known to be affected by DST toxins; however, there are practically no studies that have explored the potential subtle effects of *Dinophysis* spp. on bivalve molluscs. In a previous study, we reported mortalities of bivalve molluscs fed *D. caudata*. In the present study, adult Japanese scallops, *Mizuhopecten yessoensis* from the northern island of Hokkaido, and noble scallops, *Mimachlamys nobilis* from the western Prefecture of Mie, and Pacific oyster from Hiroshima Prefecture were fed a daily ration of a clonal culture of a strain of *Dinophysis caudata* isolated from western Japan and producing only the lipophilic toxin PTX-2 at sub-lethal daily ratios. Pathologies in nine organs and activities of four anti-oxidant enzyme (Superoxidase dismutase, Catalase, Glutathion-S-transferase, Gluthatione peroxidase) were quantified. Six pathologies were identified in six organs, with differential sensitivities of bivalve species and bivalve organs. Anti-oxidant enzymes were modulated following exposure to *D. caudata*, with increased activity of the anti-oxidant enzymes implicated in xenobiotic detoxification and in cell protection from damaging reactive oxygen species. The results compel to further assess the effects of *D. caudata* and to compare its impacts in shellfish to other *Dinophysis* species.

Disclosure of Interest: None Declared

Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)

P-265

Mass mortality of cultivated northern blue fin tuna associated with *Chattonella marina* in Baja California, Mexico

E. Garcia-Mendoza [1,*], J. Cáceres-Martínez [2], D. Rivas [1], M. Fimbres-Martinez [1], Y. Sánchez-Bravo [1], R. Vásquez-Yeoman [3]

[1]Biological Oceanography, [2]CICESE, [3]Instituto de Sanidad Acuícola, A.C. (ISA), Ensenada, Mexico

Abstract: An episode of mass mortalities of cage-cultured tuna in the northwest coast of Baja California occurred from the end of May to August of 2016. Nine events occurred from May 31 to the 2 August at different areas on the region (Bahia Todos Santos, Salsipuedes Bay and Coronado Islands). The organisms were disoriented, swan erratically, gasping and died hours after these signs were detected. Necropsies and histopathological analyses were performed on dead organisms. Abundant mucus and congestion was observed in the gills where, in some cases, phytoplankton species (c.f. *Chattonella* sp. and *Ceratium* sp.) were detected. Histopathological analysis of the gills showed hyperplasia, fusion of gill filaments and lamellae, telangiectasia, edemas, increased numbers of mucus cells and in some cases severe hemorrhage. Water samples were analyzed and a sampling camping was implemented in the cultivation area to evaluate the presence of *ichthyotoxic* algae. *C. marina* was detected in the water column during the period of the die-offs. At the end of May abundances of 5 x 10^3 Cel L^{-1} were detected in samples collected on the surface and *C. marina*, represented approximately 20% the phytoplankton collected by net tows. Maximum abundances in samples from Todos Santos Bay (TSB) increased to 33 x 10^3 Cel L^{-1} in June and this species represented 85% of phytoplankton in net tow samples. No other environmental stressful variable was detected during the mass mortality episode. *C. marina* in the water column explains the dead of the tuna since behavior, necropsies and histopathological analyses of the gills indicates a severe reaction to an environmental noxa that could be related to the characteristic toxic effect of this species. Before 2016, ichthyotoxic species were rarely detected in the phytoplankton community of the region. The presence of *C. marina* was associated with an increase in surface water temperatures, low stratification of the water column and a reduction in the intensity and duration of upwelling events in the region. Surface temperature anomalies (4 ^{o}C) were registered during winter of 2015 and spring of 2016. Global and regional mesoscale oceanographic and atmospheric phenomena such as the North Pacific basin anomaly ("The Blob") and the ENSO brought the environmental conditions for a change in the phytoplankton community in the region and the appearance and dominance of the *C. marina* during spring-summer of 2016.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-266

Occurrence of Yessotoxins in Canadian Shellfish from 2012-2017

W. Rourke [1,*], N. Haigh [2]

[1]Dartmouth Laboratory - Chemistry Section, Canadian Food Inspection Agency, Dartmouth, NS, [2]Microthalassia Consultants Inc, Nanaimo, BC, Canada

Abstract: Yessotoxins (YTXs) are a group of lipophilic shellfish toxins that are predominantly produced by the dinoflagellate *Protoceratium reticulatum* and various other species of phytoplankton. The European Union has regulated a maximum limit of 3.75 mg/kg YTX equivalents, but currently there is no maximum limit in Canada. The Canadian Food Inspection Agency (CFIA) has monitored Canadian shellfish samples for YTXs since 2003 in support of export activities and to generate data for a Canadian risk assessment. A six-year dataset (2012-2017) is presented, showing the presence of YTX compounds in Canadian shellfish and accompanying phytoplankton data when available. Although *P. reticulatum* has been detected on both the east and west coasts of Canada, YTX levels in shellfish have been higher and more prevalent on the west coast. Mussels (*Mytilus* sp.) were most commonly sampled, representing 73% of all samples and 94% of west coast samples. YTXs were detected in 65% of west coast samples (ranging from 43-85% in a given year), but only 2% of east coast samples had detectable YTX concentrations (with a maximum of 3% in a given year). During this time period, 3.8% of monitoring samples would have exceeded the EU maximum limit; only one of these samples was from the east coast. The highest total YTX concentration detected was 12 mg/kg YTX equivalents, which is one of the highest shellfish contamination levels reported in the literature to date. YTX and 45-OH YTX were the analogues detected. YTX was most commonly the dominant analogue, but samples were more likely to exceed the EU maximum level when 45-OH YTX concentrations were equal to or greater than YTX concentrations. Temperature and salinity data were available for selected sites, but no trends were observed linking YTX concentrations to either of these factors. Limited monitoring of phytoplankton revealed that concentrations of *P. reticulatum* cells peaked at the same time as shellfish YTX concentrations in two nearby areas. In general, YTX concentrations in shellfish rose rapidly and then decreased more slowly, although some variability was observed in rate of decrease. During the time covered by this dataset, no known human illnesses were linked to high YTX concentrations in shellfish properly harvested under the conditions of Canada's federal food safety program.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-267

Harmful Algal Blooms Events (HABs) in the Gulf of California in 2015 and 2017.

J. A. Barreiro [1,*], A. Pichardo [1], P. Suárez [2]

[1]Official inspector, [2]Executive Director, COFEPRIS, MINISTERY OF HEALTH, MEXICO, México, Mexico

Abstract: Title: Harmful Algal Blooms Events (HABs) in the Gulf of California in 2015 and 2017.

Keywords: COFEPRIS, Harmful Algal Blooms (HAB), Phytoplankton, Marine Biotoxins, Molluscan Shellfish, Gulf of California, Paralitic Shellfish Poison (PSP).

Summary:

Since 2015, the molluscan shellfish's growing areas of the Gulf of California have being affected by Harmful Algal Blooms characterized by the presence of *Gymnodinium catenatum*, an organism which produces Saxitoxin or PSP. A HAB event was presented in January 2015, causing the closure of the molluscan shellfish's growing areas where it took place. This event lasted 8 months, during which the Federal Commision for the Protection against Sanitary Risks (COFEPRIS) and the State Sanitary Authorities carried out sanitary control actions to avoid damage to public health. At the beginning of January 2017, a new massive phenomenon took place in that same area and it lasted until the month of October, affecting the same growing areas than the previous ones and in the same way, COFEPRIS an state authorities they took action again to keep it under control. In this paper, we present the similarities and differences between both events, regarding the presence of phytoplankton levels, PSP concentrations, their extension, percentage of marine animals mortality and its duration among other aspects.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-268

Lipophilic Toxins in Galicia (NW Spain): Spatial and temporal distribution and its incidence on the main molluscan species

J. Blanco [1,*], F. Arévalo [2], J. Correa [2], Á. Moroño [2]

[1]CIMA. Xunta de Galicia, Vilanova de Arousa, [2]Biotoxins, INTECMAR. Xunta de Galicia, Vilagarcía de Arousa, Spain

Abstract: Galicia is an area with strong mussel *Mytilus galloprovincialis* aquaculture that additionally has important fisheries of other bivalve molluscs, as the cockle *Cerastoderma edule*, several clams (*Ruditapes philippinarum*, *Venerupis pullastra*, *Ensis arctuatus*, *Polititapes rhomboides, Venerupis pullastra*), and the queen scallop *Aequipecten opercularis*. The area is frequently affected by lipophilic toxins. Since 2014, 18862 samples were analysed for OA, DTX1 and DTX2 and in 5984 of them, also for regulated PTXs, YTXs and AZAs. Approximately 2/3 of the analysis corresponded to cultured mussels and the remaining 1/3 to natural beds of several bivalve species. OA (above LOQ) was found in 68.9% of the analyzed samples, followed by YTX (10%), DTX2 (7.2%), 45-OH-YTX (2.5%) and PTX2 (0.2%). DTX1 was marginally detected (less than 0.05%) and neither of the regulated AZAs nor PTX1 were found above the LOQ. Generally, there was not a temporal coincidence between the outbreaks of the main toxin groups. For OA and YTX groups of toxins, in many cases only the main toxin of the group was detected, but when two toxins of each group were involved in the outbreak, then they were related. The presence of 45-OH-YTX was overwhelmingly linked to mussels. Only OA produced toxicities above the regulated limit, the levels of DTX2 toxicity and YTXs were one and three orders of magnitude below the banning threshold, respectively. Mussels attained the highest level for all toxins followed by cockles. All other species attained substantially lower levels. Regarding spatial distribution of toxic episodes, the rías of Pontevedra and Muros were the most affected by OA and DTX2 and the one of Ares by YTXs. In general, the outer parts of the rías were more affected by OA and DTX2 than the inner ones, but YTXs were more abundant in the inner part of the Ría of Ares. The incidence of the toxins varied both seasonally and between years. During 2014-2017 the incidence of OA was similar between years being the smallest in 2017. Due to the different abundance of *Dinophysis acuta* —to which DTX2 is linked in the area— between years, 2016 was the year with the minimal incidence of DTX2. In each year, OA level showed a maximum in April, a minimum from January to March and a second (less pronounced) maximum in October-November. The incidence of DTX2 was nearly restricted to autumn and winter months with no, or extremely rare, presence between March and July. YTXs peaked in August decreasing afterwards until the next August and its levels decreased progressively during the four sampling years. The toxins of the OA group were esterified at different degree in different bivalves. In all the studied bivalves with the exception mussel and queen scallop the esterification was nearly complete in most cases.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-269

CO-OCCURENCE OF GREEN TIDE, GOLDEN TIDE AND RED TIDE IN THE YELLOW SEA IN SPRING AND SUMMER OF 2017

T. Yan [1 2,*], F. Kong [1 2], P. Jiang [1 2], F. Liu [1 2], Q. Zhang [1 2], R. Yu [1 2], M. Zhou [1 2]

[1]Qingdao National Laboratory for Marine Science and Technology, [2]Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China

Abstract: The co-occurrence of large-scale green tide and golden tide in the south Yellow Sea, together with red tides in some areas at 35N°, was observed and investigated in spring and summer of 2017. Macro-algae biomass was determined based on surface trawling method on three cruises in coastal area of Subei Shoal and in the south Yellow Sea from April to June in 2017. The results showed that 1, The floating green algae *Ulva* and brown alga *Sargassum* started to appeared in late April sporadically, increased in both biomass and distribution in late May, then drifted northward and each reached approximately 300,000 tons of total biomass in June. However, they were different in distribution pattern, which they had a shared geographical distribution in E120°30′-122°30′. To the east of co-existing area was mainly golden tide and to the west was mostly green tide. The *Sargassum* showed golden in color offshore, while brown nearshore. 2, Green tide and golden tide co-occurred in coastal area of Subei Shoal, which is the source for *Ulva* and nearshore *Sargassum*, reached 4,100 and 19,700 tons in biomass in May, respectively. 3, Red tide of about 50 KM^2 caused by *Karenia mikimotoi* was observed at N35° E120°19.8′ in Haizhou Bay mouth in late May. Bloom of *Heterosigma akashiwo* co-existed with *Ulva* and *Sargassum* simultaneously at N35° E 121°18.8′ in late June.4, Golden tide of *Sargassum* showed increasing hazards in 2017 along China coast, costing more than 500 million RMB loss to *Pyropia* aquaculture in Jiangsu Province in January, although the green tide of *Ulva* was significantly reduced in 2017. The green tide of *Ulva* occurs in the south Yellow Sea every spring and summer since 2007, causing huge social and economic loss to south Shandong peninsula each year. Since floating *Sargassum* has wide spatial and temporal distribution along China coast, the origin, key process and formation mechanism of golden tide, as well as its mutual effect with green tide are quite complicated and need further exploration. The recent HAB variation under the influence of global climate change and human activity in China seas is also discussed in this paper.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-270

A brief Ligurian HABs story: state of the art for a smart HABs management

V. Giussani [1,*], R. Bertolotto [1], N. Melchiorre [2], A. Novellino [3], A. Pagano [4], A. M. Bassi [4], G. Rose [5], S. Alloisio [6]

[1]ARPAL Liguria - Regional Environmental Protection Agency, Genoa, [2]ARPAL Liguria - Regional Environmental Protection Agency, La Spezia, [3]ETT S.p.A., [4]DIMES, University of Genova, Genoa, Italy, [5]Otto von Guericke University - Institute for Medical Engineering, Magdeburg, Germany, [6]ETT S.p.A. - CNR-Institute of Biophysics, Genoa, Italy

Abstract: Harmful Algal Blooms (HABs) represent a significant and expanding threat to human health and fishery resources with relevant socio-economic impact. This increase in frequency, intensity and dispersion of mass proliferation of phytoplankton (cyanobacteria, diatoms, dinoflagellates) occurs both in marine and freshwater ecosystems and has been associated with climate changes and anthropogenic impact.

Since the intensification of the phenomenon and the related health consequences, the World Health Organization formulated specific guidelines (Guidelines for safe recreational water environments, WHO 2003) and the European Community issued a directive on the management of the quality of bathing water (Directive 2006/7/EC). Currently, implemented systems for monitoring and managing HABs permit to mitigate some impacts on human health and on economic activities, but are not adequate for the rapid increase of the phenomenon.

The project @lgaWarning (EuroTransBio 2017), started in January 2018, stems in this context with the aim of creating an innovative integrated system for fast cell counting and detection of algal toxicity.

As the first steps of the projects, we analysed data of monitoring campaigns carried out from January 2015 to October 2017 along Ligurian coast: 1586 sea water samples, collected in 64 sites, have been prosessed in order to describe and update the state of the art of HABs phenomenon in our pilot region.

As preliminary results, we report a list of potentially toxic algal species composed by: nineteen taxa of Dinophyceae, tow genus of Diatoms and 1 species of Raphidophyceae. Furthermore, for each listed taxa, information such as frequency, maximal concentration and periods of maximal proliferation are provided.

The overall project will offer the opportunity to rapidly advance our knowledge by providing multi-disciplinary input, meeting the needs of environmental managers and commercial enterprises worldwide.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-271

The analysis of the geographical distribution of algal blooms in Russia using Cyanohab.ru

Z. Namsaraev [1,*]

[1]NRC "Kurchatov Institute", Moscow, Russian Federation

Abstract: Russia is ranked second in the world in renewable freshwater resources. However, these resources are unequally distributed across the country. The majority of water resources of Russia are concentrated in the eastern part of the country in the Lake Baikal and rivers of the Eastern Siberia and the Far East. As a result, the European Russia, where 80% of the country's population and industry is concentrated, periodically suffers from the shortage of water resources and eutrophication of water bodies. In order to collect up-to-date information on algal blooms in Russia and analyze their geographical distribution, I developed a website https://cyanohab.ru. The major sources of information are scientific papers, governmental reports, reports from users of the website and media publications on cases of algal blooms. Currently, the database contains information on more than 100 outbreaks, which allowed me to make preliminary conclusions about the problem of algal blooms in Russia.

The algal blooms were observed in all climatic zones of Russia from subtropical to subarctic. The major problems were observed in the European part of Russia and the southern Urals region. These are the most populated regions with developed industry and agriculture. Nevertheless, a massive development of algae was also observed in regions with colder climates and low population density such as Siberia, Russian Far East and Arctic zone of Russia. Also, the intensive growth of algae was observed since 2011 in the littoral part of Lake Baikal, which contains 20% of the world's surface freshwater reserves. The most common algal genera observed during blooms were *Microcystis, Anabaena, Aphanizomenon*, and *Dolichospremum*.

It was reported that algal blooms led to the deterioration of the quality of drinking water, clogging of pipes and cessation of water supply, poisoning of people, fish and animals, and a decrease in tourist attractivity. The major causes of algal blooms were the discharge of wastewater due to outdated or absent wastewater treatment facilities, high temperature, low rainfall, a decrease of the water exchange in the reservoirs due to the erosion of the shores and the construction of dams. It should be noted that in Russia up to 30% of phosphates are still allowed in the composition of synthetic detergents, which also stimulates the development of nitrogen-fixing cyanobacteria in water bodies.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-272

Recent case study of anomalous cyanobacteria bloom in the Sau-Susqueda-El Pasteral system

C. Flores [1,*], J. Caixach [1]
[1]Mass Spectrometry Laboratory/Organic Pollutants, IDAEA-CSIC, Barcelona, Spain

Abstract: The system of hydrographic exploitation of Ter River, formed by the reservoirs of Susqueda, Sau and Pasteral, between Osona and La Selva regions, constitute the water reserve of the highest strategic interest in Catalonia. The water of this system of reservoirs is mainly destined to the water supply of cities such as Barcelona and Girona, and also to the production of electricity, irrigation, bathing and recreational activities.

The appearance of a massive growth of cyanobacteria in the Sau-Susqueda-Pasteral system in the autumn of 2015 has been the most recent episode of bloom. Historically, some cyanobacteria detected in this system of reservoirs can present mechanisms of resistance while waiting for more adequate conditions. Specifically, in the years 1998 and 2000, two episodes of massive growths of cyanobacteria were already observed, with the detection of microcystin-LR and -RR dissolved in water at levels ranging between 0.189 and 270 ng/L. From then on, the improvement of sanitation in the upper Ter River basin and the progressive reduction of the nutrient supply had caused a reduction in the frequency and scope of these blooms, to the point that they had not been detected for more than a decade, at least with an important extension.

The taxonomic study of bloom samples in autumn of 2015 confirmed the presence of *Microcystis* cyanobacteria. Large colonies were observed, with a high number of cells, present on the surface of the water and up to more than 15 meters of depth. Toxicity and analysis studies were performed by enzyme-linked immunosorbent assay (ELISA) and liquid chromatography-high resolution mass spectrometry (LC-HRMS). In some of the samples, low and high toxicity was detected in the extracellular and intracellular fraction, respectively. The results for the ELISA test and the LC-HRMS analysis were negative (<0.3 µg/L) in all the surface samples. Only traces of microcystin-LR and -dmLR were detected in concentrations estimated to be close 10 ng/L at 11 meters of depth. Unusually, unlike other blooms, the presence of different anabaenopeptins and oscillamides at high concentrations, in the order of mg/L was observed. The identification of these peptides was achieved by HRMS. Although these peptides are not included in any regulations or recommendations and they are considered less toxic than microcystins, recent data show that they can give a positive response in some toxicity tests such as phosphatase inhibition tests.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-273

First record of the genus *Azadinium* (Dinophyceae) from Puget Sound, Washington State

J.-H. Kim [1], B. Bill [2], N. Adams [2], U. Tillmann [3], B. Krock [3], J. Deeds [4], N. Harrington [5], M.-S. Han [1], C. Sloan [6], W. Stutts [4], V. Trainer [2,*]

[1]Hanyang University, Seoul, Republic of Korea, [2]NOAA Northwest Fisheries Science Center, Seattle, USA, [3]Alfred Wegener Institute, Bremerhaven, Germany, [4]US Food and Drug Administration, Silver Spring, MD, [5]Jamestown S'Klallam Tribe, Sequim, [6]NOAA Northwest Fisheries Science Center, Seattle, WA, USA

Abstract: Lipophilic toxins, in particular those associated with diarrhetic shellfish poisoning (DSP), are an emerging threat to shellfish harvesting in Washington State. While Washington was the first state in the U.S. to implement the routine testing of shellfish for DSP toxins in 2012 after the occurrence DSP in Sequim Bay, WA in 2011, there continue to be occasional reports of DSP-like illnesses likely tied to the consumption of shellfish from Puget Sound. To address these illness of unknown etiology, we began a Monitoring and Event Response to Harmful Algal Blooms project in 2015 to identify whether species of the genus *Azadinium* were present in Puget Sound. This small dinoflagellate, in particular *A. poporum*, *A. spinosum, and A. dexteroporum*, has been shown in other parts of the world to produce azaspiracids, lipophilic toxins which can produce DSP-like symptoms. The presence of the genus *Azadinium* was confirmed in whole water samples collected from several SoundToxins sites in Puget Sound based on the use of molecular probes. Here we report the establishment of *Azadinium* cultures from sediment samples from Puget Sound including *A. obesum*, *A. cuneatum*, *A. poporum*, *A. dalianense*. The production of a new azaspiracid, named AZA-59, was confirmed by liquid chromatography mass spectroscopy in several isolates of *A. poporum*, in shellfish, and in solid phase resin deployed in the field. This first confirmation of the presence of the genus *Azadinium* in Puget Sound and the first report of azaspiracid on the west coast of the U.S., underline the potential risk of azaspiracid shellfish poisoning in this region.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-274

Pollution evaluation in the Oum Er Rbia River (Morocco) using diatom-based indices and physico-chemical parameters.

F. Souilmi [1,*], M. malki [2], K. Ghedda [2]

[1]student, [2]Department of biology ,Université Hassan II ,Faculté des Sciences Ain Chock, Casablanca, Morocco, MOHAMMEDIA, Morocco

Abstract: The Oum Er Rbia River originates on the Middle Atlas at 1800 m and flows into the Atlantic Ocean at Azemmour city, many dams and reservoirs have been constructed on the Oum Er Rbia River to generate hydroelectric power and to provide water for irrigation.

To diagnose the state of pollution of the Oum Er Rbia River, several sampling points located on the section between the downstream of the sources the Oum Er Rbia River (north of the city khénifra) and the city Settat, made the subject to seasonal monitoring. The choice of these points is determined by their representativeness when assessing the impact of pollution on the quality of water and they are located upstream and downstream of the urban centre discharge point.

A physicochemical analysis of the waters was made and the results show that the mineralization of the waters of the Oum Er Rbia River is quite high.

To study more closely the relationship between pollution and diatom, four indices have been tested to assess the water quality in the Oum Er Rbia River (Morocco). The indice diatomique pratique (IDP). The indice diatomique générique (IDG) and the trophic diatom index (TDI). These indices are different by the assigned indicatives values and pollution sensitivity coefficient relative to every species and by the taxonomic list integrated in the calculation of every index. The spatiotemporal variation of these indices reflects clearly the deterioration of water quality downstream the site 6, which is situated downstream the abattoir wastewater in Khenifra (IDP=2,86± 2,5 IDG= 5,05±1,31 IDT=5,67±1,59). Among these indices ,only the IDP presents a significant correlation (0,66 ; α >5%) with the IPO (reference index).

These indices proved to be able to suspect sources of pollution where the physicochemical techniques are ineffective.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-275

Nutrients supply derived from variable water masses and its relationship to *Phaecystis globosa* blooms in Beibu Gulf, China

Y. Yuan[1 2,*], X. Lv[1 3], W. Wang[1 2], Z. Yu[1 2], X. Song[1 2], X. Cao[1 2], Z. Wu[1 2], L. He[1 2]

[1]CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, [2]Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao, [3]University of Chinese Academy of Sciences, Beijing, China

Abstract: *Phaecystis globosa (P. g.)*, which was well known as a "nuisance foam producer" that dominated several HABs along North Sea and related European coast during 90s, dominated HABs much frequently in Beibu Gulf, China since 2010. Differing with blooms occurred along European coast, size of each colony derived from *P. g.* in Beibu Gulf could be 3 to 5 cm large. During the bloom from 2015 to 2016, the colonies were gathered together and dispersed great part of the gulf, thus potentially blocked the cooling water input of a nuclear power plant in such area. However, the reasons why *P. g.* became dominating these years in Beibu Gulf are still pending. Some believed that it could be a consequence of rapid development of local offshore aquaculture, and others argued that such species was transported from other area, like Vietnam coast via currents. In this research, we focused on the supply of nutrients to the blooms frequently occurred area in Beibu Gulf, and the relationship between changes of nutrients and *P. g.* dominated HAB occurrences, based on 9 cruises all through the period since *P. g.* was initially observed till such bloom disappeared during Sep, 2016 to Aug, 2017. It's showed that *P. g.* blooms occurred mainly in the area between 10m isobaths and 30m isobaths, 10 kilometers long away from the coast. During the bloom period, such area is controlled by Zhujiang diluted water (ZDW), in which NO_3 is as rich as 6.41uM in average and the average N/P ratio is close to the redfield ratio. Meanwhile, such bloom only occurred when ZDW expanded itself to the blooms frequently occurred area and the temperature of ZDW decreased below or not increased above 20 degrees. During the bloom period, NO_3 in the euphotic layer was greatly consumed, average N/P ratio decreased to 6.39 thereof, while the concentration of NO_3 and PO_4 in the deep layer did not change much provided by continuous and steady input of N and P rich ZDW. Meanwhile, as another potential source of nutrients, the upwelled deep South China Sea water (UW) could not be transported beyond 30m isobaths to the blooms frequently occurred area all through the investigation. However, before the bloom, there is a similar fluctuating trend between scale changes of *P. g.* observed area and UW controlled area. So it might be hypothesized that *P. g.* organisms are transported via both UW upwelling and ZDW transportation to the bloom frequently occurred area, but only became dominating since the temperature is suitable for colonies' proliferation. Meanwhile, ZDW provided sufficient nutrients continuously to maintain the bloom until the temperature of ZDW exceeded 20 degrees. The local coastal water, although is rich in nitrogen and phosphate, could not be transported over 10m isobaths because of weak river discharges, thus did not play a key role in *P. g.* blooms in Beibu Gulf.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-276

Exploitation of multi-decadal phytoplankton monitoring data to study changes in Baltic Sea cyanobacterial blooms

S. Suikkanen [1,*]

[1]Finnish Environment Institute, Helsinki, Finland

Abstract: Phytoplankton monitoring data, collected by the joint Baltic Marine Monitoring Programme of HELCOM since 1979, was utilized to study biomass trends of filamentous, N-fixing cyanobacteria, the main organisms forming extensive and recurring phytoplankton blooms in the Baltic Sea. During almost 40 years of monitoring, the Baltic Sea ecosystem has undergone substantial, mainly human-induced environmental changes. The late-summer biomass of cyanobacteria showed an increase in the northern but not in the southern Baltic Sea. The potential causes and consequences of the changed cyanobacterial biomasses in the ecosystem were analyzed by examining parallel changes in and relationships between environmental factors (hydrography, nutrients) and other biota (zooplankton, benthos, fish). Multivariate statistics indicated that surface water warming was the most important single factor with respect to the observed changes in phytoplankton community structure. Other factors included surface water desalination and increased stratification, eutrophication (including changed nutrient ratios), and community or biomass changes in other biota. Pronounced effects of increased cyanobacterial biomass on diversity, mean organism size and production of plankton communities were not obvious, in contrast to the findings of previous experimental studies.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-277

A probabilistic approach to the relation between *Dinophysis* cell densities and DSP-related harvesting bans in the Galician Rías

Y. Pazos [1,*], B. Reguera [2]

[1]Oceanography and Phytoplankton, INTECMAR, Vilagarcía, [2]Toxic phytoplankton, IEO, Vigo, Spain

Abstract: The historic database of phytoplankton and shellfish toxin monitoring was used to estimate densities of *Dinophysis* species leading to toxin accumulation in shellfish in the Galician Rías above regulatory levels (RL). These estimates need to consider the inter-specific differences in toxin profile and content per cell (e.g. *D. acuta* is larger and more toxic than *D. acuminata*) as well as the relative contribution of *Dinophysis* to the whole phytoplankton community (for a given density of *Dinophysis*, the higher the ratio *Dinophysis*:chlorophyll, the faster shellfish contamination proceeds). Another difficult-to-monitor variable is shellfish condition (spawning cause a sudden increase of toxicity due to a fast and drastic gonadal weight loss). The co-occurrence of *Dinophysis* with other toxin producing species, such as *Pseudo-nitzschia* spp. (ASP) and *Gymnodinium catenatum* (PSP), adds complexity. Likewise, it is difficult to establish the criteria to open a previously closed production zone making without risks for seafood safety. There is a lag time between detection of toxic cells and shellfish toxicity above RL levels and also from the day when toxic cells are no longer observed and that when toxins accumulated in shellfish from previous events are cleared. A single cell-density result from an integrated water-column sample is but a snapshot from the sampling moment, whereas shellfish toxicity results from a continuous process of accumulation and toxins biotransformation. Decisions on openings of previously closed zones based on cell counts without additional toxin testing is not a secure practise. Bloom development is modulated by the local hydrodynamics. A representative sampling of the water column is quite difficult when *Dinophysis* populations aggregate in thin layers. In upwelling systems, oceanographic processes change very quickly during downwelling events and a total water renewal of the rías may proceed in less than a week, leading to massive accumulation of toxic microalgae affecting cultures in the water column as well as infaunal shellfish. Despite all the above, a significant relationship between cell counts and shellfish toxicity may be found, which cannot be extrapolated to different geographic areas with distinct hydrodynamic regimes. Using partial conditional probability, harvesting closure probability curves have been generated for each group of toxins on the basis of cell densities estimated from hose-integrated water column samples. These curves simplify the accumulated information and could be integrated into more complex predictive models to help health authorities and decision-makers. Nevertheless, phytoplankton counts of the target species in the water column does not allow for the establishment of local, or even less, universal trigger levels for the same species.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-278

Insights from the Norwegian monitoring program for harmful algae

E. Gustad [1], A. Engesmo [2], K. Steinhovden [3], M. Chauton [3], M. Kleiven [1], L. Valestrand [2], W. Eikrem [2,*]

[1]Institute of Marine Research, Arendal, [2]Marine Biogeochemistry and Oceanography, Norwegian Institute for Water Research, Oslo, [3]SINTEF, Trondheim, Norway

Abstract: The Norwegian monitoring program of harmful algae was started in 1981, following a large bloom of *Karenia mikimotoi,* which caused mortalities in caged fish. Aquaculture had been establishing itself as an industry in Norway since the 1970's, but was plagued by almost yearly blooms of ichthyotoxic phytoplankton. Later, it also became evident that certain microalgae produce toxins which accumulated in shellfish, making them unfit for human consumption. The Norwegian monitoring program of harmful algae has been modified and reorganized many times since its beginning, and today we monitor 36 stations along the Norwegian coast from Vollen in Inner Oslofjorden to Kjempebakken in Troms county in the North. The program is funded by the Norwegian Food Safety Authority and it is divided in two parts: direct identification of harmful microalgae in light microscopy of preserved samples (net-hauls and water samples). We monitor species of the genera *Alexandrium*, *Dinophysis*, *Azadinium* and *Pseudo-nitzschia.* We also report *Protoceratium reticulatum*, *Protoperidinium curtipes*, *P. crassipes* and *P. divergens*. The second part is screening of mollusk meat for known toxins such as: ASP = Amnesic Shellfish Poisoning caused by *Pseudonitszhia*-species; AZP = Azaspiracid Poisoning caused by *Azadinium*-species; DSP = Diarrhetic Shellfish Poisoning; caused by *Dinophysis*-species; PSP = Paralytic Shellfish Poisoning, caused by *Alexandrium*-species. The sampling is carried out weekly throughout the growth season, approximately from February to November and results are made available to the public on a weekly basis at: www.algeinfo.hi.no and www.matportalen.no/verktoy/blaskjellvarsel.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-279

Factors influencing eutrophication and the presence of nuisance algae in Lake Buchanan, Texas, USA

T. E. Gámez [1,*], S. Manning [2]

[1]Aquatic Biology, Texas State University, San Marcos, [2]Plant Biology, University of Texas, Austin, USA

Abstract: Freshwater lakes are experiencing magnified harmful and nuisance algal blooms with increased drought periods attributed to global climate change. Central Texas, USA, went through an extended drought from 2011-2015. During this period, Lake Buchanan, a large inland reservoir, experienced rapid shifts in the phytoplankton community as the lake transitioned from mesotrophic to eutrophic. Samples were taken bimonthly and included measuring water quality parameters, macro- and micro-nutrients, along with the identification of living and preserved phytoplankton to assess the impacts of the transition. The phytoplankton community was evaluated by cell counts and DNA barcoding using 23S ribotyping to verify the presence and abundance of different strains. All factors were evaluated to determine which variables contributed to the formation of harmful algal blooms. DNA sequencing analysis confirmed potentially bloom-forming cyanobacteria. Overall, this study shows that *Planktothrix*, *Aphanizomenon*, and *Cylindrospermopsis* were more competitive in drought conditions whereas *Limnothrix* and *Pseudanabaena* were not as robust. The diatoms *Fragilaria* and *Lindavia*, increased in their abundance with a decrease in micronutrients and conductivity. Towards the end of the sampling period, the harmful bloom-forming genus *Planktothrix* became dominant, comprising more than 75% of the cyanobacterial community, with the rest being the nuisance bloom-forming alga, *Limnothrix*. Following the drought, cyanobacteria decreased in average abundance by 452.9%, and diatoms increased by 793.2%. At this time, *Aphanizomenon ovalisporum* and *Phormidium tenue* were present along with additional potentially harmful yet rarely studied species. Our results suggest that drought-induced eutrophication, including elevated conductivity, micronutrients, and organic nitrogen from decreased rainfall lead to the dominance of harmful cyanobacteria in Lake Buchanan. Thus, subtropical reservoirs should be monitored closely during extended drought periods, as the risks associated with eutrophication and harmful cyanobacterial blooms are predicted to be higher.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-280

PHYTOPLANKTON BIOTIC INTEGRITY INDEXES: WATER QUALITY AND CYANOBACTERIAL OCCURRENCE IN LAKE PARANOÁ, BRAZIL

A. P. de Souza [1], R. Tezini Minoti [2], C. H. Lima [2], L. Nunes Ludolf Gomes [2,*]
[1]National Water Agency (ANA), [2]Civil and Environmental Engineering, University of Brasília, Brasília, Brazil

Abstract: The aim of this study was to evaluate the applicability of phytoplankton biotic integrity indexes to estimate water quality and occurrence of cyanobacterial blooms in a tropical reservoir. The indexes were applied for data from 35 years of limnological monitoring program of Lake Paranoá, an important water resources of the Brazil's capital city, Brasília. Five indexes were selected from the literature. The indexes applied were: P-IBI-G - *German Phytoplankton Index of Biotic Integrity*; P-IBI-I - *Phytoplankton Index of Biotic Integrity for Iraq*; IPI - *Integrated Phytoplankton Index*; PSI – Phyto-See-Index and the Q Index. All the indexes represented the 5-grade qualification required by Water Framework Directive (WFD). The indexes responses were analyzed based on spatial and temporal variations, and also according to different depths in the water column. In order to verify the capability of the indexes to validate the changes historically observed in water quality and cyanobacterial occurrence, the monitoring data was divided in two periods, 1976-1998 and 1999-2011. The first period, was characterized by frequent occurrence of cyanobacterial blooms. The indexes results classified the water for this period as "Poor" or "Unsatisfactory", emphasizing the eutrophication process. The exception was the Q Index, which classified the water as "Moderate" and "Good". The Q Index considers the ecological status of the lake based on the distribution of the phytoplankton functional groups. Therefore, the groups of indicator species will be defined according to physiological demands in several combinations of physical, chemical and biological properties. In the present case, the Q Index evaluated the cyanobacterial species found as indicators of oligotrophic to mesotrophic levels, even though the biomass was expressive (cyanobacteria density: 3.0×10^6 cells L^{-1} / chlorophyll *a*: 70.0 µg L^{-1}). In the second period, the P - IBI - G, P - IBI - I and IPI varied from "Moderate" to "Unsatisfactory" and PSI and Q Index varied from "Moderate" to "Excellent". The difference was probably due to the decrease of the genera *Cylindrospermopsis*. In this case, the cyanobacterial density reduction and the species succession were responsible for the indexes responses. Although each index presented some differences in the representation of the lake water quality condition, the results were able to differentiate the trophic state of the two periods studied. But the indexes were not effective in differentiate the water quality among the monitoring stations (spatial variation). The statistical analysis applied to verify the power of the indexes's metrics applied to Lake Paranoá data showed that the indexes sensitivity would be probably improved if the metrics's weights were different, probably due to the difference observed in tropical regions.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-281

APPLICATION OF GLM-AED HYDRODYNAMIC-ECOLOGICAL MODEL: WATER QUALITY AND CYANOBACTERIAL COMMUNITY OF LAKE PARANOÁ, BRAZIL.

T. de Souza Fernandes [1], L. N. L. Gomes [1,*], R. Tezini Minoti [1]

[1]Civil and Environmental Engineering, University of Brasília, Brasília, Brazil

Abstract: Hydrodynamic and ecological modeling has been used to improve the understanding of Lakes ecosystems, regarding the physical, chemical and biological characteristics of the environment. The results of hydrodynamic-ecological modelling can give support to the management of water resources, as well as, increase the comprehension of climate change impacts on hydrological processes and water quality. The modeling tools can also be applied to estimate occurrence and distribution of potentially toxic cyanobacteria species found in water supply reservoirs. The Lake Paranoá is an urban reservoir located in Brasília (Federal District/Brazil), with superficial area around 38 km² and total volume of 498 x 10^6 m^3. In the 80s and 90s the Lake presented several episodes of cyanobacterial blooms. Since then, different management strategies were applied to improve water quality. Nevertheless, nowadays the cyanobacterial biomass has been considerably increased. The study was conducted in one of the compartments of the Lake planned to be source of water for human consumption during a water supply crisis in Brasília. The GLM-AED model (General Lake Model - Aquatic EcoDynamics), was used to simulate the water temperature profile, mixing and cyanobacterial dynamics in the study area. The data bank was composed by hydrological, meteorological, limnological and morphometric data obtained from several public databases, such as CAESB (Environmental Sanitation Company of the Federal District), INMET (National Institute of Meteorology), INPE (National Institute for Space Research) e CEB (Energy Company of Brasília). The water quality data, including cyanobacteria density estimation, were obtained from the CAESB monitoring program, carried out since 1976. The simulation period chosen was from 2006 to 2015, represented by the return of higher densities of cyanobacteria in the Lake. A sensitivity analysis was carried out to determine which parameters were most sensible and the optimization of the calibration was done through an algorithm created in Rstudio software. For the hydrodynamic modelling, three parameters showed greater sensitivity, two of them linked to thermodynamics, coefficient for latent heat transfer (CE) and for sensible heat transfer (CH) and one to the properties of the Lake, the extinction coefficient for photosynthetically active radiation (Kw). The simulation of the temperature presented good results, showing good agreement with the *in situ* measurements. For the cyanobacterial results, the model showed good results when it was considered the periods of worse water quality. The ecological modelling had better results considering elevated trophic conditions. This fact could be related to the water quality differences observed among the dry season (winter) and the wet season (summer), and their influence in the phytoplankton succession.

Disclosure of Interest: None Declared

New major events & exploitation of longtime series (monitoring & case studies)

P-282

Wavelets analysis of ferrybox data in the Northern Baltic Sea to study cyanobacteria blooms

I. Puillat [1,*], J. Seppälä [2], T. Tamminen [2], J. Ruohola [2], P. Ylöstalo [2], P. Maunula [2], L. Laakso [3 4], S. Kaitala [2]

[1]LOPS, IFREMER centre de Brest, Plouzané, France, [2]Finnish Environment Institute (SYKE), Helsinki, Finland, [3]Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa, [4]Finnish Meteorological Institute, Helsinki, Finland

Abstract: Our work focuses on cyanobacterial HAB observed in the Baltic Sea with objective to investigate influence of associated environmental conditions on blooms. We mainly analyse spatio-temporal hydrographic data with chlorophyll-a and phycocyanin fluorescence data from ferry M/S Silja Serenade (part of the Baltic Sea Alg@line network) operating on the route Helsinki-Mariehamn-Stockholm. To study the relations between biology and physics parameter we mainly apply the wavelets analysis, including the wavelet coherence transform, to each data transect, or route of the ferry. Data sets acquired since 2008 will be presented first. The wavelets method is applied to the 2015 data set to exemplify how the method help detecting the timing and location of the most energetic signals of the observed frequencies (in power density spectrum), of their coherence and relations. Finally the method will be applied to a multiyear data set to explore the possibilities.In addition, complementary data sets help describing environmental conditions: fixed point data acquired on Ut Atmospheric and Marine Research Station located at the outer edge of the Archipelago Sea, Baltic Sea towards the Baltic Sea Proper, with additional supporting meteorological data from the marine meteorological stations along the ship route. Silja Senerade and Ut station belong to JERICO-RI Research Infrastructure and the Finnish Marine Research Infrastructure FINMARI.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

P-283

Characterisation of Azaspiracid producers in New Zealand waters using novel molecular tools

J. Steynen [1 2,*], L. Rhodes [1], K. Smith [1]

[1]Cawthron Institute, Nelson, New Zealand, [2]Liège University, Liège, Belgium

Abstract: Azaspiracids (AZAs) are lipophilic polyether marine shellfish toxins which have caused massive economic damage to the European seafood industry, in particular Ireland. AZAs may induce acute intoxications in humans consuming contaminated seafood and are cause for both public health concern and scientific interest. Given the worldwide distribution of AZAs, an improved risk assessment based on the dinoflagellate producers is required. To that end, novel molecular tools have been developed to improve the understanding of AZA producers, species from the genera *Azadinium* and *Amphidoma,* in the marine environment. Real-time PCR assays have been designed to screen seawater and sediment samples for potential AZA-producing species. *Azadinium* cells from positive samples were isolated and cultures grown. Identifications were verified through DNA sequencing of clonal cultures. Toxin production was analyzed by liquid chromatography and mass spectrometry (LC-MS/MS) and toxin profiles were determined. The presented results will include the correlation between environmental conditions and the presence of toxic *Azadinium* strains. The implications of these findings on the improvement of health risk assessments for New Zealand coastal waters will be discussed.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

P-284

EMERTOX: New EU project for the detection of emergent toxins and the species that produce them

L. K. Medlin [1,*], G. Mengs [2]

[1]MBA, Plymouth, United Kingdom, [2]Natural Biotec, Madrid, Spain

Abstract: EMERTOX is a new EU project in the Horizon 2020 RISE program that aims to map the actual situation in emergent marine toxins and the producing organisms, develop new approaches to assess their occurrence and predict the possible future scenarios in the framework of global warming. Our task in this new project will be to develop probes for emerging species using an electrochemical detection method with a new detection system, apply these first in manual set up in a microtiter plate and later incorporate them into an automated device with multiplexed laboratory on a chip units of 16 electrodes. Targeted species will be *Gambierdiscus, Coolia, Ostreopsis, Lingulodinium*, *Vulcanodinium,* among others. Once optimized, the species on new device will be increased to make it a global universal early warning system with very low costs, about 15€ per sample. Using the reverse transcriptase microarray developed in the EU mAqua, we will extend ITS barcodes for *Pseudo-nitzschia* directly on the microarray to detect these toxic diatoms, which are not easily separated using LSU genes. Historical mapping of the change in distribution of these species will be done using samples from the Hardy continuous plankton recorder.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

P-285

Rapid analysis of nutrient deficiency in red tide-forming diatoms based on nutrient-induced fluorescence transients (NIFTs)

R. Yano [1,*], S. Ohara [2], K. Koike [2]
[1]Faculty of Applied Biological Science, [2]Graduate School of Biosphere Science, Hiroshima University, Higashihiroshima, Japan

Abstract: To predict the expansion or decline of harmful algal blooms, assessments of the nutritional status of the causative algae are needed; however, the inorganic nutrient levels in water are not always indicative markers that control potential blooms. Traditional assessments, e.g., the algal growth potential (AGP), require time and effort that are not suitable for rapid measurements in the field. Nutrient-induced fluorescence transients (NIFTs) are short-term changes in chlorophyll *a* fluorescence that have been proposed to show nutrient limitations in phytoplankton species (Turpin and Weger, 1988; Wood and Oliver, 1995). Here, we determined whether this principle could be applied to assess nutritional status in two red tide-forming diatoms, *Skeletonema* sp. and *Trieres mobiliensis* (= *Biddulphia mobiliensis*). Both diatoms were cultured at 20 °C and 100 µmol photons m^{-2} sec^{-1} in f/2 media, and the nitrogen and phosphorus levels were decreased to 1/10. Growth was monitored using *in vivo* chlorophyll *a* measurement and cell counting. At the logarithmic growth and stationary phases, the cultures were subjected to NIFT analysis: a 3 ml aliquot of the culture was placed in a pulse-amplitude modulation fluorometer (Water PAM, Walz GmbH), and dark-adapted maximum fluorescence (*Fm*) was initially obtained. Then, under the illumination of actinic light (100-150 µmol photons m^{-2} sec^{-1}), light-regulated maximum fluorescence (*Fm'*) was obtained every 30 sec. Once the *Fm'* value reached a constant level, the control solution of the reaction (Milli-Q; 10 µl) was added, followed by additions of NH_4Cl and NaH_2PO_4 solutions, which gave final concentrations of 100 µM and 10 µM. A more than two-fold shift in the *Fm'* compared to that obtained from the Milli-Q addition was regarded as a distinct NIFT reaction. Obvious NIFT reactions were obtained in the phosphate-depleted *Skeletonema* sp. with added NaH_2PO_4 and in the nitrogen-depleted *T. mobiliensis* with NH_4Cl. In both cases, decreases in the *Fm'* value and the effective photochemical efficiency (Φ_{PSII}) and an increase in non-photochemical quenching (NPQ) were notable. Hence, these diatoms may allocate energy from photosynthesis to the functions of nutrient uptake and/or heat dissipation from absorbed light, as suggested by the NPQ increase. This technique enables the prompt estimation of depleted nutrient species in phytoplankton cells within a short time period and may be applicable in field monitoring.

Reference

1. Turpin, D.H., Wegar, H.G., Plant Physiol. 86, 688-692 (1988)
2. Wood, M.D., Oliver, R.L., Aust. J. Plant Physiol. 22, 331-340 (1995)

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

P-286

Recombinant nanobodies as cheap and customizable reagents for unicellular algae detection

E. Mazzega[1,*], A. de Marco[1], M. Cabrini[2], A. Beran[2]

[1]Laboratory for Environmental and life Sciences, University of Nova Gorica, Nova Gorica, Slovenia, [2]Istituto Nazionale di Oceanografia e di Geofisica Sperimentale (OGS), Trieste, Italy

Abstract: At the present, the identification of planktonic species in coastal water mainly relies on light microscopy observations. This kind of analyses is performed by highly trained personnel, requires lab equipment and long processing time. High-throughput and easy-to-perform methods are instead highly needed for routine costal and ballast water monitoring.

Immuno-reagents are widely employed in the medical field for routine diagnostics, where they provide the necessary sensitivity and specificity, as for example for cancer subtype characterization. Reagents of similar grade are so far not widely available for both diagnostics and basic research of microalgae. We describe the first successful isolation of a single-domain antibody (nanobody or VHH) from a pre-immune library, its engineering into application-ready reagents, and its inexpensive production as recombinant fusion protein.

Alexandrium minutum was chosen as a model organism to test the feasibility of the procedure. The procedure foresees the panning of a pre-immune phage library of VHHs that was used for *in vitro* selection against directly the target cells. Monoclonal nanobodies specific for *A. minutum* cells were identified and optimized for recombinant production as fusion with fluorescent proteins in bacterial hosts. Such fluorescently-tagged VHHs were validated by immunofluorescence and cytofluorimetry for their selectivity by testing unicellular algal species that can be found in the same environment of *A. minutum.* Two nanobodies were found to be highly specific for the target cells, were able to bind also cysts of *A. minutum* and they gave no cross-reaction, even for a not-toxic strain of the closely related *A. tamutum*.

Different tags can be then fused to the selected nanobodies and used instead of the fluorescent proteins to obtain a reagent immediately applicable to further techniques, such as cell Enzyme Linked Immuno Sorbent Assay (ELISA) or biosensor surface functionalization. The newly produced reagents can be applied for direct whole-cell detection in seawater, bypassing the need of cell processing required for DNA or RNA diagnostics, and can be used for both alive and fixed cells, guaranteeing the possibility to check old samples and to perform confirmatory morphological studies.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

P-287

The use of an sxtA qPCR assay for on-farm management decisions in Tasmania, Australia

R. Ruvindy [1,*], P. Lamb [2], M. van Asten [3], S. Murray [1]

[1]Climate Change Cluster, University of Technology, Sydney, Ultimo, [2]Spring Bay Seafoods, Triabunna, [3]Diagnostic Technology, Sydney, Australia

Abstract: Over the past 5 years, *Alexandrium catenella* (Group 1) has bloomed on an almost annual basis in waters off the east coast of Tasmania, Australia. During 2017, blooms reached an unprecedented level of 250,000 cells L^{-1} and 140 mg kg^{-1} PST in mussel tissue. A commercialised qPCR assay has been developed based on the gene sxtA, the first step in saxitoxin biosynthetic pathway. We have implemented this method as an early warning system on a mussel farm on the East Coast of Tasmania. We have also developed a sample collection and handling pipeline that is able to detect PST-producing species at low concentrations. This platform has been specifically designed to be used on farm. It consisted of a standardised sampling device involving a gravity filtration method, a simple cell lysis, and a commercially available sxtA-based qPCR assay (DinoDtec, Diagnostic Technology). In 2017, this method was used to make a management decision, which led to an early voluntary closure and the switching of production to another site. The use and validation of this pipeline in a farm in Tasmania will be presented.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

P-288

Semi-automated method for detecting and counting cells of cyanobacteria colonies and filaments

H. Nelson [1,*], P. Lehman [2]

[1]Vice President, Fluid Imaging Technologies, Scarborough, [2]California Department of Fish and Wildlife, California Department of Fish and Wildlife, Stockton, CA, USA

Abstract: Various technologies utilize fluorescence measurements to detect cyanobacteria and estimate biovolume or cell counts within a water system. While useful in determining trending applications, results from fluorometers can be significantly skewed by turbidity and the presence of other fluorescing pigments, and little if any taxonomic information can be obtained. Fluid Imaging Technologies has recently adapted their imaging flow cytometer, FlowCam, so that it can detect the presence of the phycocyanin pigment in cyanobacteria and, through digital imaging and the instrument's image recognition software, provide for organism identification, classification, size distribution, biovolume calculation, and more. In addition, the abundance of cells within colonies and filaments can be counted using a simple Excel based formula, enabling monitoring agencies and researchers to rapidly enumerate cells in large sample volumes. Along with detecting the phycocyanin pigment, the instrument is also able to detect and characterize chlorophyll, allowing for one instrument to be used in the detection and identification of all chlorophyll microalgae. Here we present an overview of the technology along with field data from natural samples.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

P-289

A new method to probe allelopathic interactions in phytoplankton mixtures

A. Peltekis [1,*], A. Micoulaud [1], L. Guillou [2], A. Méjean [3], P. Cardol [4], F.-A. Wollman [1], B. bailleul [1]
[1]UMR7141 IBPC CNRS, paris, [2]Station Biologique de ROscoff, Roscoff, [3]LIED, paris, France, [4]Université de Liège, Liège, Belgium

Abstract: Little is known about allelopathy in marine microalgae, in spite of the competitive advantage that these interactions could provide to invasive species. This is mainly due to a number of methodological difficulties, including the lack of information on the relative physiologies of two microalgae in mixture.

We chose a new approach to explore these interactions based on a physical phenomenon, the electro-chromic shift (ECS) of photosynthetic pigments, when subjected to the electric field generated across the thylakoid by photosynthesis. On the one hand, the ECS shows a different spectral signature in each photosynthetic clade of microalgae, allowing the extraction of the photosynthetic responses of each species in an assembly. We can therefore measure the photosynthetic activity of a species alone or in a mixture with another species, and highlight an allelopathic interaction targeting photosynthesis. On the other hand all complexes involved in photosynthesis contribute to the generation of a trans-thylakoïdal electric field (PSII, PSI, cytochrome *b6f*) or to its consumption (ATP-synthase). This allows to define the exact target of the released secondary metabolites on the photosynthetic apparatus. With this innovative and fast method we were able to screen allelopathic interactions that affect photosynthesis between different clades of phytoplankton.

We observed for example that the photosynthetic activity of some diatoms (*Thalassiosira pseudonana*, *Phaeodactylum trichornutum*) and prasinophytes (*Bathycoccus prasinos*, *Nephroselmis pyriformis*) was almost fully inhibited when mixed with the dinoflagellate *Amphidinium carterae*. We were further able to identify the target of the unknown secondary metabolites on the photosynthetic apparatus of the target species. It appears to affect the electrochemical proton gradient necessary for the production of ATP by the ATP-synthase during photosynthesis. We believe that this new method could help understanding better the role of allelopathy on the structure of the phytoplankton communities.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

P-290

Unusual winter harmful algal bloom dynamics detected in northern Monterey Bay with automated imaging flow cytometry

A. D. Fischer [1,*], K. Hayashi [1], A. McGaraghan [1], R. Kudela [1]

[1]Ocean Sciences, University of California, Santa Cruz, Santa Cruz, USA

Abstract: Monterey Bay is the largest open embayment along the U.S. West Coast, and is subject to intense dinoflagellate blooms, particularly between August and November. Highest bloom frequency and intensity occurs in the northern bay, coincident with warm surface waters, low wind stress, and retentive circulation. During long-term deployments at Santa Cruz Municipal Wharf, in the northern bay, the Imaging FlowCytobot (IFCB) documented a characteristic fall dinoflagellate bloom in 2016 and an unprecedented winter–spring bloom in 2018. The IFCB records images of cells with chlorophyll fluorescence above a trigger threshold, which are of sufficient resolution to identify many organisms to genus and even species level. Manual cell counts of weekly water samples from the site confirmed the cell concentrations calculated from IFCB data. In September 2016, a massive, monospecific *Akashiwo sanquinea* bloom initiated during a prolonged warm period with relaxed winds in the northern bay near the end of the annual upwelling season. The bloom peaked at >400 cells ml^{-1} in mid-October, at which point foam produced from vertical mixing of the bloom concentrated on coastal shorelines (links between foam and a seabird mortality event are inconclusive). In February–April 2018, a time of year when dinoflagellates do not occur in high abundances, oscillating pulses of *A. sanguinea* and *Prorocentrum* spp. cells were detected by the IFCB. Maximum concentrations exceeded 30 cells ml^{-1} in mid-March following an upwelling event. Upwelling was anomalously strong in early 2018, while wind stress in the northern bay was anomalously weak, leading to warmer surface waters and greater retention than would normally occur – ideal incubation conditions for dinoflagellates. This unusual winter dinoflagellate bloom may be a return to a more normal ecosystem state after the warm Pacific Blob (2013–2015) and the California drought (2011–2017). Weekly water sampling for microscopy provided a useful overview of these blooms but IFCB sampling was necessary to capture bloom onset and the dynamic interplay between the different species and physical forcings. As the frequency and amplitude of harmful dinoflagellate blooms have increased in Monterey Bay, high-resolution, real-time IFCB data has great utility to inform time-sensitive monitoring decisions.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

P-291

The development of a Genus specific qPCR assay for the detection of *Alexandrium*

R. Hatfield [1,*], D. Lees [1], T. Bean [1], J. Lowther [1], A. Lewis [1], A. Turner [1], C. Baker-Austin [1]
[1]Aquatic Health and Hygeine, Cefas, Weymouth, United Kingdom

Abstract: The use of qPCR as a tool for the identification and enumeration of HAB species is well documented, with assays generally being both highly sensitive and selective. Toxic groups of algae, such as *Alexandrium* are diverse, meaning that any method to detect their presence in an environment must be inclusive of the whole Genus rather than specific to a single species or sub species and therefore do not commonly use qPCR techniques. In an attempt to address this, we developed an inclusive, 18s rDNA, TaqMan, qPCR assay for the amplification and identification of any species within the Genus *Alexandrium*. Preliminary validation of the assay was conducted whereby DNA extraction and qPCR reactions were tested, refined and optimized using laboratory cultured *Alexandrium*. The assay was then run in parallel with phytoplankton and flesh testing routine monitoring programmes to assess its performance. The study consisted of samples taken from two locations on the South Coast of England over a two-year period, within which four toxic events occurred. The developed qPCR assay was highly sensitive, able to identify a single *Alexandrium* cell within water samples containing a diverse mix of other micro-algae. Both in-silico and in-vitro tests provided evidence for adequate degrees of selectivity, although, methodological factors had to be refined to achieve this. Also, a notable variability in copy number between *Alexandrium* species was identified, which poses issues with accurate estimations of cell numbers. The qPCR assay was however, able to provide good correlation with both flesh testing and microscopy-based methods. Our findings indicate that a qPCR-based assay can provide a highly sensitive, fast and cost-effective technique capable of identifying and tracking population changes of *Alexandrium* cells in marine environments.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

P-292

Biosensing toxic algae in natural environments from Adriatic and Aegean Sea

E. Villa [1,*], J. FRANCE [2], K. Klun [2], N. Kovac [2], D. Guillebault [1], P. Mozetic [2], C.-L. Manes [1] and EU-SMS project
[1]R&D, Microbia Environnement, Banyuls sur mer, France, [2]Marine Biology Station, Piran, Slovenia

Abstract: Harmful Algal Blooms (HABs) affect both water quality and aesthetics, but also represent a threat to human health associated with the transfer of toxins in the human food chain through toxin-affected shellfish and fish. Monitoring and time series acquisition of HABs occurrences and associated toxins are essential not only to understand both natural and anthropogenic driven marine ecosystem change but also to build an effective early warning system for a better management of fisheries and aquaculture production sites. High-frequency water quality monitoring using biosensors is the key for ensuring blue-based economy sustainability.

We have optimized an RNA sandwich hybridization assay coupled to a colorimetric detection as an easy-to-use, fast, sensitive and accurate biosensor for high-frequency water sample for the detection of the main PSP, DSP and ASP toxin-producing HAB species from the genera *Pseudo-nitzschia*, *Dinophysis* and *Alexandrium* respectively.

The biosensor is a species-specific ribosomal RNA/DNA probe-based assay of potentially toxic species and is calibrated to equivalent cell numbers. Here we present the field validation tests results performed in two relevant environments : the aquaculture production site in the Slovenian part of the Gulf of Trieste (Adriatic Sea) and the pristine Marine Park in the Greek Aegean Sea. The results were compared to the microscopic counts of toxic phytoplankton species at both sites.

Within the framework of the EU FP7-OCEAN-2013.1 SMS project (www.project-sms.eu), the sampling campaigns took place from July-September 2016 on Slovenian coast and in May 2017 at the Alonissos Island in Greece.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

P-293

Sensitive biosensing of potentially toxic cyanobacteria for risk-anticipation in freshwater environments

S. Sauvagere [1,*], F. Herard [1], S. Moreira [2], D. Guillebault [1], C. Laplace-Treyture [2], C.-L. Manes [1]
[1]R&D, Microbia Environnement, Banyuls sur mer, [2]Aquatic Systems & Global Change Unit, Irstea, Cestas, France

Abstract: Harmful cyanobacterial blooms are a major threat to freshwater ecosystems globally weighing down the Good Environmental Status of waters and risk for human health. Biosensors are a highly promising technology that can produce near real-time measurements that could be used in early warning systems safeguarding human and freshwater ecosystem health. We developed a rapid, sensitive, simple and economical test to identify and daily monitor the blooms of potentially toxic cyanobacteria in aquatic environments based on their genetic identity. This innovative solution comes in a colorimetric biosensor format to quickly and efficiently monitor and sustainably manage the contamination risks of potentially harmful cyanobacteria. The biosensor is a genus-specific ribosomal RNA/DNA probe-based assay of potentially toxic genus and is calibrated to equivalent cell numbers. Here we present the field validation tests results performed in a recreative bathing area in south Paris concerning the detection of *Microcystis* at different sampling points during summer 2017 campaign. The validation was performed by taxonomic cyanobacteria cell counting present in the samples.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

P-294

Underwater Surface Plasmon Resonance Imaging sensor for the detection of marine biotoxins

E. Justine [1], A. Bocher [2], B. Forest [3], M. Tardivel [1], S. Laurent [4], F. Colas [1,*]
[1]REM/RDT/LDCM, [2]REM/RDT/SI2M, [3]REM/RDT/CSM, [4]REM/EP/LM2E, Ifremer, Plouzané, France

Abstract: Toxic species of the genus Pseudo-nitzschia are particularly dangerous as they produce domoic acid (DA), a neurotoxin that can bioaccumulate and rapidly transvector throughout the food chain via phytoplankton feeder organisms. The production of toxins within and among toxigenic phytoplankton species depends on environmental parameters such as temperature, salinity, nutrients and trace elements concentrations. Cell number cannot be easily related to toxicity. Systems able to detect the toxin at the early stage of the bloom would be of great interest.

For the last decades, biosensors have demonstrated their ability to detect several kinds of molecule at very low concentration [1]. Recently, an underwater biosensor of DA was reported [2]. It was based on an optical technique called Surface Plasmon Resonance (SPR) and used monoclonal antibodies. This system enabled us to detect DA at concentration of the order of 0.1 ppb during laboratory and shipboard experiments. These first results were very promising. However, the detection of very low concentration of DA requires several controls as well as replicates. In addition, special techniques such as SPR imaging now enable researchers to detect many compounds at the same time [3] and open very nice prospects.

The aim of this presentation will be first to expose the new SPRI biosensor as well as first results that we obtained during laboratory experiment and mesocosme facility deployment.

<u>References</u>

[1] J. Dostálek, J. Ladd, S. Jiang and J. Homola, in SPR biosensors for detection of Biological and Chemical Analytes, eds, J. Homola and O. S. Wolfbeis (Springer Verlag, Heidelberg, 2006).

[2] Colas F, Crassous M-P, Laurent S, Litaker RW, Rinnert E, Le Gall E, et al. A surface plasmon resonance system for the underwater detection of domoic acid: Domoic Acid Detection Using SPR. Limnology and Oceanography: Methods. 2016. 14(7):456–65.

[3] Sereda A, Moreau J, Roulade M, Oliver A, Canva M, Maillart E. Compact 5-LEDs illumination system for multi-spectral surface plasmon resonance sensing. Sensors and Actuators B: Chemical. 2015. 209:208–11.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

P-295

Untargeted high resolution mass spectrometry approaches for identifying novel microcystins

C. O. Miles [1,*], D. G. Beach [1], E. J. Wright [1], A. J. Foss [2], S. Uhlig [3], I. A. Samdal [3], M. Sandvik [3], I. Ine Van Nieuwenhove [4], K. E. Løvberg [3]

[1]Measurement Science and Standards, National Research Council Canada, Halifax, Canada, [2]GreenWater Laboratories, Palatka FL, USA, [3]Norwegian Veterinary Institute, Oslo, Norway, [4]Laboratory of Food Analysis, University of Ghent, Ghent, Belgium

Abstract: Microcystins (MCs) are a group of hepatotoxic heptapeptides that inhibit protein phosphatases, and are produced by many species of cyanobacteria. Most MCs contain the unusual β-amino acid "Adda^5 ", γ-linked D-Glu^6, and *N*-methyldehydroalanine (Mdha^7) at positions 5–7, with more than 250 different microcystin congeners having been reported in the literature, and with new congeners being discovered regularly. In addition to methods capable of measuring total MCs or targeting known congeners, improved methods are needed for identifying new candidate MCs , to ensure the safety of recreational and drinking waters. Untargeted high resolution mass spectrometry (HRMS) methods allows for simultaneous analysis of a tens of thousands of known and unknown chemicals in complex biological or environmental samples. Typically, identification of novel MCs involves only LC–MS/MS analysis in positive ionization mode with detection of a characteristic fragment from Adda^5 at *m*/*z* 135.0804. However, some MCs contain modified Adda-moieties, or are otherwise modified to change the MS/MS conditions under which this fragment is generated, and are not readily detected in this manner. We have recently shown that thiol-derivatization of the Mdha^7/Dha^7 group in MCs is a highly effective method for identifying even trace amounts of novel MCs in complex matrices by LC–MS. Here, we present a new approach using metabolomics software for semi-automated detection of novel MCs based on mercaptoethanol derivatization, together with accurate mass detection of precursor and characteristic product ions in negative and positive ionization modes. Taken together, this approach targets any molecules containing Adda^5, D-Glu^6 or Mdha^7/Dha^7, one or more of which is present in every microcystin reported to date. Furthermore, it is these three residues that are in closest contact with the catalytic centre of protein phosphatases, and which appear to be most important for toxicity. HRMS methods using data independent acquisition scan modes were particularly well suited for this purpose, allowing for simultaneous acquisition of MS/MS data on all compounds detected. This type of data is also well suited to retrospective analysis of newly discovered MCs from previously acquired data. The application of microcystin immunoaffinity columns containing antibodies with broad specificity to the whole family of MCs (presentation by Samdal et al.) further enhances the utility of this metabolomics workflow. We demonstrate the power of the combination of these novel approaches with the identification of numerous novel MCs in field and culture samples as well as a blue-green algal matrix reference material.

Disclosure of Interest: None Declared

New tools (omics, lab-on-a-chip, ecotron…)

P-296

Low levels detection of diarrheic toxins using the recombinant catalytic subunit of Mytilidae PP2A: A natural receptor.

D. A. Rojas [1], F. Urbina [1], J. M. Sepúlveda [2,*], M. del Campo [2], N. Lagos [1], E. Maldonado [1]

[1]ICBM, Program of Molecular and Cell Biology, [2]ICBM, Program of Biophysics and Physiology, University of Chile, Faculty of Medicine, Santiago, Chile

Abstract: In every spring-summer period occur harmful algae blooms in southern Chile, one of them produce diarrheic toxins that accumulate in shellfish and this is and endemic phenomenon. In order to prevent people intoxication and to support the Chilean shellfish industry, the levels of these toxins must be monitored. Since most methods to measure okadaic acid and its derivatives are costly and time consuming, we have developed a qualitative and quantitative assay based on the inhibition of the activity of the PP2A catalytic subunit of Mytilus chilensis, a common Chilean mussel. The catalytic subunit was cloned and expressed in insect cells producing large amounts of recombinant protein, which can be inhibited by nanomolar concentrations of okadaic acid and microcystin LR, similar to the mammalian PP2A counterparts. Since the recombinant protein is produced in large quantities and can be easily purified, it could be used for the routine detection of diarrheal toxins in the field or in the laboratory.

Disclosure of Interest: None Declared

Eco-physiology & cellular biology of harmful algae and cyanobacteria

P-297

Fishing neurotoxins acting on nicotinic acetylcholine receptors by using « immobilized » and « in-solution » Torpedo-electrocyte membranes.

P.-E. Girault-Sotias [1], J. Echterbille [2], S. Longo [3], D. Servent [1], L. Quinton [2], M. Chinain [3], R. Aráoz [4,*]

[1]CEA/DRF/ JOLIOT/SIMOPRO/ Toxines Récepteur et Canaux Ioniques, Gif-Sur-Yvette, France, [2]Laboratory of Mass Spectrometry- MolSys, Department of Chemistry, University of Liege, Liege, Belgium, [3]Laboratoire des Micro-algues Toxiques, Institut Louis Malarde, Papeete-Tahiti, French Polynesia, [4]CNRS, Neuro-PSI, UMR9197, Gif sur Yvette, France

Abstract: Current monitoring of known and regulated neurotoxins from marine and continental water is performed by LC/MS-based methods or by using dedicated ELISA assays, although as alkaloid toxins are very small, it is usually very difficult to raise good antibodies against them. Mouse bioassay remains the only detection method for unknown and unanticipated neurotoxins from aquatic environmental samples (dinoflagellates, cyanobacteria, diatoms, shellfish, fish, crustaceans, etc). Therefore, novel functional assays are necessary for rapid detection of known, unknown and unanticipated neurotoxins from aquatic environments. This work describes a methodology developed to discover new ligands directed against nicotinic acetylcholine receptors (nAChR) from complex mixtures containing small size alkaloids or large peptides. The methodology uses purified electrocyte membranes from *Torpedo marmorata* in solution or immobilized on the bottom of 96-well microplates. Advantageously, the nicotinic receptor is in its native form at high concentrations and surrounded by its natural lipids and clustering proteins. Thereby, *Torpedo*-nAChRs are stable and functional for years if properly conserved. The proposed technology addresses the question of the chemical nature of the toxin or ligand detected. This is a novel concept. Most bioassays enable the detection of any given analyte, but the chemical nature of the binder remains unknown. The present method allows the direct capture of a given toxin by the nicotinic acetylcholine receptors in solution or coated on the wells of plastic microplates. After wash and elution, the chemical nature of the captured toxin could be determined by mass spectrometry. By using this method « in-solution », a novel conotoxin never described to date, was captured, identified and sequenced from the venom of *Conus ermineus*. Radio-ligand competition binding assay showed that EIIB (m/z = 1753.704) binds to *Torpedo*-nAChR with a K_i of 2.2 ± 0.7 nM. We applied the microplate receptor binding assay to capture low molecular weight alkaloid compounds from cultured dinoflagellate extracts that were active on nAChRs as evidence by electrophysiological recordings on *Xenopus laevis* oocytes expressing muscle and neuronal nAChRs subtypes. Taken together, the proposed technology (WO 2012/101378 A1) is suitable for discovering unknown and unanticipated neurotoxins from marine and continental aquatic environments.

Acknowledgements. The authors acknowledge the funding support of the GDR-PHYCOTOX for the mini-project EPSOM-AO 2016-GDR, andof the Interreg/ Atlantic Area for ALERTOX-NET EAPA_317/2016 project.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-298

Detection of azaspiracids using an immunosensor

M. Subrt [1], J. Kilcoyne [2], J. Silke [2], C. Elliott [1], K. Campbell [1,*]

[1]Institute for Global Food Security, School of Biological Sciences, Queen's University, Belfast, United Kingdom, [2]Marine Institute, Rinville, Ireland

Abstract: Azaspiracids (AZAs) are a group of lipophilic polycyclic ether marine algal toxins produced by the dinoflagellates genera Azadinium and Amphidoma. They can accumulate in bivalves and cause Azaspiracid Shellfish Poisoning (AZP) in human upon digestion of contaminated shellfish. Acute symptoms include diarrhoea, nausea, vomiting and abdominal cramps. Deformation of intestinal epithelial villi, damage to T and B lymphocytes, lung tumours, cytotoxicity and neurotoxicity were reported in *in vitro/vivo* studies. The presence of AZA, first reported in Ireland in 1995, is now widespread. A European action limit has been established as 160 mg/kg of AZAs in shellfish meat. In the present study, a monoclonal antibody was raised to azaspiracid and the MBio planar waveguide biosensor was utilised to develop an assay for the detection of AZA. The applicability to natural samples was investigated whereby minimal matrix effects were observed and good toxin recovery observed. The comparison of this method with LC-MS results for contaminated mussel and oyster samples has shown the compatibility and feasibility of this immunosensor to be able to support monitoring programs and research activities for evaluation of the toxin occurrences and levels of exposure.

Disclosure of Interest: None Declared

Mitigation of HABs

P-299

Adsorptive removal of nitrate and phosphate from aqueous solution using granular drinking water treatment residuals

S. Park [1,*], S.-J. Lee [1], J. Kim [1], W. Noh [1], C.-M. Kang [1]
[1]Korea Institute of Toxicology, Jinju-si, Republic of Korea

Abstract: Eutrophication, caused by the release of nitrate and phosphate ions into the water stream, is responsible for the harmful algal bloom. This can increase treatment costs at the drinking water treatment plant. Thus, it is very important to control the nitrate and phosphate ions from water to solve these environmental and economic problems. In this study, drinking water treatment residuals (DTWRs) were used for the adsorptive removal of nitrate and phosphate ions from aqueous solution. Due to the low hardness of calcined DWTRs, the adsorbents were prepared by the combination of calcined DWTRs and three granulating agents, alginate (AL), agar (AG) and chitosan (CH). The effect of granulating agents and calcined DWTRs content on the removal of nitrate and phosphate ions was investigated. Initial concentration of nitrate and phosphate was 100 ppm and the adsorbent concentration was 10 g/L. The adsorption capacity of nitrate at pH 3 was higher in order of DWTRs-CH bead (2.2 mg/g bead), DWTRs-AL bead (1.46 mg/g bead), and DWTRs-AG bead (0.17 mg/g bead). In the adsorption of phosphate at pH 3, DWTRs-AL bead showed the highest phosphate adsorption capacity (8.66 mg/g bead), but the swelling of beads was occurred during regeneration step under alkaline condition. The higher content of DWTRs (5 and 10%) in both DWTRs-AL and DWTRs-CH beads decreased the adsorption capacity of nitrate and phosphate ions. From the results, DWTRs-CH bead was suitable for the adsorptive removal of nitrate and phosphate.

Disclosure of Interest: None Declared

Mitigation of HABs

P-300

Electro-flotation-oxidation for harvesting bio-flocculated algal biomass and simultaneous treatment of residual toxicants in wastewater

B.-G. Ryu [1,*]

[1]Applied Bioresources Research Division, Nakdonggang National Institute of Biological Resources (NNIBR), 137, Donam 2-gil, Sangju-si, Gyeongsangbuk-do, Republic of Korea

Abstract: This study investigated the feasibility of employing an electro-flotation-oxidation process that employs a pair of boron-doped diamond (BDD) and aluminum (Al) electrodes for electrochemical harvesting of green microalgae (*Scenedesmus quadricauda*) and treatment of residual pollutants in coke effluent, following an algal-bacterial process. Electro-coagulation-flotation with polarity exchange and with direct electro-flotation at 15 mA cm^{-2} or more for 40 min allowed almost complete harvesting of microalgae. Similar harvesting efficiencies were achieved using direct electro-flotation, without electro-coagulation, under different electrical densities because algal biomass formed flocs with the other microorganisms in the activated sludge (AS). These results also indicate that the proposed approach of inducing bioaggregation *via* floc-forming microorganisms with microalgae is an efficient alternative to chemical flocculation, because it can minimize the release of toxic metal coagulants during electrochemical harvesting. During sequential electro-oxidation, anodic oxidation using the BDD electrode simultaneously mineralized residual soluble chemical oxygen demand (SCOD) and thiocyanate (SCN^-), which are not degraded by algal-bacterial mixed cultures. Although the degradation rate of SCN^- was much higher than that of SCOD under certain current densities, further investigation is needed to clarify the mechanism of SCN^- mineralization during BDD-anodic oxidation. To satisfy the standard level of electrical power consumption for wastewater treatment, an electric current density below 15 mA cm^{-2} must be supplied. The proposed electrochemical approach involving bioflocculation could be used as an efficient post treatment of microalgae-mediated process for treating coke wastewater.

[This work was supported by the Nakdonggang National Institute of Biological Resources granted by the Ministry of Environment, Republic of Korea]

Disclosure of Interest: None Declared

Mitigation of HABs

P-301

Development of an information sharing system for broad harmful algal bloom distributions

S. Nagasoe [1,*], A. Maeda [2], T. Tokunaga [1], Y. Matsuyama [1], K. Okamura [1], K. Ichihashi [2], K. Kimoto [1]

[1]Seikai National Fisheries Research Institute, Japan Fisheries Research and Education Agency, [2]System and Development Department, ICOC Co., Ltd., Nagasaki, Japan

Abstract: In the Japanese finfish farming area, mass mortalities caused by harmful algal blooms (HABs) are still one of the serious industrial problems. To prevent the damages by red tides, finfish farmers take measures such as relocation of aquaculture rafts to lower HABs sites and stopping feeds. Therefore, finfish farmers are eager to obtain prompt and spatial information on red tides occurrence, so that they can plan the destinations of rafts and timing of stopping feeds; they desire to receive daily information by noon of each day for preparing for the following day. Considering farmers' requests, municipalities in each prefecture investigate the fluctuations of HAB species and release the results to finfish farmers. In general, field surveys of areas with red tides occurrence are held in the morning, and finfish farmers are alerted in early evening or at night. Moreover, there is a further time lag to obtain information of other waters surveys conducted by different organizations. Considering this situation, we developed a quasi real-time HABs alert system that allows fish farmers to use information published in various places by using Information and Communication Technology. In particular, species-specific distributions of HABs are automatically displayed directly on the website via Geographic Information System, when users input the data such as sampling points (latitude and longitude), species name, and cell density of phytoplankton. The possibility of a more detailed and large-scale information sharing is higher with an increase in the number of users who input data into this database. To date, registrations to the database are conducted by not only public organizations (local governmental institutions and universities) but also finfish farmers. We believe that the use of this information sharing system is not limited to red tide countermeasures, because the number of data elements to be entered in this database can be increased (temperature, salinity, dissolved oxygen concentration, nutrient concentration, and so on). We hope that this will allow fishermen to adapt it for various other purposes for a better understanding of the coastal fishing environment. In this presentation, we intend to introduce this information sharing system and provide some examples of use case.

This work was supported by Japan Fisheries Agency-commissioned project for addressing red tides and oxygen-depleted water masses.

Disclosure of Interest: None Declared

Mitigation of HABs

P-302

Removal effects on harmful algal bloom species of eco-friendly algicidal substance ‘GreenTD’

J. H. Kim [1,*], S. H. Baek [1], H. Cho [2], Y. K. Lim [1], M. Lee [1]
[1]Korea Institute of Ocean Science and Technology, Geoje, [2]Chosun University, Gwangju, Republic of Korea

Abstract: Since the 1960s, the frequency of harmful algal bloom (HAB) events has gradually increased, and it has been causing serious ecological and economic problems in Korea. Particularly, massive blooms of *Cochlodinium polykrikoides* caused and severely increased economic losses of fishery industries due to fish mortality in the coastal area, and blooms of Microcystis have been causing serious water quality problems in fresh water system. Many physical, chemical, and biological algicidal techniques have been developed to combat HAB, however there are not many appropriate methods for field application. A novel algicidal thiazolidinediones derivate substance, ‘GreenTD’ was developed, and tested its algicidal activity on HAB species, *C. polykrikoides* and *Microcystis*, and evaluated the biological toxicity using zooplankton. From the results of screening test using *C. polykrikoides* culture, the suitable concentration of GreenTD was 1 ppm for field application. The active chlorophyll *a*. of *C. polykrikoides* were dramatically decreased from 5.8 to near 1 μg/L and the values of cell activity (*Fv/Fm*) were decreased from 0.52 to near 0.1 after one hour of GreenTD inoculation in 0.1–5 ppm range, however, there was no striking effect < 0.01 ppm of GreenTD. In concentration of 1 ppm or more, fatal cell mortality of *C. polykrikoides* was postulated. In microcosm test (20 liter), chlorophyll *a.*, active chlorophyll *a.*, *Fv/Fm* and cell density of bloomed *Microcystis* was rapidly decreased after one day of GreenTD inoculation of 1 ppm. Algicidal efficiency was 93% at 4th day of microcosm, but it was decreased as 75.7% at 6th day. The 1 ppm of GreenTD was inoculated at 6th day again, then the algicidal efficiency remained at 86.5% during 12 days of experiment. Acute toxicity tests were performed using *Artemia* and natural marine zooplankton during 48 hours. Toxicity effects were observed from 1000 ppm to *Artemia* and 10 ppm to natural marine zooplankton, all of *Artemia* were decimated at 8000 ppm and natural marine zooplankton were at 100 ppm, however there was no toxicity ranged in < 1 ppm which was our targeted concentration for application. Developed algaecidal substance has had a remarkable HAB removal effect, moreover it showed non-toxic effect on zooplankton.

Disclosure of Interest: None Declared

Mitigation of HABs

P-303

Combatting harmful cyanobacteria with hydrogen peroxide is more effective at high light

G. Sandrini [1,*], T. Piel [1], E. White [1], T. Xu [1], M. Schuurmans [1], J. Huisman [1], P. Visser [1]

[1]Department of Freshwater and Marine Ecology, Institute for Biodiversity and Ecosystem Dynamics, Amsterdam, Netherlands

Abstract: Harmful cyanobacteria cause ecological and economical problems worldwide. A promising short-term method to selectively combat harmful cyanobacterial blooms in lakes is the application of a low concentration of hydrogen peroxide (H_2O_2). Cyanobacteria are much more sensitive to H_2O_2 than eukaryotic algae, because cyanobacteria use a Mehler-like reaction that does not produce H_2O_2. We have applied H_2O_2 treatments to several cyanobacteria-dominated lakes in The Netherlands since 2009 (Matthijs *et al.*, 2012). Currently, we investigate under which environmental conditions the H_2O_2 treatment method will be most successful.

To study effects of light on the effectivity of the H_2O_2 treatment, we performed lab experiments with the toxic cyanobacterium *Microcystis aeruginosa* PCC 7806. The axenic strain was cultured in chemostats and samples of the steady state cultures were subjected to different H_2O_2 concentrations and light conditions in 24 hour experiments in batch cultures.

Our results show that light has a very strong effect on the effectivity of the H_2O_2 treatment. In the dark, cells were hardly affected by 1-10 mg/L H_2O_2, while cells were increasingly more affected under increasing light conditions. Furthermore, H_2O_2 was degraded at a higher rate by cells exposed to light compared to cells exposed to darkness. Extracellular microcystin concentrations increased with increasing H_2O_2 concentrations and higher light intensities, while the sum of intracellular and extracellular microcystins decreased after addition of H_2O_2.

In conclusion, harmful cyanobacteria are more sensitive to H_2O_2 at higher light intensities. Hence, H_2O_2 treatments of lakes should be performed preferably on sunny days, when the treatment is already effective at a low dosage. Conversely, treating lakes after sunset or at night is not recommended.

Matthijs H.C.P., Visser P.M., Reeze B., Meeuse J., Slot P.C., Wijn G., Talens R., Huisman J. (2012). Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide. *Water Research* **46:** 1460–1472.

Disclosure of Interest: None Declared

Mitigation of HABs

P-304

Mitigation of cyanotoxins with magnetic nanoparticles

A. Alfonso [1,*], J. M. González-Jartín [1], I. Rodríguez [1], M. J. Sainz [2], M. R. Vieytes [3], Z. Vargas Osorio [4], L. de Castro Alves [4], Y. Piñeiro-Redondo [4], J. Rivas [4], L. M. Botana [1]

[1]Farmacología, [2]Producción Vegetal , [3]Fisiología, Universidad de Santiago de Compostela, Facultad de Veterinaria, Lugo, [4]Física Aplicada, Universidad de Santiago de Compostela, Facultad de Física, Santiago de Compostela , Spain

Abstract: Cyanobacteria have a global distribution and are frequently found in freshwater like rivers or lakes. Certain conditions lead to excessive growth of this microorganisms which results in the formation of harmful algal blooms (HABs). Freshwater HABs are a major environmental problem causing severe impacts on public health, aquatic ecosystems and the economy. HABs can produce toxins known as cyanotoxins including, among others, microcystins (MCs), nodularins (NODs), cylindrospermopsins (CYNs), saxitoxins (STXs) and anatoxin-a (ATX-a). These compounds have been associated with animal and human poisonings after water ingestion. Thus, the presence of cyanotoxins in drinking-water poses a public health threat. Conventional water treatments like coagulation, sedimentation or filtration are not effective for removing high amounts of these toxins. Therefore, mitigation strategies and treatment options must be developed to avoid human and animal exposure to cyanotoxins. The applicability of nanoscale materials for environmental remediation is a new tool in development stage. In comparison with traditional sorbents, nanomaterials have a large surface area and allow combining several reactive components. In this context, the aim of this work was to establish the potential of magnetic nanoparticles for adsorbing cyanotoxins from water. Toxin content in solutions with mixtures of MC-LR, MC-RR, NOD, ATX-a and CYN was determined by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MSMS) before and after treatment with different types of nanoparticles. These particles were magnetically separated from solutions and, afterward redispersed in organic solution by vortex mixing and sonication to enforce an effective extraction of the adsorbed toxins. Tested magnetic nanoparticles show good results reducing the cyclic peptides MC-LR, MC-RR and NOD, with extraction efficiencies raged from 25 to 80%. Therefore, the implementation of magnetic nanomaterials in water treatments could be a promising approach to remove cyanotoxins and, hence, to avoid the presence of these compounds in drinking water.

Disclosure of Interest: None Declared

Mitigation of HABs

P-305

The success of hydrogen peroxide treatments to combat cyanobacteria depends on the timing of the treatment and the species composition

T. Piel [1,*], E. Weenink [1], G. Sandrini [1], J. M. Schuurmans [1], M. van Herk [1], H. C. P. Matthijs [1], J. Huisman [1], P. M. Visser [1]
[1]Department of Freshwater and Marine Ecology, Institute for Biodiversity and Ecosystem Dynamics, Amsterdam, Netherlands

Abstract: The preferred ultimate solution for the prevention of reoccurring toxic cyanobacterial blooms, i.e. the reduction of external and internal nutrient loads, is often a long-term process and may not be applicable in all lakes. Methods that work effectively against cyanobacteria on a short term are also needed. One of the major challenges in acute situations is to rapidly tackle harmful blooms by selectively killing toxic cyanobacteria while keeping unwanted side effects on non-target species to a minimum.

Hydrogen peroxide, a compound that degrades to water and oxygen without leaving chemical traces in the environment, has been shown to be a highly effective and selective cyanocide, when applied in low concentrations. To obtain more experience with its application in various lakes and with different cyanobacterial species, we treated several lakes in The Netherlands with low concentrations of hydrogen peroxide. The lakes varied in size and depth and were dominated by different cyanobacteria, e.g. *Aphanizomenon* s. l., *Dolichospermum* spp., *Planktothrix agardhii* and *Planktothrix rubescens*.

In almost all treatments, we observed that cyanobacteria were indeed selectively targeted and instantly removed from the lake while other phytoplankton profited. The success of the treatment and the duration of the absence of cyanobacteria in the lake was dependent on the dominant species, the abundance of algae, and the timing of the treatment. We therefore conclude that depending on the aim of the treatment and the field conditions, a treatment with hydrogen peroxide can be a rapid, highly effective and low-cost method to combat cyanobacterial blooms.

Disclosure of Interest: None Declared

Mitigation of HABs

P-306

First Evaluation of Antialgal activity of Macrophytes extracts against *Microcystis aeruginosa* and *Chlorella* sp. in solid medium

Z. Tazart [1 2,*], S. El Amrani Zerrifi [1], M. Douma [1], L. Tebaa [1], K. Mouhri [1], A. T. Caldeira [2], M. Loudiki [1]

[1]Laboratory of Biology and Biotechnology of Microorganisms, Faculty of Sciences Semlalia Marrakech,Cadi Ayyad University, Marrakesh, Morocco, [2]HERCULES Laboratory, Évora University, Largo Marquês de Marialva 8, 7000-809. , Évora, Portugal

Abstract:

Introduction

Many studies have demonstrated the effectiveness of isolation and allelochemicals compounds produced by macrophytes against pathogenic aquatic microbes. However, the elucidation of their antialgal properties in solid medium has received less attention.

This study aims to evaluate the potential of allelochemicals activities of ethyl acetate extracts from 10 different aquatic plants *Ceratophyllum sp., Enteromorpha sp., Rannanculus aquatilis, Myriophyllum spicatum, Ceratophyllum submersum, Potamogeton natans, Rannanculus sp., Nasturtium officinale, Nasturtium sp.* and *Elodea sp*. by investigate their inhibitory effects on *Chlorella sp.* and *Microcystis aeruginosa* in solid medium

Methodology

The macrophytes antialgal activity was assessed in vitro by the paper disc diffusion method. Ethyl acetate extracts of macrophytes were compared to several organic compound standards used as positive controls. Also, total phenolics, flavonoids and tannin contents in the macrophytes were determined to reveal the potentially allelochemicals.

Results

The highest antialgal activity against Chlorella sp. was observed for *Ceratophyllum* sp, *Myriophyllum spicatum* and *Ceratophyllum submersum* ethyl-acetate extracts with 31 ± 1 mm, 35,33 ± 1,53 mm and 33 ± 1 mm respectively. *Enteromorpha sp. Rannanculus aquatilis* and *Myriophyllum spicatum* ethyl-acetate extracts presents the most inhibition zone of *Microcystis aeruginosa* compared to the control with 30 ± 1 mm, 29,33 ± 1,53 mm and 30,33 ± 1,15 mm respectively. The phytochemical analysis results revealed that the phenolic, flavonoids and tannin compounds were present in all the investigated plants.

Conclusion

It was concluded that the paper-disc method can be used to evaluate the antialgal activity of macrophytes extracts against cyanobacteria and microalgae *in vitro*.

Disclosure of Interest: None Declared

Mitigation of HABs

P-307

Control of HABs using modified clay: the technology and application

X. Cao [1 2,*], Y. Yuan [1 2], X. Song [1 2], Z. Wu [1 2], L. He [1 2], Z. Yu [1 2]

[1]CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, [2]Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China

Abstract: As Harmful Algal bloom (HAB) is the proliferation of microalgae whose rapid growth is matched with suitable environmental factors, such as radiation, nutrients, water exchange, etc., the concept “controlling HABs” means the effective interruption to the matching relationship between biological factors representing its growth and environmental factors supporting its proliferation for a specific HAB occurrence. Clay flocculation is one of promising measures that has been used to control HABs in field but still has crucial bottlenecks for its popularizing in real-world cases, including insufficient efficiency for clay to remove HAB organisms, great amount of clay in use, huge cost and plenty deposit thereof. According to our research, surface modification is an effective way to increase the removal efficiency for natural clay, thus we proposed Modified Clay (MC) technology. By using MC in field, 70%>80% HAB organisms could be flocculated directly to the bottom while the growth of residual is inhibited and would be ultimately settled down by repetitive sprayings. There are 3 characteristics of such technology. Initially, it’s safe and environmental friendly. MC is produced through a series of purifying procedures from the row mineral which is the major component of nature soil. Meanwhile, MC tech. is proved safe to use in field after careful and plenty evaluations for its ecological effects on typical aquaculture, water quality, benthic, planktonic organisms, and so on. Secondly, it’s highly efficient to control HABs in field. The water clarity could be visibly improved in 30 minutes while the removal rate of MC to different HAB organisms could reach 90% averagely. Meanwhile, the average amount of MC used in field is only 4-10 tons/km^2, which is adequately as 4-10 g/m^2, with the purpose of effective control of HABs. Last but not least, it’s such a technology being easy to use. The automatic specialized equipment for MC mixing and spraying has been developed in several types according to varied scale of waters. By using the equipment, a well-mixed, thin MC slurry can be obtained and sprayed with good dispersion. Consequently the removal efficiency of MC in field application can be sustained over repetitive sprayings. Since 2005, as a “fire extinguisher” for HAB emergencies, MC has been successfully applied in over 20 different waters of China, including cooling water of nuclear plants, typical aquaculture areas and waters for some important activities, such as Olympic sailing regatta, BRICs summit, etc. It has been listed in the National Standards of China for HAB control, and successfully introduced to Chile and demonstrated in field, with the purpose of mitigating negative impacts of HABs to the aquaculture of Chile. As a summary, MC technology is a promising method to control HABs in field and proved to be effective and environmentally safe through dozens of successful applications.

Disclosure of Interest: None Declared

Mitigation of HABs

P-308

Eco-environmental effects of using modified clay to control harmful algal blooms

X. Song [1 2 3,*], J. Li [1 2], Y. Zhang [1 3], P. Zhang [1 3], Z. Wang [1], Z. Yu [1 2 3]

[1]CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, [2]Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao, [3]University of Chinese Academy of Sciences, Beijing, China

Abstract: Presently, modified clays (MC) application is the most widely used method for the mitigation of harmful algal blooms in the field in China. Although all the raw materials selection and the preparation procedures of MC are based on the consideration with harmless and minimal adverse environmental influences, there is still concern they might impact the ecosystem following their application. Therefore, potential effects of MC disposal on water quality (such as nutrients, DO, pH etc.) , typical aquatic organisms (such as fish, shrimp, scallop/ shellfish and sea cucumber etc.) , benthic environments, and ecosystems was examined. Based on numerous studies, including laboratory trials and long term monitoring in the field after application, several conclusions can be made:(1) MC could decrease the nutrients concentration of water column, especially phosphate and ammonium. The adsorption capacity for phosphate could reach to 80%, depend on the initial concentration of phosphate and the MC dosage, which can effectively decrease the chance of second-bloom of the target organisms. (2) Recommended MC dosages used in field (4-10 t/km^2) are too low to cause mortality or adverse effects to several nektons and benthos at both observed life history. (3) MC has a minimal impact on some common chemical parameters, including oxidation-reduction potential, total organic carbon and typical heavy metal (e.g. copper, zinc, chromium, and lead), within the benthic environment in relatively long term. (4) MC can effectively reduce the ingest of filter feeding shellfish on toxic algae through remove algal cells from the water column, and consequently reduce the accumulation of algal toxins in their tissue. Specifically, MC at concentration of up to 100 g/m^2 (eq. 100 t/km^2) has no adverse effects on Atlantic salmon (*Salmo salar*), which was the major aquaculture product of Chile, and suffering from *Pseudochattonella spp.* blooms in recent years. In summary, both laboratory and field results have shown that appropriate dosage of modified clay is capable of controlling HAB efficiently with negligible negative effects on aquatic ecosystems while achieving marked improvement in water quality and aquatic animal welfare.

Disclosure of Interest: None Declared

Mitigation of HABs

P-309

Algicidal effects of Bioionix's electro-chemical technology on *Alexandrium catenella*: Laboratory and field experiments

M. Seguel [1,*], D. Varela [2], J. Paredes [2], A. Aguilera [3], H. Kossmann [4], J. Vogel [5], C. Martinez [2]
[1]CERAM, Universidad Austral de Chile, [2]Centro i-mar, Universidad de Los Lagos, [3]Universidad San Sebastian, [4]Patagonia Wellboat, Puerto Montt, Chile, [5]Bioionix Inc, McFarland, USA

Abstract: The development of salmon farming in the southern regions of Chile has made it necessary to transport salmon in well boats, from farming areas to processing plants. One of the problems with this means of transport is the transfer of the vegetative cells of *Alexandrium catenella* from areas of greater risk, especially in spring and summer which is the period of greatest occurrence of Harmful Algal Bloom (HAB). The main objective of this work was to evaluate the effect of Bioionix's electro-chemical technology on the vegetative cell of *A. catenella* viability. In June 2016 an experiment was carried out under controlled conditions, where *A. catenella* (2000 cells mL^{-1}) was subjected to four doses: 0.13, 0.33, 0.79, 1.59 Ampere / liter * minutes, using Bioionix electrochemical technology. After 21 days of culture of *A. catenella*, the growth rates were negative for the doses of 0.79 and 1.59 $A \cdot L^{-1} \cdot min^{-1}$. In February 2018, given the presence of a bloom of *A. catenella* in the region of Aysén, an experiment was carried out to test these doses under field conditions, in Seno Canalad (44° 35' 26''S, 73° 18' 51''W). The phytoplanktonic community was constituted by diatoms and dinoflagellates, where the concentration of *A. catenella* varied 24 and 79 cells mL^{-1}. In the samples collected through the water intake of the well boat (Patagón IV), where the bioionix 6500 equipment was installed, three doses were tested: 0.79, 1.59 and 3.18 $A\ L^{-1}\ min^{-1}$. The samples were then filtered and placed in flasks with L1 culture medium, with and without germanium dioxide. The flasks were taken to the laboratory and kept in controlled conditions for 11 days, observing the growth and morphology of *A. catenella*. The results indicate that the dose of 3.18 $A\ L^{-1}\ min^{-1}$ produced inactivation or death of all cells of *A. catenella* and the phytoplankton community, while the other doses partially affected the growth of *A. catenella*. It is concluded that this dose could be used to inactivate vegetative cells of *A. catenella* in the well boat.

Disclosure of Interest: None Declared

Mitigation of HABs

P-310

Application of molecular methods for efficient monitoring of HAB dinoflagellate resting cysts in harbour sediments

S. Casabianca [1], M. Bastianini [2], S. Capellacci [1], E. Di Poi [3], M. Cabrini [3], M. Pompei [4], M. Marini [5], A. Penna [1 5,*]

[1]DISB, University of Urbino, Pesaro, [2]ISMAR, Consiglio Nazionale delle Ricerche, Venezia, [3]OGS, Trieste, [4]Centro Ricerche Marine, Cesenatico, [5]ISMAR, Consiglio Nazionale delle Ricerche, Ancona, Italy

Abstract: It is known that toxic dinoflagellates produce resting cysts as part of their life cycle depositing in the bottom sediments. These resting stages play an important role in bloom initiation as the seed population can persist for long time in the sea floor. Monitoring programs for cyst abundance determination in coastal areas are fundamental to prevent, minimize and forecast damages by potential toxic blooms. Resting stages of marine HAB species can survive in ballast waters that are loaded at source port, transported over long distances and discharged at destination port, representing a major vector for introduction and dispersal of those species. The aim of this study was to develop and apply a rapid, specific and sensitive molecular assay based on the qPCR (quantitative PCR) for the detection and enumeration of toxic dinoflagellate cysts in sediment samples collected from harbour mud during the survey activity in the Adriatic ports within the EU Interreg Project BALMAS. One of the main purpose of this project was to identify and control the discharge of non-indigenous and noxious organisms through ballast waters of ship cargos. Cyst quantification by the qPCR assay was also compared with the traditional microscopy determinations. The molecular standard curves of various target species allowed obtaining the rDNA copy number per cyst. The analytical sensitivity was set at 2 or 10 rDNA copy number per reaction. This assay revealed to be a highly sensitive and specific method for toxic dinoflagellate resting cysts detection and quantification in a complex matrix, such as marine sediments of ports also allowing the detection of cyst abundance at concentrations that are higher or not detectable by light microscopy. These molecular tools coupled with efficient monitoring through accurate and more adequate sampling will be fundamental to manage and prevent health and economic risks related to the toxic and non-indigenous species blooms, potentially transported by ballast waters, in coastal areas.

Disclosure of Interest: None Declared

Mitigation of HABs

P-311

Effect of different algicidal on the viability of *Alexandrium catenella* at laboratory conditions

M. Seguel [1,*], D. Varela [2], J. Paredes [2], A. Aguilera [3], C. Martinez [2], R. Alarcon [4], A. Zuñiga [5], M. Diaz [6]
[1]CERAM, Universidad Austral de Chile, [2]Centro i-mar, Universidad de Los Lagos, [3]Universidad San Sebastian, [4]Servicio Nacional de Pesca y acuicultura, [5]Subsecretaria de Pesca y Acuiucltura, [6]Atlanthium Technoligies Ltda, Puerto Montt, Chile

Abstract:

In order to find the mechanism of destruction of the vegetative cells of *A.lexandrium catenella,* to prevent the dispersion of this microalgae from geographical areas with high cells concentrations to free zones, experiments were carried out under laboratory conditions, allowing to determine the concentration of hydrogen peroxide and chlorine dioxide generated in situ and the dose of ultraviolet radiation with algicidal effect. A culture of *A. catenella* at a concentration of 2.000 cells mL^{-1} were assay to the following treatments: 4 concentrations of hydrogen peroxide (100, 200, 400 and 800 ppm) for 30 minutes; 5 concentrations of chlorine dioxide generated in situ (5, 20, 30., 50 and 75 ppm) for 2.5 and 5 minutes; and 7 doses of ultraviolet radiation, 9, 25, 37, 65, 122, 160 and 172 mJ cm^{-2}. Once the treatments were finished, the cultures were placed in flasks with culture medium, and kept in optimal growth conditions for 21 days. The parameters used to evaluate the algicidal effect on *A. catenella* were the growth rate, the maximum cells concentration and morphology of the cells. The concentrations and UV doses that produced negative growth rates and cellular destruction were: 200, 400 and 800 ppm for 30 minutes of hydrogen peroxide; 20, 30, 50 and 75 ppm for 2.5 and 5 minutes of chlorine dioxide; 160 and 172 mJ cm^{-2} of ultraviolet radiation. Despite these results, similar experiences should be evaluated in the field, to know its effects on real scales, i.e. in the fish tanks of the ships that serve for the aquaculture activity (wellboat) and in the salmon processing plants.

Disclosure of Interest: None Declared

Mitigation of HABs

P-312

Effects of immobilized algicidal bacteria on harmful dinoflagellates

Y. Wang [1,*], K. Coyne [1]

[1]College of Earth, Ocean, and Environment, University of Delaware, Lewes, USA

Abstract: *Shewanella* sp. IRI-160 is a bacterium that can secrete water-soluble compounds that inhibit the growth of harmful dinoflagellates, while not having negative impacts on other algal species. Previous research demonstrated that *Shewanella* could be immobilized on different porous matrices, including agarose, alginate hydrogels, cellulosic sponges, and polyurethane foam, allowing the diffusion of algicidal compounds into the surrounding medium. In this study, immobilized bacteria at concentrations up to 10^8 cells/ ml were prepared in agarose cubes and alginate hydrogel beads, and deployed into microcosm experiments using water samples collected during dinoflagellate blooms in two separate experiments. Dinoflagellate abundance was determined over 5 days. When using agarose cubes, results indicate the immobilized bacteria were able to control dinoflagellate blooms effectively, and showed that the growth of the dominant species, *Gyrodinium instriatum*, was inhibited significantly by the initial 24 hours of the experiment when immobilized bacteria were in the highest concentration. The relative abundance of the entire dinoflagellate community at the final time point (Day 5) was also measured using quantitative real-time PCR (qPCR), showing a significantly lower abundance of dinoflagellates in the group treated with the highest concentration of immobilized bacteria in agarose cubes than in other groups. Analysis of dinoflagellate response in the second experiment using immobilized bacteria in alginate hydrogel beads is ongoing. The eukaryotic community structure in treatment vs. control microcosms for each experiment will be examined by terminal restriction fragment length polymorphism (TRFLP). The results of this study will increase our knowledge of mitigating harmful dinoflagellates using immobilized algicidal bacteria, and its implications for coastal ecosystems.

Disclosure of Interest: None Declared

Biotechnology or adding value to toxic and harmful algae

P-313

Testing agar as substrate for growing *Gambierdiscus* spp. Preliminary results

P. Assuncao [1,*], E. Portillo [1]

[1]Biotechnology Department, Instituto Tecnológico de Canarias, Pozo Izquierdo, Spain

Abstract: Different culture medium are used for growing *Gambierdiscus* strains, such as K, f/2 and L1 medium with variation of strength and including (ex: selenio) or excluding (ex: Tris and Copper) specific reagents. Agar formula is $C_{14}H_{24}O_9$, and can be an organic carbon source for cells' nutrition or physical support for its growth. The aim of this work was to test the effect of agar included in f/10 medium (without copper) as a substrate to support the growth of *Gambierdiscus* cells. For this matter, four concentrations of agar (0.25, 0.1, 0.05 and 0.01%) were tested to evaluate *Gambierdiscus* growth of two species. *Gambierdiscus* cells were able to grow in medium with agar but cell number was not higher than the control. Cells in agar medium had a higher motility rate and where mobile until de end of the experiment (42 days), whereas cells from the control (without agar) stopped moving after 28 days. Cells grown in medium with agar produced cells with a lighter color than the control. In conclusion, *Gambierdiscus* cells were able to grow in medium with agar but the expected benefit of this component to increase *Gambierdiscus* cell number was not observed.

Disclosure of Interest: None Declared

Biotechnology or adding value to toxic and harmful algae

P-314

Biotransformation of pharmaceuticals products by bloom-forming cyanobacterial species

A. TEUCILIDE RIVERA [1], D. BUISSON [1], S. MANN [1], K. COMTE [1], A. Méjean [2,*]
[1]AVIV MCAM UMR7245, MNHN, [2]LIED, Paris, France

Abstract: Abstract: During last decades, pharmaceuticals and personal care products (PPCPs) and other emerging contaminants (ECs) have been constantly released at low concentrations (ng/L to mg/L) through sewage treatment plant (STP) which are unable to eradicate or efficiently removed them. These chemicals are known to persist in the aquatic systems as parent compounds, metabolites, or transformation products which eventually impact all the aquatic organisms. Up to now, the occurrence and the fate of these pharmaceuticals products remain underestimated and unresolved. In particular, the involvement of cyanobacteria in their biotic environmental transformations is poorly studied despite the decisive (and harmful) role of these microorganisms in the ecosystem functioning.

This study provides a unique focus on the potential bioactivity of several bloom-forming cyanobacterial species in the biotransformation of five pharmaceutical molecules (Furosemide, Diclofenac, Ranitidine, Propanolol, Carbamazépine) commonly found in freshwaters. Here we show that up to four strains (including *Leptolyngbya, Planktothrix, Oscillatoria* species) have the ability to modify the chemical initial PPCPs in several metabolites. The LC-MS/MS and NMR methods were applied to specifically determinate the chemical structure of each isolated transformation compounds.

Disclosure of Interest: None Declared

Biotechnology or adding value to toxic and harmful algae

P-315

Exploitation of the biotechnological potential of microalgae: the AlgaeCeuticals project

P. Madesis[1], N. Labrou[2], L. Cerasino[3,*], N. Salmaso[3], L. Palmieri[3], S. Martens[3], S. Fluch[4], P. F. Ruiz[5], A. M. San Martin[5], J. Mullor[6], E. Bakoula[7], M. Chatzikonstantinou[7]

[1]Institute of Applied Biosciences, CENTRE FOR RESEARCH AND TECHNOLOGY HELLAS - CERTH, Thessaloniki, [2]Department of Biotechnology, Agricultural University of Athens, Athens, Greece, [3]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy, [4]ecoduna AG | eparella GmbH, Bruck an der Leitha, Austria, [5]Centro Tecnológico Nacional de la Conserva y Alimentación, Molina de Segura, [6]Bionos Biotech S.L., Valencia, Spain, [7]Fresh Line Company, Koropi, Greece

Abstract: Microalgae and cyanobacteria represent a formidable source of metabolites: some are toxic, but many others have potential beneficial applications and are attracting a lot of interest from different industrial sectors (food, drugs, cosmetics) seeking for innovative, active and “natural” products.

Microalgae biomass represents a rich source for discovery. The potential for algae-based ingredients in the industry relies on the manipulation and targeting of ingredients to fit increasingly niche product specifications. Microalgae are exposed to extreme environment and thus have developed unique mechanism for protection. Furthermore, these organisms produce different metabolites which we need to identify and exploit in a sustainable way for the production of food, drugs and cosmetics.

The AlgaeCeuticals project (Project N. 778263, H2020-MSCA-RISE-2017) will take advantage of the native microalgae and cyanobacteria strains producing high added value products and through the application of novel omics technologies (genomics, metagenomics, transcriptomics, proteomics, enzymomics and metabolomics) as well as algae culture technologies and production of novel products. AlgaeCeuticals will screen and characterize microalgal biodiversity, develop and optimize algae culture systems, develop omics resources for algae and also develop downstream processing strategies and novel products. For this reason and in order to achieve its object, 4 academic and research centers from Greece (Centre for Research and Technology Hellas; Agricultural University of Athens), Italy (Fondazione Edmund Mach) and Spain (Centro Tecnológico Nacional de la Conserva y Alimentación) will collaborate for four years with 3 industrial R&D partners from Greece (Fresh Formula), Spain (Bionos Biotech ND) and Austria (Ecoduna AG). Through this collaboration the academic partners will work closely with the industrial R&D and form a complementary and highly competitive team that will promote transfer of knowledge and excellence to industrial partners. This will strengthen the industrial competitiveness in the field of food and cosmetics in the process of the design, development, testing of the products proposed by the project.

Disclosure of Interest: None Declared

Biotechnology or adding value to toxic and harmful algae

P-316

Evaluation of the total extract of *Heterosigma Akashiwo* on the model copepod Tisbe longicornis: Applications in salmon industry.

A. Astuya [1 2,*], L. Muñoz-Sobarzo [1], M. P. Rivas [1], J. Silva [3], A. Llanos [1], J. J. Gallardo [4]

[1]Departamento de Oceanografía, [2]Programa COPAS SUR AUSTRAL, [3]Departamento Zoologia, [4]Departamento de Ingeniería Química, Universidad de Concepción, Concepción, Chile

Abstract: Control of ectoparasite copepod infestation is one of the main challenges for salmon industry. Ectoparasites are associated with large economic losses, which are estimated reached US$350 million annually in Chile and close to US$1,500 million worldwide. In Chile, *Caligus rogercresseyi* is the ectoparasite copepod responsible for most of the losses. This copepod infestation results in a lower meat quality and the loss of the protective function of the skin, which reduces efficiency in feed conversion and growth. Currently, the industry has addressed this problem by using many chemicals for controlling infestations. However, the development of resistance, the high treatment costs and the negative effect on the environment due to the persistence of these compounds has prompted the industry to seek new strategies. Previous works focusing on biological relation of raphidophytes and copepods -which could feed on them- leaded to the conclusion that this kind of microalgae use their toxicity as a protection measure. For instance, our group has observed inhibition *in vivo* in *Artemia salina* and *Tisbe longicornis* using a strain of *Heterosigma akashiwo*. *T. longicornis* is used as a model copepod in ecotoxicology studies and has also been used to test control drugs for caligus-like ectoparasites. As a result, we now have a functionally-characterized crude extract capable of interrupting its growth (by inhibiting the molting process). *H. akashiwo* cultures were obtained in agitated reactors and harvested in exponential phase. For exponential biomass we observed low toxicity with both *in vitro* and *in vivo* models (< 20% zebra fish) by contrast, the stationary biomass was toxic. Thus, the exponential biomass extract dissolved in DMSO showed effects on the viability of *T. longicornis*, determining a NOEC of 10µg/mL and LOEC of 20 µg/mL for all exposure times (24, 48 and 72 hours). In the bioassays with a 24-hour exposure (a later washing step was performed) survival percentage after 48h was of 100% (for 10 to 40 µg/mL), while with 80 µg/mL was 20%. On the other hand, the effects of this extract on the growth of *T. longicornis* were analyzed. The exposure of nauplii at concentrations of 20 and 40 µg/mL showed a significant decrease in the height reached with respect to control individuals (24, 48 y 72 hours of exposure). This effect proved to be persistent since it is maintained after the washing step. Together, these results would indicate that the evaluated raphidophyceae microalgae extract provides an opportunity to create a novel natural solution for the salmon industry.

Disclosure of Interest: None Declared

Biotechnology or adding value to toxic and harmful algae

P-317

Stable isotopic labelling of marine biotoxins through small scale culturing using 13C labelled sodium bicarbonate and carbon dioxide

S. Burrell [1,*], J. Kilcoyne [1], R. Salas [1], U. Tillmann [2], C. O. Miles [3]

[1]Marine Institute, Galway, Ireland, [2]Alfred-Wegener-Institut für Polar und Meeresforschung, Bremerhaven, Germany, [3]National Research Council, Halifax, Canada

Abstract: The accumulation of biotoxins in shellfish, produced by some species of phytoplankton can have a serious effect on human health and can cause significant economic hardship to producers through closures. The EU regulated reference method for the analysis of azaspiracids (AZAs) and okadaic acid (OA) group toxins is based on LC-MS/MS, a highly sensitive and selective technique. However, LC-MS/MS can be prone to matrix interferences that can be problematic where accurate quantitation of the target analyte is required, particularly when dealing with complex matrices, such as shellfish. The use of isotopically labelled standards would correct for the inaccuracies caused by these interferences. Currently, there are no suppliers of isotopically labelled internal standards for marine biotoxins, despite the advantages this technique provides.

The isotope ^{13}C was selected for labelling due to the high number of C atoms in the target compounds, AZA1, AZA2, OA and DTX1. Labelling was performed using the producing organisms, *Azadinium spinosum* (AZAs) and *Prorocentrum lima* (OA and DTX1) under conditions enriched with ^{13}C. The cultures were grown in a modified glove box, purged of atmospheric CO_2 and replaced with carbon-free purified air and $^{13}CO_2$, using a re-circulation pump fed into both culturing chambers. To minimise further sources of ^{12}C, decarbonated seawater was used. The growing medium was enriched through the use ^{13}C labelled sodium bicarbonate. Preliminary results using the ^{13}C labelled sodium bicarbonate with no $^{13}CO_2$ showed approximately 12% uptake by the molecule.

Disclosure of Interest: None Declared

Biotechnology or adding value to toxic and harmful algae

P-318

The Algal Resources Collection: an asset for the cultivation, maintenance, and identification of toxic microalgae

C. Wingert [1,*], R. York [1], C. Tomas [1], C. Alves-de-Souza [1]

[1]Algal Resources Collection, MARBIONC at Crest Research Park, University of North Carolina Wilmington, 5600 Marvin K. Moss Lane, Wilmington, NC 28409, USA

Abstract: The Algal Resources Collection (ARC) was formally established as a public service collection in September 2016, although it had been operating as a private research collection since its initial inception in 1987. Our main goal is to be a resource for both the HAB research community and biotechnology endeavors. Presently, the collection is a major supplier of toxic algal strains for researchers all over the world, with many collaborations, loans, and exchanges initiated each year. Focused on the growth and maintenance of toxic microalgae, the ARC currently houses 412 strains distributed across 10 taxonomic groups, 50 genera, and 102 species. Although the majority of the collection is comprised of Dinoflagellates (63%) and Raphidophytes (23%), other taxonomic groups are also represented, including Haptophytes (7%), Cryptophytes (2%), Dictyochophyceans (1%), Diatoms (1%), Cyanobacteria (1%), Eustigmatophyceans (1%) and a few others (1%). ARC strains originate from diverse temperate and tropical locations around the globe and about 80% of our cultures have been classified as harmful taxa with known toxin production, found in association with toxic species, or related to known toxin producers. These include type cultures used in the description of new species and a new algal class currently under description. ARC strains have been used in various applied research studies, some of which led to the development of advanced bioassays used for detecting and quantifying marine algal toxins. ARC strains have also been the source from which new bioactive compounds have been discovered and utilized to produce new pharmaceuticals that now treat or cure a variety of diseases. The collection was recently awarded a National Science Foundation (NSF) grant that will facilitate the necessary adjustments towards its consolidation as a research resource and to achieve the self-sustainability essential for its maintenance and expansion. An overview of the species diversity, photobioreactor culturing, and collaborative opportunities will be presented.

Disclosure of Interest: None Declared

Epidemiology, reports of human outbreak cases

P-319

Records of Harmful algal poisoning symptoms in coastal communities of Nigeria, West Africa

M. O. Kadiri [1,*], O. Omoruyi [1]
[1]University of Benin, University of Benin, Benin City, Nigeria

Abstract: A socio-economic study was conducted along Nigerian coast (latitude 4°10′ to 6°20′ N; longitude 2°45′ to 8°35′ E and situated in the Gulf of Guinea within the Guinea Current Large Marine Ecosystem), using questionnaires and interview, to investigate the health symptoms of harmful algae experienced by coastal communities on consumption of sea foods. Eighteen symptoms were recorded. Of the respondents who experienced symptoms after consumption of sea foods, overall, more people (33.5%) experienced vomiting as a symptom, followed by nausea (14.03%) and then diarrhea (13.57%). Others were headache (9.95%), mouth tingling (8.6%) and tiredness (7.24%).The least were muscle pain, rashes, confusion, chills, burning sensation, breathing difficulty and balance difficulty which represented 0.45% each and the rest (dizziness, digestive tract tumors, itching, memory loss, & stomach pain) were less than 3% each. In terms of frequency, the most frequent symptom was diarrhea (87.5%), followed by vomiting (81.3%), tiredness (75%), nausea (62.5%) and headache (50%). Others such as dizziness, Itching, memory loss, mouth tingling and stomach pain had ≤40% occurrence. The least occurring symptoms were muscle pain, rashes, confusion, chills and balance difficulty and burning sensation occurring only once i.e 6.3%. Examining the seasonal influence on visible symptoms revealed that vomiting occurred more in the month of January with 5.5%, while headache and itching were predominant in October with (2.8%). Nausea had 3.1% in January than any season of the year, and 2.6% of the entire respondents opined to have experienced diarrhea in October than in any other season of the year.

Disclosure of Interest: None Declared

Epidemiology, reports of human outbreak cases

P-320

HAB in the Aysen region of Chile: a challenge for public health

A. Rivera Belmar [1,*], M. A. Tocornal Ríos [2]

[1]Food and Nutrition, Ministry of Health, Santiago, [2]Laboratorio de Salud Pública, SEREMI de Salud Región de Aysén, Puerto Aysén, Chile

Abstract: *Alexandrium catenella* is the main responsible microalgae of Paralytic Shellfish Poisoning (PSP) in Chile and is, therefore, an important concern of the authorities since it affects humans and animals, as well as some seafood. As is widely known, the consumption of seafood contaminated with STX-like is the cause of paralytic shellfish poisoning, whose clinical picture ranges from mild symptoms such as tingling in the mouth and extremities to patient death due to respiratory paralysis. Considering the role of the Ministry of Health of Chile in the protection of the health of people, in 1995 the National Program for the Prevention and Control of Poisoning by Harmful Algal Blooms (PNFAN) was implemented. Its application has been successful, achieving a remarkable level of food safety. Due to the lack of antidotes for the treatment of intoxications and the lack of models to predict the appearance, duration and place of occurrence of these blooms, the public policies implemented are based on the prevention of poisoning by consumption of contaminated seafood products. During the summer of 2018, the Aysen region was affected by a large bloom of *A. catenella*. The rapid progression and toxicity recorded put the Chilean prevention system to test. PSP values over the permitted limit (80 µg STX eq./100g meat) were recorded in almost all the phytoplankton and marine toxin monitoring stations in the region. During the event, the Ministry of Health was notified of 11 cases of contaminated seafood consumption. Patient presented symptoms ranging from mild to severe, although without fatal consequences. On January 29th, the regional PNFAN station in the Aysen region reported a result of 143,130 µg STX eq./100g of meat in mussels (*Mytilus chilensis*) located in the Ninualac Channel, Melchor Island. This result is the highest value recorded in Chile since the program was executed. Given the obtanined results, the Health Authority of the region of Aysen issued a resolution banning the extraction and marketing of all seafood and called on citizens to apply sanitary and self-care standards, both to consumers and to those who extract, elaborate and commercialize these products, in order to prevent poisonings by PSP. The contingency plan included communication and dissemination actions, response from the public and private healthcare network, epidemiological surveillance actions and control measures which included certification of suitability for consumption of all seafood marketed in the region by the Laboratory of Public Health of the Sanitary Authority, increase in the frequency of sampling and intersectoral coordination for access to isolated areas. The absence of fatal cases confirms the importance of maintaining quality surveillance programs that protect consumers, since a FAN of these characteristics could cause great mortality in the exposed population.

Disclosure of Interest: None Declared

Socio-economic impacts of HABs

P-321

The impact of algae bloom on drinking water treatment and distribution

L. Vanysacker [1,*], I. Keupers [2], R. Jenne [3], L. Verdickt [3]
[1]Research and Development, [2]Resource and Environment, [3]Water Technology, De Watergroep, Brussel, Belgium

Abstract: De Watergroep is a major water company of Flanders (northern part of Belgium), producing around 155 million m^3 of high quality drinking water every year for 2.8 million inhabitants. In addition to 57 groundwater extraction plants, De Watergroep uses surface water as a drinking water source. Due to the high population density and the high level of industrial and agricultural activities, there is an increase in water stress in Flanders, especially in the western part where most of the surface treatment plants are located. Last year, after a long period of drought, an increase in salinity was observed in different watercourses. A drinking water treatment plant located in West Flanders, “De Blankaart”, treats lowland water that is stored in a reservoir of 63 hectares by means of conventional treatment techniques. Yearly, the reservoir is affected by different algae blooms. Last summer, intake of fresh water into the reservoir was impossible due to a long period of drought, which was accompanied by high concentrations of pesticides, high load of nutrients and an increase in salinity in the raw water sources. As a result the level in the water basin dropped to 1.5m and a high *Microcystis* bloom was observed. In this period, high concentrations of toxins were found in the untreated water. In addition, different taste and odour compounds like MIB and geosmin were found in the treated and distributed water. The *Microcytis* bloom had a serious impact on the water treatment. To prevent breakthrough of turbidity through the decantation and rapid sand filtration steps, the coagulant dose ($FeCl_3$) was almost doubled and polydadmac was used as a coagulant aid. In addition, powdered activated carbon was dosed prior to the decantation step to solve the odour problem. Increased monitoring of the plant resulted in additional personnel costs. At the moment the cost of this bloom is estimated to be as high as over €500,000. In parallel with the large scale treatment plant, a 50 m^3/h pilot plant is operated to investigate the combination of ion exchange and coagulation/flotation. In contrast to the full scale decantation plant, the pilot scale flotation plant did not require an increased coagulant dose to achieve optimal turbidity removal during algae bloom. However, the presence of a high level of autochthonous natural organic matter (NOM) resulted in an elevated DOC content of the flotate. The autochtonous NOM was removed poorly by the ion exchange process. With this abstract, we would like to demonstrate the important negative impact of algae blooms on drinking water production, mainly the stress it causes on water treatment facilities and the consequences in terms of customer satisfaction and complaints. Moreover, we expect that due to climate change, i.e. increase in temperature and longer drought periods, the algae problems will increase.

Disclosure of Interest: None Declared

Socio-economic impacts of HABs

P-322

Traditional preparation methods to examine the distribution of Paralytic Shellfish Toxins in whole and partial butter clams

M. Peacock [1,*], R. Hunter [1], M. Hatch [2]

[1]Salish Sea Research Center, Northwest Indian College, [2]Huxley College of the Environment, Western Washington University, Bellingham, USA

Abstract: The Salish Sea is home to numerous shellfish species which are harvested for commercial, recreational, subsistence, and cultural uses. Shellfish bioaccumulate biotoxins which can cause harm to human health. Paralytic shellfish toxins (PSTs) and *Alexandrium* spp., the marine phytoplankton that produce them, are regularly present in the Salish Sea, with the earliest written record from 1793. Indigenous people have experienced the effects of PSTs and other biotoxins for millennia. Traditional Indigenous knowledge and practices used to reduce biotoxin exposure include seasonal timing of harvest, observation of wildlife, and shellfish preparation methods. To further reduce the risk of shellfish poisoning, Coast Salish People traditionally prepare and clean butter clams (*Saxidomus gigantea)* by cutting off the tip of the siphon and cleaning out the stomach of the clam before eating. Scientific literature also indicates that PSTs are more likely to be sequestered in the tip of the siphon of the butter clam. In June 2017, butter clams were dug from Birch Bay State Park, WA, USA during a Washington Department of Ecology PST closure of this area, and tested for PSTs using Enzyme-Linked Immunosorbent Assay. Some clams were analyzed for PSTs by homogenization of the whole clam, while others were split into siphon tip, siphon neck, gills, digestive gland, and the rest of the clam to identify if removal of the siphon tip and cleaning of the stomach reduces human exposure to PSTs. The siphon tip and gills had the greatest toxic load per gram, but when scaled to the whole clam weight accounted for 18.2% +/- 7% of each clam's toxic load. Full removal of the neck (including the tip), gills, and digestive gland accounted for ~30% removal of the toxic load. Climate change, agricultural and urban runoff, and drought events are increasing the likelihood of shellfish exposure to biotoxins, which can have a disproportionate impact on subsistence harvesters. This is a serious food sovereignty and security issue for people who rely on shellfish and needs to be forefront in conversations about harmful algae mitigation.

Disclosure of Interest: None Declared

Integrated ecosystem assessment and foresight planning (scenario building, HABs and multi-use offshore platforms, HABs and desalination…)

P-323

Integrating various data products to predict risk and impact of HAB events on the Aquaculture Sector (PRIMROSE)

J. Silke [1,*], W. Schmidt [1], L. Hastie [1], J. Maguire [2], K. Davidson [3], R. Henderson [4], P. Miller [5], M. Sourisseau [6], M. Mateus [7], M. Ruiz [8], L. Maman [9], L. Ferrer [10]

[1]MEFSS, Marine Institute, Oranmore, [2]Indigo Rock, Bantry, Ireland, [3]Scottish Association for Marine Science, Oban, Scotland, [4]Seafood Shetland, Lerwick, Shetland Islands, [5]Plymouth Marine Lab, Plymouth, United Kingdom, [6]IFREMER, Brest, France, [7]Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal, [8]Instituto Español Oceanografía, A Coruna, [9]Agencia de Gestion Agraria y Pesquera de Andalucía, El Rompido, Huelva, [10]Fundación AZTI, Sukarrieta, Spain

Abstract: The future supply of fish according to the most recent projections from the OECD-FAO 2014 will derive more from aquaculture (Agricultural Outlook 2013-2022). This will demand responsible and sustainable approaches to its economic development. Every year approximately 1.25 million tonnes of products are produced by aquaculture in the EU, much of it in the European Atlantic Arc. Aquaculture has led to the establishment of more than 14,000 local enterprises in the EU, 90% of which are micro-enterprises with less than 10 employees. The negative impact on the industry of human illness is indiscriminate, and given the uncertainties insurance is often not available. Vulnerable coastal communities require better governance and management of their livelihood, this includes the empowerment of concerned communities through the provision of better forecasting of impending threats. A newly established project funded by Interreg Atlantic Area (PRIMROSE) will build on existing forecasting systems conceived developed and implemented by the project consortium.

PRIMROSE will deliver:

- Improved forecasts with increased resolution, combining ocean models with satellite Earth observation data.
- A wider suite of parameters.
- New index based risk assessment.
- Encompass aquaculture from Shetland to Canary Islands.

The long terms effects of the project will be the improvement of the economic resilience of the Aquaculture industry within European Atlantic Arc region to HAB and pathogen impacts. This will be achieved through cross border collaboration and methodology exchange leading to early warning risk methodologies and alerts that produce: a) A reduction in the risk of shellfish toxicity to humans; b) A reduction in the business impact/losses though either shellfish product harvesting delays or product recalls and c) The opportunity to take mitigating action and prevent potential farmed fish kills.

Disclosure of Interest: None Declared

Optical sensors and drone systems for the monitoring of harmful blooms

P-324

Detection by drone of eutrophication of freshwater

L. BRIENT [1,*], Y. LE MEDEC [2], S. DUFOUR [3], A. COMAR [4], E. VENTURINI [5]

[1]UMR ECOBIO, University of Rennes 1, RENNES, [2]Minyvel-Environnement, PORNIC, [3]UMR6554 LETG, University of Rennes 2, RENNES, [4]HIPHEN-PLANT, AVIGNON, [5]UMR 6554 LETG, University of Rennes 2, RENNES, France

Abstract: Drones and the development of new sensors at specific wavelengths can provide information for day-to-day and more or less long-term management.

At the single pixel of the classical sample is obtaining a image with a million pixels that integrates a multitude of samples.

This digitization, realized at low level, supplants the human eye and its limits in the current cartography, for example those of satellite images dependent on cloud cover or the difficulty of accessing lakes and ponds or that of aquatic macrophytes that are indicators of eutrophication and whose measurements remain very subjective. Aerial drones, equipped with sensors at specific wavelengths, indicate a trophic status of the whole lake by detecting phycocyanin for cyanobacteria and/ or chlorophyll for macrophytes and phytoplankton. This technology determines new health management tools for the recreational activities, drinking water production and ecology. Nautical drones, equipped with sensors fixed to a winch, measure and collect in the water column enslaved to the measurement of the sensors. They determine the areas of development of cyanobacteria in the water column and other information becomes available to further understand their ecology in lakes. The extent of eutrophication in 3D of a water body by automaton becomes accessible.

Disclosure of Interest: None Declared

Optical sensors and drone systems for the monitoring of harmful blooms

P-325

A platform for real time observation of the diversity, dynamics, and vertical distribution of harmful algae

M. Brosnahan [1,*], B. Karlson [2], D. Anderson [1], F. Correia [1], D. Ralston [3], R. Olson [1]

[1]Biology, Woods Hole Oceanographic Institution, Woods Hole, MA, USA, [2]Oceanographic Research, Swedish Meteorological and Hydrological Institute, Västra Frölunda, Sweden, [3]Applied Ocean Physics and Engineering, Woods Hole Oceanographic Institution, Woods Hole, MA, USA

Abstract: Real-time monitoring technologies for harmful algal blooms (HABs) promise to transform the management of coastal resources and aquaculture. Several technologies are now available that can provide continuous, automated descriptions of phytoplankton abundance, dynamics, and toxin content. Through deployments of support platforms that host combinations of these sensors, species diversity, vertical distribution, and dynamics can be described in near real-time and in extraordinary detail. Here we describe one such observatory platform that has been used to couple two of these next generation plankton sensors - the Imaging FlowCytobot (IFCB) and Environmental Sample Processor (ESP) - with a continuously profiling conductivity-temperature-depth (CTD) logger. The system has been assembled and operated at two locations – Salt Pond, a drowned kettle pond within the Nauset Marsh estuary (Eastham, MA USA) and a mussel farm in Tångesund, Sweden. Essential elements are its ability to provide power, Internet connectivity, and remote management/repositioning of sensors. The subsystems addressing these needs have been developed from commercial off the shelf components over 7 years, iteratively improving the system's overall capability and reliability through mission- and site-specific modifications. Data collected by this platform point to new opportunities for improved biotoxin safety and aquaculture management. These opportunities and ongoing development of the platform are described.

Disclosure of Interest: None Declared

Optical sensors and drone systems for the monitoring of harmful blooms

P-326

Application of Current Technologies for the Detection and Monitoring of Toxins in Marine Environments

J. Sreekumar [1,*], S. Taylor [1 2]
[1]Q-Technologies Ltd, [2]University of Liverpool, Liverpool, United Kingdom

Abstract: With the growing concern and pressure to prevent, control and mitigate the chemical and microbiological compounds in marine environment, technologies are involved in various stages from prediction to mitigation of harmful species in water. To aid in this process, analytical systems are commonly used to measure the amount of toxins that is present in the source water. Harmful algae blooms (HABs) in water can produce toxins that could harm humans, marine organisms and ecosystems. HABs are a global problem that significantly alters the ecosystem and suffer substantial economic losses. There is an urgent need to develop and validate rapid, reliable, cost effective and robust diagnostic tools for identification and detection of algal toxins in marine environment. However, the problem of limited measurement features of most real-time HABs monitors has restricted its use to handle different algal species.

Now, a reliable, truly portable, field measurement instrument capable of fluorometric technique has been developed for both solvent extraction and direct measurements of algal toxins in marine environment. An innovative arrangement in ALGAE-600P capable of providing accurate and precise measurements, and offers the flexibility to detect wide range of toxins type. The instrument is capable to distinguish and quantify HABs by analysing their fluorescence spectra. We also supply the AquaMMS analyser for Volatile Organic Compounds (VOCs) analysis. This instrument technology uses mass spectrometry technique to allow monitoring of organic contaminants in water to an extremely high sensitivity. As a mass spectrometric technique, the technique is information rich and provides both qualitative data (mass) and quantitative data (concentration) of toxins in water. Implementation of these techniques would help the operators to make a corrective treatment decision on time.

ALGAE-600P analyser provides accurate and timely information on the occurrence of different species without a need of re-calibration. Powerful HexaSoft interactive software builds intelligence and decision-making capabilities into the instrument. All data processing is performed in the analyser touch screen panel. AquaMMS and ALGAE-600P analysers have been designed for both field and laboratory bench top analysis.

Disclosure of Interest: None Declared

Optical sensors and drone systems for the monitoring of harmful blooms

P-327

SURVEILLANCE OF CHANGING BLOOM CONDITIONS THROUGH THE APPLICATION OF OPTICAL REMOTE SENSING AND ECOLOGICAL ASSOCIATIONS

M. C. Tomlinson [1,*], D. Dupuy [2], R. P. Stumpf [1], J. L. Wolny [3], K. S. Reece [4], T. A. Egerton [5], W. C. Holland [6], R. W. Litaker [6], M. E. Ondrusek [7], W. K. Vogelbein [4], A. R. Place [8]

[1]NOAA National Centers for Coastal Ocean Science, Silver Spring, MD, [2]CSS-Dynamac, Fairfax, VA, [3]Maryland Department of Natural Resources, Annapolis, MD, [4]Virginia Institute of Marine Science, Gloucester Point, VA, [5]Virginia Department of Health, Richmond, VA, [6]NOAA National Centers for Coastal Ocean Science, Beaufort, NC, [7]National Environmental Satellite, Data, and Information Services, NOAA, College Park, MD, [8]University of Maryland Center for Environmental Sciences, Baltimore, MD, USA

Abstract: High frequency monitoring using satellite remote sensing has been beneficial to protecting public and environmental health in several key US waterways. In most instances, the success of these tools relies on strong optical signatures of some high biomass harmful algal bloom species (HABs). The near monospecific nature of blooms, like *Karenia* in the Gulf of Mexico and *Microcystis* in Lake Erie, make them suitable for optical detection. In southern Chesapeake Bay, monospecific blooms of *Alexandrium monilatum* and *Margalefidinium polykrikoides* are also amenable to detection with satellite data. In contrast, a majority of blooms in northern Chesapeake Bay occur as mixed assemblages that are optically complex, making it difficult for direct detection of individual species. However, these mixed assemblages may produce a unique signature if properly validated. An approach using optical detection combined with ecological associations may aid in further classification of blooms (e.g., *Heterocapsa rotundata* blooms during the winter). This presentation demonstrates how fluorescence and high biomass algorithms applied to the 300 m Ocean and Land Colour Imager (OLCI) on the Sentinel-3 satellite have improved our ability to detect and characterize algal blooms at higher resolution. Statistical models constructed with information regarding the ecological niche of individual species (time of year; bloom succession; salinity, temperature and nutrient regimes), combined with remote detection of blooms can potentially provide a real-time monitoring system for HAB events in Chesapeake Bay. This type of integrated system would be a valuable tool for resource managers responsible for safeguarding fisheries and public health within the region.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

P-328

Harmful algal bloom events in the Philippines: temporal and spatial trends

A. Yñiguez [1,*], R. Azanza [1]

[1]Marine Science Institute, University of the Philippines Diliman, Quezon City, Philippines

Abstract: As we experience changes in climate together with localized environmental shifts, it becomes more important to understand how these hierarchy of factors potentially affect the occurrence of harmful algal blooms. Long-term time series data is an essential component to help tease out these patterns. In the Philippines, HAB event records spanning the years 1983 – 2016 were used to investigate trends in harmful algal bloom events. These records come primarily from the national monitoring program of the Bureau of Fisheries and Aquatic Resources (BFAR) supplemented with published literature. While the BFAR data represent only the occurrence of a shellfish ban, it is the only information available in the long-term and on a larger spatial scale. These were recorded as standardized data within the HAEDAT information system. Twenty sites were used to represent all areas where HAB events have historically occurred. Most of these HAB events have been due to the bloom of the toxic dinoflagellate *Pyrodinium bahamense* var. *compressum.* Overall, the frequency of HAB events as well as the duration of these events do not seem to be increasing through time, although there has been an increase if data were partitioned into before and after the mid-1990s. Patterns of periods of high and low occurrences can be observed and are explored relative to environmental and climatic variations. More sites though are being affected by HABs through time. As recently as 2015, a new site had a HAB event. The most commonly affected areas have spanned the country from Zambales up north to southern Mindanao in Zamboanga del Sur.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

P-329

Blooms of Gymnodinium catenatun and *Cochlodinium catenatum* in Santa Elena Bay, Ecuador: importance of implementing monitoring programs

G. Torres [1], S. Recalde [1], M. Borbor-Cordova [2], O. Carnicer [3,*]

[1]Instituto Oceanográfico de la armada, [2]Escuela Superior Politécnica del Litoral (ESPOL), Guayaquil, [3]Escuela de Gestión Ambiental, Pontificia Universidad Católica del Ecuador, Sede Esmeraldas, Esmeraldas, Ecuador

Abstract: Harmful algae blooms are a symptom of the imbalance being suffered by marine ecosystems caused, in many cases, by environmental changes arising from anthropogenic impacts. In the Ecuadorian coasts, these proliferations have been mainly registered in the southern half, in the Gulf of Guayaquil and Santa Elena peninsula. In this regard, this study presents additional data collected in two aquaculture hotspots located in Santa Elena Bay. Samples of surface water fixed with lugol were collected the 25th March in Salinas-La Libertad beach (8 samples) and the 16th April 2018 in San Pedro beach (2 samples). Microalgae were counted under inverted microscope in 25 mL Utermohl chambers. Results highlights the presence of high abundances of the dinoflagellate *Gymnodinium catenatum* (from $9x10^3$ to $1.6x10^6$ cell. L^{-1}) in Salinas-La Libertad, registered for the first time in the area. The bloom observed in San Pedro beach correspond to *Cochlodinium catenatum* (from $1.2x10^5$ and $1.8x10^5$ cell. L^{-1}). These episodes occurred at 23.83°C and 23.16°C respectively, which are temperatures colder than usual for this period. This is confirmed by the ONI Index of -1.27, in the region NINO 1+2, that is linked to La Niña event for this year. Fish mortality was not registered during the presence of HABs. There is evidence of higher abundances of these species during warm and rainy season (February-April), observed in the monitoring programme performed by INOCAR since 1992 at 10 miles distance from the coast. However, there is scarce data from coastal areas in the country that do not count with national legislation regarding monitoring vigilance for marine aquaculture. Occurrence of *G. catenatum* (saxitoxin and analogues producer) is a matter of concern since blooms are involved in negative impacts on marine ecosystem, human health and local economy. Therefore, it is necessary the implementation of suitable monitoring strategies programs involving the quantification of toxic species of phytoplankton as an early stage. Further investigations need to be performed in order to design preferential locations to avoid potential economic, ecological and sanitary impacts related to seafood safety in the aquaculture sector.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

P-330

CIGUA-PIRE Advancing Global Networks for Ciguatera in Tropical Oceans through Partnerships in International Research and Education

A. Robertson [1,*], D. M. Anderson [2], A. Capper [3], L. L. Chan [4], D. Erdner [5], C. Faeste [6], C. A. Hernandez [7], K. Heck [8], M. Huggett [9], G. Hyndes [10], L. Ivanova [6], P. Lavery [10], P. McCarron [11], K. McMahon [10], C. Miles [11], M. Miller [8], M. L. Parsons [12], M. L. Richlen [13], F. Rise [14], I. A. Samdal [6], M. Sandvik [6], T. B. Smith [15], S. Uhlig [6]

[1]Marine Sciences, Dauphin Island Sea Lab & the University of South Alabama, Dauphin Island, [2]Biology, Woods Hole Oceanographic Institute, Woods Hole, USA, [3]Marine Sciences, International Centre for Island Technology, Heriot Watt University , Orkney, United Kingdom, [4]State Key Laboratory in Marine Pollution , City University of Hong Kong, Kowloon, Hong Kong, [5]University of Texas Marine Sciences Institute, Port Aransas, USA, [6]Norwegian Veterinary Institute, Oslo, Norway, [7]Center for Environmental Studies of Cienfuegos, Ministry of Science, Technology, and the Environment of Cuba , Cienfuego, Cuba, [8]Dauphin Island Sea Lab , Dauphin Island, USA, [9]University of Newcastle, Newcastle, [10]Edith Cowan University, Joondalup, Australia, [11]National Research Council, Measurement Science and Standards, Halifax, Canada, [12]Florida Gulf Coast University, Fort Meyers, [13]Woods Hole Oceanographic Institute, Woods Hole, USA, [14]Chemistry, University of Oslo, Oslo, Norway, [15]University of the Virgin Islands, St. Thomas, Virgin Islands, U.S

Abstract: Coral reef ecosystems are among the most biodiverse habitats on earth, providing societal benefits and fishery resources critical to coastal communities. Ciguatera is a significant threat to utilizing these resources. While the mechanisms and environmental drivers of toxigenic phenotypes of the *Gambierdiscus* spp. remain largely unknown, many other toxigenic algae live in the same community and capable of producing toxic metabolites that can enter the food web. The elucidation of these metabolites is critical to the development of monitoring approaches for public health protection. Management strategies for CFP lag behind other seafood safety issues due to four major deficiencies: 1) the inability to easily identify and monitor for the toxigenic Gambierdiscus species responsible for CFP; 2) a lack of data on the toxicity and structure of secondary metabolites produced by benthic micro-algae in some regions; 3) a limited understanding of the food web dynamics and biotransformations of these metabolites; and, 4) the inability to predict when and where CFP outbreaks are most likely to occur. Based on these interlinked needs, we have developed a large cross-regional partnership in collaborative research and education (PIRE) funded by the National Science foundation, Research Council or Norway, and others. The overarching hypothesis is that toxigenic benthic dinoflagellates produce a stable suite of secondary metabolites (meta-metabolome) allowing them to prosper in an otherwise unstable environment driven by shifts in the epiphytic flora, available substrates, marine biota, and environmental conditions. Moreover, the stability and advantages gained in this meta-metabolome are universal across toxigenic genera and applicable across (sub)tropical reef environments. CIGUA-PIRE will approach this across multiple levels of biological organization and in a cross regional and interdisciplinary research and education program. PIRE links with the community and provides exchange opportunities to middle-school students, undergraduates, graduates, and postdoctoral scientists. Scientists, educators, and stakeholders from the US, Canada, Norway, Hong Kong, Australia, Cuba, Canada, United Kingdom are engaged in this network and has extended to collaborations in the Philippines, France, Vietnam, Nigeria, with many opportunities for others via educational and harmonization workshops. The purpose of these connections is to build knowledge and leverage infrastructure and resources so that we can collectively develop best practices and harmonize strategies in risk assessment models and methods for ciguatera based on the best available and integrative science. This program addresses the global nature of CFP by working across hyper-endemic regions, and develops unique and lasting educational experiences for students in the US and abroad.

Disclosure of Interest: None Declared

P-331

Overview of New Zealand HAB species and HAEDAT Events, with a comparison to Australian events

E. Jaffrezic [1 2,*], L. Rhodes [1], L. Mackenzie [1], G. Hallegraeff [3]

[1]Cawthron Institute, Nelson, New Zealand, [2]Institut Universitaire Europeen de la Mer, Plouzane, France, [3]University of Tasmania, Hobart, Australia

Abstract: As part of the project for a Global Harmful Algal Bloom Status Report, and to complete missing data for the Australasian region, New Zealand harmful algae blooms (HAB) events with negative impacts on human society have been added to the IOC-ICES Harmful Algae Events Database (HAEDAT). The New Zealand occurrences of toxic microalgae species appearing on the reference list of IOC-UNESCO have also been catalogued. All these data come from the published literature. The similarities and differences of HABs and toxic species between Australia and New Zealand are reported and key factors highlighted to demonstrate differences between the two countries. For example, the differences in phytoplankton and biotoxin monitoring efforts and utilisation of coastal waters for aquaculture are presented. The first referenced New Zealand HAB event was a 'slime event ' in the 1860s. Since then, many harmful and/or toxic events with varied impacts have occurred in New Zealand coastal and freshwater areas. One of the biggest toxic events, in terms of distribution, number of reported illnesses, and economic losses, was an unexpected neurotoxic shellfish poisoning (NSP) event at the beginning of 1993 in the Hauraki Gulf. Since this event, weekly monitoring of harmful phytoplankon and biotoxins has been carried out. Overall, there are 110 Australasian HAB events catalogued in HAEDAT, 65 for Australia and 45 for New-Zealand. New Zealand differs from Australia in the number of 'slime events' which have occurred in Tasman Bay (mainly due to a *Gonyaulax* sp.) and Hawke Bay during the 1900-1960 period. In New Zealand, paralytic shellfish poisoning (PSP) is a predominant syndrome and has caused 35 human illnesses; PSP has also caused either illnesses or shellfish harvesting closures in Australia. NSP reports, including illnesses caused by *Karenia brevisulcata* aerosols, have reached 617 reported human illness in New Zealand, while none NSP event have been reported in Australia. In Australia, ciguatera fish poisoning (CFP) is the major reported syndrome and has caused 96 human illnesses, but no CFP has been recorded from consuming New Zealand fish. Also in Australia, two hundred diarrhetic shellfish poisoning (DSP) illness events have been recorded whereas in New Zealand only four illnesses have been recorded, probably due to the extensive monitoring.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

P-332

RAMOGE, an International Agreement in the NW Mediterranean Sea with a working group dedicated to *Ostreopsis* sp.

R. Lemee [1,*], E. Berdalet [2], M. Chiantore [3], L. Mangialajo [1], A. Vissio [4]

[1]LOV, Villefranche-sur-Mer, France, [2]CSIC, Barcelona, Spain, [3]DisTAV, Genoa, Italy, [4]RAMOGE, Monaco, Monaco

Abstract: As early as the 1970s, Prince Rainier III of Monaco set up a pilot zone for the protection of the marine environment in order to co-ordinate joint actions to limit marine pollution in the boundary area between Italy, Monaco and France. This initiative, materialized on May 10, 1976, through the official signature of the «RAMOGE Agreement» by three governments: French, Italian and Monegasque.

This agreement on the protection of Mediterranean coastal waters falls within the framework of the Barcelona Convention and the Action Plan for the Mediterranean Sea. The Agreement represents an instrument for scientific, technical, legal and administrative co-operation in order to implement actions to promote integrated coastal management and thus preserve the marine environment. In 2014, the RAMOGE agreement integrated Spanish scientists in order to extend its influence.

A working group, dedicated to *Ostreopsis* outbreaks development in the Mediterranean Sea, was set up in 2010. In addition to promote data exchange and strategies to monitor this benthic toxic dinoflagellate in the Mediterranean Sea, the RAMOGE-*Ostreopsis* working group supported (a) scientific meetings (International Conference on *Ostreopsis* Development, Villefranche, France, 2011; Regional Workshop on Monitoring and Management Strategies for Benthic HABs - with IAEA, GlobalHAB and NOAA-, Monaco, 2018), (b) scientific missions, such as the Mare Nostrum expedition from Gibraltar to Marmara Sea, (c) informations via scientific bibliography available at the RAMOGE website (http://www.ramoge.org), and elaboration and distribution of an outreach flyer for the general public and managers about *Ostreopsis* blooms impacts; and (d) the ongoing international exercise (2018-2019; with IAEA and GlobalHAB) in order to improve sampling methods of benthic dinoflagellates, including *Ostreopsis* but also *Gambierdiscus* species.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

P-333

Summary Report on Harmful Algal Blooms in Latin America and The Caribbean (1956-2018).

S. M. Méndez [1,*], J. E. Mancera Pineda [2], M.-Y. Dechraoui-Bottein [3], H. Enevoldsen [4], C. Alonso-Hernández [5], I. Sunesen [6], R. Alonso Rodríguez [7], O. Amaya-Monterrosa [8], R. Quintanilla [8], H. L. Carrillo-Ovalle [9], N. Arbeláez [10], M. Vargas [11], N. F. Chow-Wong [12], L. Rosario Valerio-Gonzalez [13], J. I. Mardones [14], G. Pizarro [15], L. Proenca [16], S. Sanchez [17], G. Torres [18], G. Arencibia Carvallo [19], G. Delgado Miranda [19]

[1]Dirección Nacional de Recursos Acuáticos (DINARA), Montevideo, Uruguay, [2]Universidad Nacional de Colombia, , Sede Bogotá, Colombia, [3]International Atomic Energy Agency, Environment Laboratories, Monaco, Monaco, [4]IOC Science and Communication Centre on Harmful Algae, University of Copenhagen, Copenhagen, Denmark, [5]Centro de Estudios Ambientales de Cienfuegos (CEAC), Cienfuegos, Cuba, [6]CONICET and UNLP -División Ficología, Facultad de Ciencias Naturales y Museo, La Plata, Argentina, [7]Unidad Académica Mazatlán, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Mazatlán, Sinaloa, Mexico, [8]Marine Toxins Laboratory of the University of El Salvador LABTOX-UES, Faculty of Natural Sciences and Mathematics, San Salvador, El Salvador, [9]Centro de Estudios del Mar y Acuicultura (CEMA), Universidad de San Carlos de Guatemala (USAC), Guatemala, Guatemala, [10]Instituto de Investigaciones Marinas y Costeras (INVEMAR), Santa Marta, Colombia, [11]Universidad de Costa Rica, Centro de Investigación en Estructuras Microscópicas, San José, Costa Rica, [12]Centro de Investigación y Recursos Acuáticos de Nicaragua,,Universidad Nacional Autónoma de Nicaragua-Managua (CIRA/UNAN-Managua), Managua, Nicaragua, [13]Escuela de Ciencias Aplicadas del Mar, Universidad de Oriente, Boca de Río, Venezuela, Bolivarian Republic Of, [14]Centro de Estudio de algas Nocivas (CREAN), Instituto de Fomento Pesquero (IFOP), Puerto Montt, [15]Instituto de Fomento Pesquero (IFOP), Punta Arenas, Chile, [16]Instituto Federal de Santa Catarina, Florianópolis, Brazil, [17]Instituto del Mar del Perú, Lima, Peru, [18]Instituto Oceanográfico de la Armada, Guayaquil, Ecuador, [19]Centro de Investigaciones Pesqueras (CIP), La Habana, Cuba

Abstract: The study of the historical records from 1956 to 2018 was done with the aim of compiling and for the first time to have an overview of the main impacts and trends of Harmful Algal Blooms in the Latin America and the Caribbean regions, as a summary easy to understand by decision makers, scientists and interested public. Different syndromes caused by microalgae have been identified; the most relevant for the Latin American and Caribbean region (LAC) are Paralytic Shellfish Poisoning (PSP), Diarrheic Shellfish Poisoning (DSP), Amnesic Shellfish Poisoning (ASP), and Ciguatera Fish Poisoning (CFP). This report uses data from HAEDAT (Harmful Algae Event Database), which includes HAB events recorded, by each country in Latin America and the Caribbean (LAC). HAEDAT records, based on monitoring programs, reports and scientific publications, are available online, and are managed by IODE (International Oceanographic Data and Information Exchange) as a project of the IOC - UNESCO. It is important to point out that only events that have generated any type of negative impact at a social, economic, environmental or health level are registered in HAEDAT. This base is not exhaustive to the totality of HAB events occurred in the LAC region, however, it represents an important number of historical records (265) until August 2018. Some known HAB events already published or disclosed, are not part yet of this database. A big number of records had been updated through the cooperation networks between countries in the context of the International Atomic Energy Agency (IAEA) ongoing projects. The information analysis is presented both, globally for Latin America and the Caribbean (LAC) and sub-regional. Given that the FANs in the Caribbean region have particularities regarding the causative species and the toxins produced, which differ quali and quantitatively from those registered in South America, this report analyse globally for LAC and at the same time for regions, according to with the regionalization determined by the UNESCO IOC- HAB program: FANSA (Harmful Algal Blooms of South America) and ANCA (Harmful Algal Blooms in the Caribbean).This study shows that in recent years there has been a clear trend of increase in the occurrence of harmful algal blooms in Latin America and the Caribbean. This increase is worrying considering the negative impacts of HAB. This historical analysis allows identifying the main syndromes, their geographical distribution, and their trends, as well as the main causal organisms.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

P-334

The power of comparative ecosystem approaches by the North Pacific Marine Science Organization

N. Haigh [1,*], C. Guan [2], X. Dai [2], S. Sakamoto [3], W.-A. Kim [4], T. Park [5], T. Morozova [6], T. Orlova [6], H. Enevoldsen [7], V. Trainer [8]

[1]Microthalassia Consultants, Inc., Nanaimo, B.C., Canada, [2]National Marine Environmental Monitoring Center, State Oceanic Administration, Dalian, Liaoning, China, [3]National Research Institute of Fisheries and Environment of the Inland Sea, Japan Fisheries Research and Education Agency, Hiroshima, Japan, [4]Oceanic Climate and Ecology Research Division, National Institute of Fisheries Science, Busan, [5]Southeast Sea Fisheries Research Institute, National Institute of Fisheries Science, Tongyeong, Republic of Korea, [6]National Scientific Center of Marine Biology, FEB RAS, Vladivostok, Russian Federation, [7]Intergovernmental Oceanographic Commission, UNESCO, Copenhagen, Denmark, [8]NOAA Northwest Fisheries Science Center, Seattle, WA, USA

Abstract: The North Pacific Marine Science Organization (PICES) member countries enter annual data on harmful algal bloom (HAB) occurrence, distribution and intensity into the global Harmful Algal Event Database (HAE-DAT) as part of the terms of reference for the Section on Harmful Algal Blooms (HAB-S). In a parallel effort, these member countries, including Russia, China, Japan, Korea, Canada, and U.S.A, have compiled data on HAB species occurrence in the PICES region which will be a cornerstone in the first Global HAB Status Report literature review.

HAE-DAT and the ICES-PICES-IOC Harmful Algal Event Status Report contain information on decadal maps of HAB occurrence, event duration, maximum concentrations, species, and exceptional events. Several workshops and special sessions held by the HAB-S during PICES annual meetings have made possible the comparison of HAB events in geographically distinct ecosystems. The collaborations have provided insights into the drivers of toxigenic *Pseudo-nitzschia* blooms, which are typically observed in the eastern Pacific but have not yet caused closures in the western Pacific. Likewise, a comparison of *Heterosigma* blooms in the North Pacific region have shown that there are distinct subpopulations that have optimal temperature and salinity windows in which these blooms are most toxic, providing clues regarding environmental drivers of toxicity. Such comparative ecosystem approaches will help us answer some of the toughest questions regarding drivers of HAB events and will help to facilitate clear and concise communications among scientists, policy makers, legislators and funding agencies.

Disclosure of Interest: None Declared

Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives

P-335

CoCliME: Co-development of CLimate services for adaptation to changing Marine Ecosystems – focus on the North Sea Case Study

K. Toebe [1,*], L. Arneborg [2], A. D. Cembella [1], M. Gröger [3], U. John [1 4], B. Karlson [2], L. Naustvoll [5], G. Vulturius [6], J. Joy West[7]
[1]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany, [2]Swedish Meteorological and Hydrological Institute, Sven Källfelts gata 15, 426 71 Västra Frölunda, [3]Swedish Meteorological and Hydrological Institute, Folkborgsvägen 17, 60176 Norrköping, Sweden, [4]Helmholtz Institute for Functional Marine Biodiversity, Ammerländer Heersstraße 231, 26129 Oldenburg, Germany, [5]Institute of Marine Research, Flødevigen Research Station, Nye Flødevigveien 20, 4817 HIS, Norway, [6]Stockholm Environment Institute, Stockholm Centre, Linnégatan 87D, 115 23 Stockholm, Sweden, [7]CICERO Center for International Climate Research, P.O. Box 1129, 0318 Blindern, Oslo, Norway

Abstract: The EU JPI Climate ERA4CS CoCliME project co-develops and co-produces bespoke, proof-of-concepts or prototype marine ecosystem climate services and a transferable framework for climate services development, to support informed decision making relevant to climate change-related ecological and socio-economic impacts across different coastal regions. To achieve these objectives the CoCliME consortium brings together a transdisciplinary team of natural and social scientists, decision makers, and users of climate services that will dynamically interact to identify common and priority climate change-related vulnerabilities and solutions in six European coastal areas (Atlantic, Baltic, Black, Mediterranean, North and Norwegian Seas case studies). In these areas, CoCliME focuses on coastal ecosystem status indicators, e.g. harmful algal blooms, marine biotoxins and pathogens and marine microbial biodiversity that can be markedly influenced by climate change and have direct impacts on human health, economic prosperity (fisheries, aquaculture, tourism) and social wellbeing (recreation). For this purpose available global IPCC climate change scenarios are selected and refined to adapt to the specific regional requirements of the case study area. A co-production and co-development approach to climate services identifies the information and knowledge needs of decision makers and users, and facilitates and accelerates local, national and European decision making concerning adaptation to climate change impacts. This marine ecosystem climate service framework will feed into mechanisms such as the UN Sustainable Development Goals, Marine Strategy Framework Directive, Marine Spatial Planning, national monitoring and reporting requirements, and climate adaptation planning to ensure the protection and sustainable use of Europe´s marine and coastal ecosystems for future generations. Here we define the first project results of an intensive hindcast data study, including preliminary analyses of the modelling component of the North Sea case study, with particular focus on *Alexandrium, Dinophysis* and *Pseudochattonella* species, and elements of newly developed climate services for German end-users. In addition, we present the first genomic results of a coordinated CoCliME sampling initiative in 2018. This study documents the presence or absence of potential toxigenic representatives of the benthic genera *Ostreopsis*, *Gambierdiscus*, *Coolia* and *Prorocentrum*, respectively, at different sites of the case study areas. Combining these findings with analysis of preliminary stakeholder consultations in Norway and Sweden, and assessment of ongoing HAB-related services in the region, we suggest some promising focus areas for further HAB-climate service development for the North Sea component of CoCliME.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-336

Paralytic shellfish toxin accumulation and metabolization in Sydney Rock Oysters (*Saccostrea glomerata*) selected for growth

M. Lassudrie [1 2,*], F. Caron [3], W. O'Connor [4], L. Parker [5], M. Dove [4], S. Murray [2]

[1]LER-BO, IFREMER, Concarneau, France, [2]ClimateChangeCluster (C3), University of Technology Sydney, [3]ClimateChangeCluster (C3), University of Technology Sydney, Broadway, [4]Port Stephens Fisheries Institute, NSW Department of Primary Industries, Taylors Beach, [5]School of Life and Environmental Sciences, University of Sydney, Camperdown, Australia

Abstract: The aquaculture industry can be strongly impacted by harmful algal blooms through toxin accumulation in shellfish, which become unsafe for human consumption, resulting in harvesting closures. Therefore, identifying the factors influencing toxin levels in shellfish is an important public-health and socio-economic challenge. Throughout the world, selection for growth is widely used to increase shellfish industry profitability, however, the effect of growth rate on algal toxin accumulation remains unclear.

In New South Wales, Australia, almost 30 years of selective breeding has led to the production of numerous families of Sydney rock oysters (*Saccostrea glomerata*) with different growth and disease resistance characteristics, providing an ideal opportunity to study the effect of selection for growth on toxin accumulation.

Experimental exposures to paralytic shellfish toxin (PST)-producer *Alexandrium* spp. at field-realistic concentrations were conducted to compare different *S. glomerata* phenotypes.

In the first experiment, oyster families selected for both fast growth and disease resistance (WMr: Winter Mortality resistance; and B2: dual resistance to Winter Mortality and QX) were compared with a non-selected wild oyster. While toxicity of all oyster types was similar, a difference in PST analogue composition indicated different abilities to metabolize the toxins.

In a second experiment, two oyster families with contrasted growth (slow *vs* fast growth) were analysed for PST content, and ecophysiological rates (respiration and ingestion) were assessed during the experiment. Toxicity was two-fold higher in the fast growth family and was associated with a higher ingestion rate. Additionally, a difference in metabolization of PST analogues was identified between the two oyster families.

Altogether, these results suggest that a higher growth rate in oysters is associated with higher feeding rates that can result in higher algal toxin uptake. This study also highlights that selection can modify the ability to metabolize toxins in oyster tissues, illustrating the metabolic differences between oyster types. Considering these results, oysters selected for fast growth may therefore be at higher risk for biotoxin accumulation.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-337

Fatty acid esters of gymnodimine in some field shellfishes and mussels (*Mytilus galloprovincialis*) fed with *Karenia selliformis*

A. Li [1 2], Y. Ji [2,*], Y. Che [2], E. Wright [3], P. McCarron [3], P. Hess [4]

[1]Key Laboratory of Marine Environment and Ecology, Ministry of Education, [2]College of Environmental Science and Engineering, Ocean University of China, Qingdao, China, [3]Measurement Science and Standards, National Research Council Canada, Halifax, Canada, [4]Laboratory of Phycotoxins, Ifremer, Nantes, France

Abstract: Bivalves could accumulate the phycotoxins gymnodimines (GYMs) from microalgae *Karenia selliformis* in marine ecosystem. Some fatty acid esters of GYMs have been documented in a previous study. In order to further understand the metabolism of GYMs, field shellfish samples and mussels fed with GYMs-producing microalgae *K. selliformis* were analyzed for the profile and content of fatty acid esters of gymnodimine A (GYM-A) in this study. Oxidative stress and activity of antioxidant enzymes were also monitored to explore the effect of toxin accumulation on physiological status of mussels. Complex components of fatty acid esters of GYMs were detected in the field shellfishes, including *Antigono lamellaris*, *Atrina pectinata*, *Crassostrea* sp. and *Batillaria zonalis*, collected from coastal regions of China. Analysis of mussels exposed to toxic microalgae showed that GYM-A was accumulated and bio-transferred to ester metabolites as well as a kind of new analogue (p-GYM) in mussels for the first time. Metabolisms of GYM-A through both 'phase I' and 'phase II' pathways were indicated here. Acylation degree of the parent toxin was species-specific in bivalves, and the relative abundance ranged from 6% to 87%. Ester metabolites constitute the majority (>90%) of total toxins while both 16:0-GYM-A and 20:1-GYM-A were the major ester derivatives in mussels. Levels of ROS were stable and no obvious variation of antioxidant enzymes were found in mussels fed with toxic (*K. selliformis*) or non-toxic microalgae (*Isochrysis galbana*). Therefore, the total toxicity of GYMs and ester derivatives should be considered in order to protect the seafood safety.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-338

Distribution and differences of diarrhetic shellfish toxins in Japanese scallops

R. Matsushima [1,*], H. Uchida [1], R. Watanabe [1], H. Oikawa [1], Y. Kosaka [2], T. Suzuki [1]

[1]National Research Institute of Fisheries Science, Yokohama, [2]Aomori Prefectural Industrial Technology Research Center, Fisheries Research Institute, Aomori, Japan

Abstract: Diarrhetic shellfish toxins (DST) are defined as Okadaic acid (OA) group and regulatory limit of DST is total OA equivalent of 0.16 mg per kg of edible tissue in CODEX STAN 292-2008. Testing method of DST using LC/MS/MS has been officially authorized from 2015 in Japan.

Distribution and differences of DST in scallops collected in Aomori prefecture were investigated. The 14-20 individuals of the scallops were divided into digestive glands, gonads, mantles, gills, adductor muscles, and the concentrations of OA groups in each tissue were quantified by LC/MS/MS after hydrolysis. The dominant toxin in the scallops was Dinophysis toxin 1 (DTX1). More than 97 % in total amounts of DTX1 was detected in the digestive glands.

Scallops and blue mussels were collected, and the concentration of DTX1 contained in the digestive glands was quantified for each individual of 30 samples. The average value of DTX1 was higher in mussels than the scallop. The numbers of individuals required to correctly reflect the DTX1 content of a sample group was estimated by resampling analysis. Using the data of 30 individuals of scallops or blue mussels, values that can be taken by the average value of samples 5 to 25 were calculated repeatedly by 10,000 times with random sampling without duplication. In scallop, using 10 individuals fell within ± 20% of 30 individual's average with a probability of 99.8%. On the other hand, in the blue mussel, average of 19 individuals and 15 individuals fell within ± 20% of 30 individual's average with 98% and 90% probability, respectively.

Although a series of studies gave us useful information for improving DST risk management, further investigation will be required.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-339

The effects of water depths and geographical distances on toxicity variability of paralytic shellfish toxins accumulated in bivalves

R. Watanabe [1,*], M. Kanamori [2], H. Yoshida [2], Y. Okumura [3], H. Uchida [1], R. Matsushima [1], H. Oikawa [1], T. Suzuki [1]
[1]National Research Institute of Fisheries Science, Yokohama, [2]Hokkaido Research Organization, Hakodate Fisheries Research Institute, Hakodate, [3]Tohoku National Fisheries Research Institute, Shiogama, Japan

Abstract: Paralytic shellfish toxins produced by certain toxic dinoflagellates such as *Alexandrium* spp. are a group of potent neurotoxins and often accumulated in shellfish through food chain. In many countries, shellfish toxicity has been monitored by official testing method such as mouse bioassay and instrumental analysis. However, sampling of shellfish for the testing have not been fully discussed because of shortage of the relating data. Therein, we investigated the effects of water depths and geographical distance on toxicity variability of paralytic shellfish toxins accumulated in bivalves such as scallop *Patinopecten yessoensis* and mussel *Mytilus galloprovincialis*. The toxicities and toxin profiles of those bivalves sampled from different locations and water depths during the closure of farm at 2015 were determined by using instrumental analysis. We determined the toxicities of scallops sampled at 5, 10 and 15 m depths. The highest toxicities were observed in samples collected at 5 m depths. At the same time, cell densities of causative dinoflagellate *Alexandrium tamarense* collected from a different location against the scallops were counted and the highest densities of 640 cells/L were observed at 10 m depth, suggesting that the discrepancy may be explainable by the difference of sampling location between the scallops and the causative dinoflagellate. Moreover, we determined the toxicities of mussels collected at three different long-line facilities. Two-fold variabilities in toxicities of the mussels were observed. At the long-line No. 3, the toxicities of the mussels taken at different depths (1, 5, and 9 m) decreased with deeper depths, whereas their toxin profiles were similar. Therefore, it was concluded that both of water depth and location with the highest toxicities should be considered when sampling point of shellfish for official testing method is decided. This work was financially supported by Ministry of Agriculture, Forestry and Fisheries, Japan.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-340

Response of CYP450 to exposure of the DSP toxins-producing dinoflagellate *Prorocentrum lima* in *Perna viridis*

X.-M. Wei [1], M.-Y. Lu [1], H.-Y. Li [1], J.-S. Liu [1], W.-D. Yang [1,*]
[1]Jinan University, Guangzhou, China

Abstract: Diarrhetic shellfish poisoning (DSP) toxins, one of the most important shellfish toxins, can lead to diarrhetic poisoning in human, and moreover, induce tumor. But bivalves have some tolerance to DSP toxins, and various bivalve species exhibit different sensibility to the toxicity of DSP toxins. Nevertheless, the metabolism and detoxification of DSP toxins in bivalve species remains unclear. In the current study, we found that the expression of CYP3L3, CYP2C8 exhibited some tissue divergence in *P. viridis*, while CYP3A4, CYP3A1 and CYP1A1-like had high expression both in gill and digestive gland. CYP3L3 performed high expression in digestive gland, however its expression was very low in gill. In contrast, CYP2C8 expressed only in gill of *P. viridis.* After exposure to *P. lima* at density of 2×10^5 cells/L, CYP450 content showed significantly changes in gill and digestive gland at 2 h. Correspondingly, the expression of CYP1A1-like mRNA significantly changes in gill and digestive compared with control counterparts ($p<0.05$). After exposure to *P. lima* at 2×10^6 cells/L, the expression of CYP3A4 mRNA significantly increased in digestive gland at 2 h and 12 h. While CYP1A1-like was down-regulated at 2 h, but up-regulated at 6 h. These data suggested that CYP3A4 and CYP1A1 might be involved in DSP toxins metabolism. After the addition of ketoconazole, the activity of CYP3A4 significantly decreased in digestive gland at 2 h and 12 h, while the okadaic acid content significantly deceased at 2 h and 6 h compared to related group without ketoconazole. These outcomes indicated that ketoconazole could depress CYP3A4 activity in bivalves, and therefore altering the metabolism process of DSP toxins in bivalves. It is likely that CYP3A4 might play an important role in DSP toxins metabolism in bivalves.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-341

Target screening for potential dioxin-like compounds in Japanese bivalves using GCxGC–TOFMS

A. Goto [1,*], M. T. Nguyen [1], S. Tanabe [1], T. Kunisue [1]
[1]Ehime university, Matsuyama, Japan

Abstract: 1,3,7-/1,3,8-tribrominated dibenzo-*p*-dioxins (1,3,7-/1,3,8-TriBDDs) can be produced by marine algal species living in the coastal environment. These natural brominated dioxins have been detected in Baltic marine organisms and notably accumulated in bivalves (e.g. mussel and oyster). Although a few studies in the Baltic Sea suggested the possibility that bivalves are chronically exposed to other dioxin-like compounds including unknown substances, their occurrence and sources remain unclear, especially in the Japanese coasts. The present study screened potential dioxin-like compounds in mussels from Seto Inland Sea: the largest semi-enclosed sea located in the western part of Japan, using comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–ToFMS). In addition to man-made chemicals such as persistent organic pollutants (POPs), various halogenated natural products (HNPs) were detected in the analyzed mussel samples. Among the HNPs identified by GC×GC–ToFMS, brominated methylindoles, which are known to be produced by marine algae, highly accumulated in the mussel tissues. Considering that these algal products have been suspected as aryl hydrocarbon receptor (AhR) agonists, further studies on their dioxin-like potency and risk assessment for marine fish and shellfish are required.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-342

Analysis of tetrodotoxin in flesh of a pufferfish, *Takifugu flavipterus*, collected from the Seto Inland See, Japan.

N. Oshiro [1,*], K. Kuniyoshi [1], S. Yamamoto [1 2], T. Yamada [1 2], A. Hotta [1 2], T. Suzuki [1 2], N. Sugita [1 2], K. Matsuura [3], A. Nakashima [4], Y. Anzai [5], H. Asakura [1]

[1]Division of Biomedical Food Research, National Institute of Health Sciences, Kawasaki, [2]Department of Pharmacy and Health Sciences, Meiji Pharmaceutical University, Kiyose, [3]National Museum of Nature and Science, Tsukuba, [4]Health and Environment Center, Hiroshima Prefectural Technology Research Institute, [5]Hiroshima City Public Health Center, Hiroshima, Japan

Abstract: A pufferfish, *Takifugu flavipterus* formerly known as *T. poecilonotus,* has been well consumed in Japan. According to the list of “Species and parts that can be processed to eliminate health risks” provided by the Ministry of Health, Labour and Welfare (MHLW), Japan, the flesh of *T. flavipterus* is edible, although the other parts of the body are not allowed to be consumed. The list also states that individuals of this pufferfish caught in Okirai, Kamaishi, and Ogatsu Bays along the Pacific coast of northern Honshu Island are inedible because of having the highly toxic flesh. *T. flavipterus* is one of pufferfishes frequently causing tetrodotoxin (TTX) poisoning in Japan. While victims consumed inedible parts including the liver, ovary and skin in most cases of poisoning, there are some cases due to consumption of the flesh. To improve the risk management of pufferfish poisoning such as the list provided by MHLW, we studied TTX value in the flesh and skin of *T. flavipterus.* Sixty-five specimens were obtained from the Seto Inland Sea, and their flesh samples were analyzed by LC-MS. The analysis showed that flesh samples from 6 specimens were toxic (>10 MU/g = 2.2 mg/kg): 3 specimens were analyzed in fresh (though, 1 was not so good in condition) and 3 were frozen and thawed before analysis. In order to elucidate why high TTX value was detected in these flesh samples, they were divided into outer and inner portions. Our analysis clearly indicated that TTX values of the outer portions were higher than those of inner portions. The TTX values in skin samples of 6 specimens were high or extremely high: the values in 3 freeze-thawed specimens ranged from 600 to 950 MU/g (132 to 200 mg/kg), 165 MU/g (36.3 mg/kg) in a fresh specimen in not good condition, and 4,500 and 6,000 MU/g (990 and 1320 mg/kg) in the other 2 fresh specimens in good condition. In addition, TTX values in the skin seemed to be regional specific. We concluded that TTX in the flesh of *T. flavipterus* migrated from the highly toxic ski. Our study strongly recommends that toxic portions of *T. flavipterus* should be removed as soon as possible just after individuals are caught in seas and it is also recommended to avoid consuming the fish caught from high toxic area.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-343

YTX AND HOMOYTX ACCUMULATION IN MUSSELS AS FOOTPRINT, RESPECTIVELY, OF PROTOCERATIUM RETICULATUM AND GONYAULAX SPINIFERA BLOOMS

R. Pistocchi [1,*], M. Cangini [2], F. Guerrini [1], L. Pezzolesi [1], M. Pompei [2], S. Vanucci [3], A. Milandri [2]

[1]Department of Biological, Geological and Environmental Sciences, University of Bologna, Ravenna, [2]Fondazione Centro Ricerche Marine, Cesenatico, [3]Department of Biological and Environmental Sciences, University of Messina, Messina, Italy

Abstract: In 1995 YTXs were detected for the first time in the Adriatic Sea mussels and since then the presence of this group of toxins has represented the cause of mussel farms closure for prolonged periods. Initially, mussels were shown to contain YTX as the prevailing compound but also different analogs, mainly represented by homoYTX and 45-OHYTX. Three different YTX producing species have been identified over time in the Adriatic Sea phytoplankton population, which are the dinoflagellates *Protoceratium reticulatum*, *Gonyaulax spinifera* and *Lingulodinium polyedrum*. *P. reticulatum* was shown to synthesize several analogs previously observed in mussels, such as YTX, homoYTX, 45-OHYTX, carboxyYTX and noroxoYTX, with YTX being 30 to 140-fold more abundant than the others and thus matching the toxin profile found in mussels. In 2004 and 2006, however, farmed mussels were found to contain high levels of homo-YTX as the dominant analog and this finding did not coincide with a bloom of *P. reticulatum*, which was present at very low levels, but with the presence of *G. spinifera* in high numbers. Subsequent studies on two strains of this species, isolated one in 2004 and one in 2006, lead to contrasting results: the former contained only YTX while, in the latter, homoYTX was the prevailing analog being 10-fold more abundant than YTX. Finally, the third producer, *L. polyedrum*, analyzed from a net haul sample where it was the dominating species, was associated with the production of homoYTX. Those above reported, were the only studies performed on cultured YTX producers from the Adriatic Sea but still YTXs represent the most relevant problem for mussel commercialization (followed by DSP toxins) even if, in 2013, the law limit was increased to to 3.75 mg/kg from the initial value of 1 mg/kg. The aim of this study was to follow the distribution pattern of the three YTX producing species at a large spatial and time scale and to carefully determine the pattern of all the YTX analogs in mussels. Samplings of phytoplankton and mussels were performed, from April to November, in 10 farms and two natural banks, along the Emilia-Romagna coast. All the three YTX producer species were detected; in particular, *P. reticulatum* and *G. spinifera* displayed a different distribution along the investigated period and their presence well correlated with the presence of YTX and homoYTX, respectively, as the prevailing toxins in mussels.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-344

Toxin accumulation in benthic marine fauna and exposure of juvenile green turtles to potentially toxic microalgae in southern Brazil

A. R. Moreira Gonzalez[1 2,*], K. Rosa[2], C. Domit[2], L. L. Mafra Jr.[2]

[1]Centro de Estudios Ambientales de Cienfuegos, Ciudad Nuclear, Cuba, [2]Centro de Estudos do Mar, Universidade Federal do Paraná, Pontal do Paraná, Brazil

Abstract: Toxin-producing microalgae can cause illness and/or mortality of marine organisms through either direct exposure to toxic cells or ingestion of toxins, which can be bioaccumulated in consumers. In this study, (i) lipophilic toxins were quantified by LC-MS/MS in benthic marine organisms representative of different trophic levels and collected in the Paraná coast (southern Brazil), from winter/2015 to autumn/2016; and (ii) the presence of potentially toxic microalgae was also investigated within the digestive contents of 38 threatened green turtles (*Chelonia mydas*) found dead-stranded in same region and period. From all lipophilic toxins investigated, only the diarrheic toxin okadaic acid (OA) was detected in selected tissues of benthic marine animals, mostly sampled during summer and autumn/2016. Free-OA was recorded at higher concentrations in the liver of the bottom-feeder fish *Genidens genidens* (24. 3 ± 31.7 ng g^{-1}) and in whole tissues of the suspensivorous bivalve *Mytella guyanensis* (21. 3 ± 2.13 ng g^{-1}), both in autumn/2016. Less frequently and at lower levels, OA was also recorded in other fishes, gastropods and crustaceans. Toxin levels accumulated in these organisms were positively correlated with the abundance of *Prorocentrum caipirignum* cells among the microphytobenthos assemblage in the outer sector of the Paranaguá estuarine complex. Additionally, 14 potentially toxic species of microalgae (7 dinoflagellates, 6 cyanobacteria and one diatom) were found in the digestive contents of green turtles, with *Dinophysis acuminata* complex being the most frequent and abundant taxon (maximum average abundance of 566 cells g^{-1} in spring/2015). Moreover, about one quarter (9/38) of the examined sea turtles exhibited detectable OA levels in washed digestive tissues – seven in the intestine (max. 24.1 ng g^{-1}) and two in stomach tissue (max. 7.40 ng g^{-1}). Toxin levels in tissues were significantly associated ($r=0.73$, $p <0.025$) to the cell abundance of *D. acuminata complex* + *Prorocentrum* spp. (*P. caipirignum* and *P. lima*) in the sea turtle digestive contents. Although OA concentrations were below the regulatory levels for acute human intoxication, possible chronic effects to the benthic marine fauna and their consumers cannot be disregarded due to the frequent presence of low toxin levels associated to both benthic and planktic toxic microalgae in this geographic region. To the best of our knowledge, this is the first study to report the detection of diarrheic toxins in a wide range of marine fauna associated with benthic ecosystems in South America, including the endangered species *Chelonia mydas* that inhabit the coast of Paraná, southern Brazil.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-345

***Prorocentrum lima*, a new diarrhetic shellfish toxins producer in northern Chile**

E. Uribe [1], G. Alvarez [1 2,*], J. Rengel [1], J. Blanco [3]

[1]Departamento de Acuicultura, [2]2Centro de Investigación y Desarrollo Tecnológico en Algas (CIDTA), Universidad Católica del Norte, Coquimbo, Chile, [3]Centro de Investigacións Mariñas, Xunta de Galicia, Vilanova de Arousa, Spain

Abstract: The benthic dinoflagellate, *Prorocentrum lima* is a cosmopolitan species distributed in tropical and temperate seas. This dinoflagellate is known to produce okadaic acid (OA), dinophysistoxin-1 (DTX-1), dinophysistoxin-2 (DTX2) and water-soluble OA esters, that cause diarrheic shellfish poisoning (DSP). In early February 2016, a bloom of *P. lima* occurred in Bahía Calderilla, a little bay located near Bahía Inglesa one of the most important scallop aquaculture sites in northern Chile. During the bloom, cells were found to be distributed in water column, sand, rocks and the surface of different macroalgae such as *Codium fragile*, *Gracilaria chilensis* and *Ulva* sp. A clonal culture of *P. lima* was established and its morphology and toxicity were studied. Morphological examination by SEM showed very good overall agreement with descriptions made for *P. lima*. Cells were slightly oval with measures 38.3 – 42.8 µm length and 26.6 – 39.7 µm width. The valvar surface was smooth, except for the central pores (77 ± 2 pores) and the row of marginal pores (60 ± 2 pores) situated at the valve periphery. The periflagellar area was a V-shaped triangle constituted by eight plates of different size. Toxin analysis of the culture extract, using liquid chromatography mass spectrometry (LC-MS) revealed that the toxic profile was dominated by OA followed by DTX1 and demonstrated the absence of DTX2. The estimated amounts of OA and DTX1 were of 3.53 pg cell^{-1} and 0.012 pg cell^{-1}, respectively. Finally, C8 and C10 OA diol-esters were detected but not quantified. Although, there is no apparent case of accumulation of DSP toxins in shellfish of the area produced by *P. lima*, their detection indicates that this species constitutes a potential risk and suggests that it is necessary to routinely monitor it in order to warrant public health.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-346

Biodilution of cyanobacterial toxin microcystin in small fish from eutrophic Nyanza Gulf, Lake Victoria, Kenya

B. Simiyu [1,*], S. Omondi Oduor [2], T. Rohrlack [3], L. Sitoki [4], R. Kurmayer [1]

[1]Research Department for Limnology, University of Innsbruck, Mondsee, Austria, [2]Department of Biological Sciences, Egerton University, Egerton, Kenya, [3]Environmental Sciences, Norwegian University of Life Sciences, As, Norway, [4]Department of Geoscience and the Environment, The Technical University of Kenya, Nairobi, Kenya

Abstract: The human health risks posed by exposure to cyanobacterial toxins such as microcystin (MC) through water and fish consumption remain poorly described. During the last two decades coastal regions of Lake Victoria such as Nyanza Gulf (Kisumu Bay) have shown severe signs of eutrophication with blooms formed by *Microcystis* producing MC. In this study the spatial variability in MC concentration was investigated which was mostly caused by *Microcystis* buoyancy and wind drifting. Small fish (< 6 cm) mainly composed of *Rastrineobola argentea* were examined for MC content by means of ELISA and protein phosphatase inhibition assay (PPIA). On average the ELISA determined MC contents exceeded the PPIA determined MC contents by a factor of 8.2 ± 0.5 (SE). Using PPIA the MC content varied from 25 – 109 (mean 62 ± 7) ng/g fish dry weight in Kisumu Bay vs. 14 ± 0.8 ng MC/g in the more open water of L. Victoria at Rusinga channel. Drying the fish under the sun showed little effect on MC content, although a higher air humidity might indirectly favor photocatalyzed MC degradation. Overall the MC content in small fish was related to the high MC concentration observed in the seston however was 1000-fold decreased.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-347

CIGUATOXIN KINETICS OF A RECONSTRUCTED CORAL REEF FOOD WEB: A CONTROLLED MULTIPATHWAY FEEDING STUDY WITH CARIDEAN SHRIMP AND DAMSELFISH

C. Bennett [1,*], A. Robertson [1]

[1]Marine Sciences, University of South Alabama; Dauphin Island Sea Lab, Dauphin Island, USA

Abstract: Ciguatoxins (CTX) are biosynthesized in coral reef organisms through the biotransformation of precursors produced by the benthic dinoflagellate *Gambierdiscus* spp. These algal metabolites biomagnify through reef food webs and human consumption of CTX contaminated seafood causes the most common non-bacterial seafood borne illness, ciguatera fish poisoning. Research on CTX has primarily focused on top and bottom trophic levels including commercially important fish species and the CTX source, *Gambierdiscus*, in both cases due to the role these organisms have in ciguatera food webs. However, intermediates such as invertebrate mesograzers and fish secondary consumers are comparatively underrepresented but are key components to the flux of CTX into the higher food web. Understanding the toxicokinetics of CTX flow between low and intermediate trophic levels may improve CTX food web models and allow resource managers to develop better methods for monitoring reef zones and predicting periods of resource fish toxicity. In this study, we exposed two common types of reef fish prey, Caridean shrimp (Palaemonetes) and damselfish (Pomacentridae), to Caribbean CTX (C-CTX) using controlled laboratory feeding to define the role these organisms may have in CTX food webs. A simple food chain involving C-CTX transfer by single step (CTX to shrimp; CTX to damselfish) and two step (CTX to shrimp to damselfish) exposure was constructed. Tissues of experimental organisms were chemically extracted using standardized methods to recover C-CTX. Analyses of sodium channel dependent ciguatoxicity was performed using the ouabain-veratridine dependent mouse neuroblastoma (N2A) assay, with confirmation by LC-MS. The toxicokinetics of CTX uptake across treatments relative to varied dose and acute versus chronic exposure scenarios will be presented.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-348

Biosynthetic enzymes related to marine polyethers in dinoflagellate extracts

M. Satake [1,*], D. Naganuma [1]
[1]Department of Chemistry, School of Science, The University of Tokyo, Tokyo, Japan

Abstract: Ladder-shaped polyethers (LSPs) such as brevetoxins, ciguatoxins, and yessotoxins are secondary metabolites produced by marine dinoflagellates and have unique structures and potent toxicities. The biosynthesis of LSPs has been proposed as follows. A *trans*-polyene precursor is polyepoxidized and a resultant epoxide is cyclized in a cascade of an *endo-tet* ring expansion process by enzymes. However, the fused ether ring formation of marine ladder-shaped polyethers has not yet been elucidated experimentally because of a lack of genetic information of dinoflagellates and the complexity of these structures.

For exploration of biosynthetic enzymes such as epoxidase and an epoxide-opening enzyme in dinoflagellates, truncated epoxide precursors of yessotoxin were designed and synthesized from D-glucose. Incubation of the model epoxides with extracts from *P. reticulatum* cells gave rise to a 6-membered ether ring products. These results strongly indicated that the epoxide-opening enzyme was found in the extract from *P. reticulatum*.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-349

Combined effects of warming and acidification on PSP toxins accumulation and genotoxicity in mussels *Mytilus galloprovincialis*

A. Braga [1], C. Camacho [1], V. Pereira [2], R. Marçal [2], A. Marques [2], S. Guilherme [2], A. Marques [1], A. Gago-Martínez [3], M. Pacheco [2], P. R. Costa [1,*]

[1]IPMA, Lisbon, [2]University of Aveiro, Aveiro, Portugal, [3]University of Vigo, Vigo, Spain

Abstract: *Mytilus galloprovincialis* under current environmental conditions and simulated climate change scenarios (warming, acidification and warming with acidification) were exposed to PSP toxins-producer *Gymnodinium catenatum*. Shellfish toxicity and DNA damage (comet assay) were assessed in mussels from all treatments. Mussels were acclimated (21 days), exposed to *G. catenatum* (5 days, uptake), and feed with a non-toxic diet (10 days, elimination). PSP analyses were carried out by Liquid Chromatography with Fluorescence Detection. The highest PSP content was observed under actual conditions, day 5 (1493.8 ± 202.4 µg STX eq. kg^{-1}), exceeding the safety limits (800 µg STX eq. kg^{-1}). Significantly lower contents were observed under climate change scenarios. The lowest, reaching 661.9 ± 22.8 µg STX eq. kg^{-1}, was found in warming, followed by acidification (761.2 ± 62.3 µg STX eq. kg^{-1}). However, warming and acidification interaction did not reveal an additive effect. After shifting to non-toxic diet, lower toxins elimination was observed in warm-acclimated mussels. Genotoxicity was assessed after each period. In non-exposed mussels, the highest damage was observed under warming and acidification interaction after 36 days. In *G. catenatum* exposed mussels, DNA damage significantly increased earlier, just after uptake phase. The treatments acidification and interaction of warming with acidification, revealed higher damage than actual conditions, highlighting synergistic impacts. DNA damage decreased during elimination, although subtly under warming and acidification interaction. This is the first study assessing the impact of combined warming, acidification and biotoxins in mussels. In conclusion, it was provided evidence that climate change may lead to lower PSP contents, but also to slower elimination rates and to synergistic effects on DNA damage implying possible consequences for the mussels populations.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-350

Presence and persistence of the amnesic shellfish poisoning toxin, domoic acid, in octopus and cuttlefish brains

V. Lopes [1], R. Rosa [1], P. R. Costa [2,*]
[1]University of Lisbon, [2]IPMA, Lisbon, Portugal

Abstract: Domoic acid (DA) is a neurotoxin that causes degenerative damage to brain cells and induces permanent short-term memory loss in mammals. In cephalopod mollusks, although DA is known to accumulate primarily in the digestive gland, there is no knowledge whether DA reaches their central nervous system. Here we report the presence of DA in brain tissue of the common octopus (Octopus vulgaris) and the European cuttlefish (Sepia officinalis), and its absence in the brains of several squid species (Loligo vulgaris, L. forbesi and Todarodes sagittatus). We argue that such species-specific differences are related to their different life strategies (benthic/nektobenthic vs pelagic) and feeding ecologies, as squids mainly feed on pelagic fish, which are less prone to accumulate phycotoxins. Additionally, the temporal persistence of DA in octopus brain reinforces the notion that these invertebrates can selectively retain this phycotoxin. This study shows that two highly-developed invertebrate species, with a complex central nervous system, where glutamatergic transmission is involved in vertebrate-like long-term potentiation (LTP), have the ability of retaining and possibly tolerating chronic exposure to DA, a potent neurotoxin usually acting at AMPA/kainate-like receptors. Here, we filled a gap of information on whether cephalopods accumulated this neurotoxin in brain tissue, however, further studies are needed to determine if these organisms are neurally or behaviourally impaired by DA.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-351

Evidence of *Noctiluca scintillans* grazing during a bloom of toxic *Dinophysis acuminata* complex, south Brazil

B. Sobrinho [1 2,*], L. Luz [2], J. Bersano Filho [2], L. Fernandes [1], L. Mafra [2]

[1]Department of Botany , Universidade Federal do Paraná, Curitiba, [2]Center for Marine Studies, Universidade Federal do Paraná, Pontal do Paraná, Brazil

Abstract: Although *Noctiluca scintillans* is not capable of producing toxins, blooms of this heterotrophic dinoflagellate may occasionally cause massive mortality of fish and marine invertebrates in coastal regions. This large-celled (200-2000 µm in diameter), flagellate species prey on different planktic organisms, including smaller dinoflagellates. Here, we investigated the possible role of *N. scintillans* grazing on bloom termination and as a possible vector of toxin transfer during a massive bloom of *Dinophysis acuminata* complex. On June 2016, the bloom affected a large area in southern Brazil, attaining high cell densities of *D. acuminata* complex (up to 5.37×10^5 cells.L^{-1}), simultaneously, high cell abundance of *N. scintilans* was also recorded (5.92×10^4 cells.m^{-3}) along the coast of Paraná State. Food vacuoles of 300 *N. scintilans* cells per sample (n=9) were examined under light microscope, revealing the presence of up to 8 algal cells per *N. scintilans* (average: 1.2 to 2.9 cells.individual^{-1}). The food vacuole content was dominated (42-89% of total content) by centric diatoms, mainly *Thalassiosira* spp. and *Coscinodiscus* spp., at early bloom, and by unidentified, partially digested material (up to 79%) at late bloom stage. Intact cells belonging to *D. acuminata* complex were found in 1 to 7% of the *N. scintillans* individuals (up to 2 cells.individual^{-1}) examined in different samples. Other potentially toxigenic dinoflagellates like *Prorocentrum* spp. and *Ostreopis* spp., as well as unidentified cysts and even intact copepodes and their remnants were also recorded in *N. scintillans vacuoles.* Concurrently with the highest ingestion rates of *Dinophysis* cells by *N. scintillans* at early bloom, okadaic acid (OA) was detected both in seston (160.09 ng.L^{-1}) and in 300-µm trawl net samples (77.53 ng.g^{-1}), dominated by *Noctiluca,* Copepoda and Cladocera. The occurrence of toxigenic dinoflagellate cells (mostly *D. acuminata* complex) within *N. scintillans* food vacuoles coupled with the detection of OA in large planktic grazers, co-dominated by *N. scintillans,* support at least a partial role of this species in harmful algal bloom termination and toxin transfer through the pelagic food web.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-352

High uptake and slow elimination of diarrheic toxins by scallops (*Nodipecten nodosus*) under simulated bloom conditions

G. Morais [1], K. Rosa [1], L. Martins [1], J. Inglez [1], I. Leite [1,*], F. Lagreze-Squella [1], L. Mafra Jr. [1]
[1]Centro de Estudos do Mar, UFPR/Brazil, Pontal do Paraná, Brazil

Abstract: Bivalves exhibit highly species-specific variation in toxin accumulation capacity, resulting from differential toxin uptake and elimination rates. We have experimentally exposed juvenile scallops (*Nodipecten nodosus*) to toxin-containing *Prorocentrum lima* cells and measured the levels of diarrheic toxins accumulated in their adductor muscles (AM) and digestive glands (DG) over 7 days of exposure followed by a 21-day depuration period in the laboratory. Scallops were placed in 4 tanks per treatment and exposed to a control non-toxic diet (T1: 4.0×10^6 *Tetraselmis suecica* cells.L^{-1}) or to biovolume-equivalent toxic diets containing either low (T2: 4.0×10^3 *P. lima* + 3.92×10^6 *T. suecica* cells.L^{-1}) or high (T3: 4.0×10^4 *P. lima* + 3.22×10^6 *T. suecica* cells.L^{-1}) cell densities of *P. lima*. During the subsequent depuration period, all scallops were exposed to a non-toxic diet as described for T1. Pooled samples (3 individuals per tank) of DG and AM were taken before (0 h) and throughout the exposure (after 6, 24, 72 and 168 h) and the depuration periods (after 6, 24, 72, 168 and 504 h). *P. lima* culture contained mostly okadaic acid (OA; 16.3±7.6 pg.$cell^{-1}$), besides two isomers of OA (iso-OA) and another of dinophysistoxin-1 (iso-DTX-1) at cell quotas of 0.5±0.2, 0.1±0.06 and 0.1±0.05 pg.$cell^{-1}$, respectively. Scallops assimilated and retained high levels of OA and traces of iso-OA during the exposure and depuration periods in both T2 and T3; iso-DTX-1 free toxin was only detected in T3. Most part of the toxin was conjugated (i.e., metabolized), especially during depuration, when free toxins generally accounted for <10% of the total content. Toxin levels were much higher in DG (up to 27,900 and 3,200 µg.Kg^{-1} in T3; and 3,200 and 500 µg.Kg^{-1} in T2 for total and free-OA, respectively) than in AM (max. 40 and 15 µg.Kg^{-1} in T3; and 30 and 14 µg.Kg^{-1} in T2 for total and free-OA, respectively). In the DG, isomers of OA contributed to ≤10% of the total-OA content, and iso-DTX-1 reached up to 230 µg.Kg^{-1} in T3. Moreover, maximum free-toxin levels were attained after 168 h of exposure, whereas conjugated forms reached the highest levels after 6 h of depuration, indicating active toxin transformation. However, toxin elimination was slow and scallops still contained 1,160±410 µg total-OA.Kg^{-1} in DG after 3 weeks of depuration. These findings are especially relevant for the management of OA-contaminated bivalves in places like Brazil, where diarrheic shellfish poisoning events have become more frequent and intense over the past years. More importantly, scallops can be sold as whole individuals in Brazil, and harvesting bans are usually suspended only a few weeks following toxic blooms, mainly based on toxin levels measured in mussels and oysters, which usually have much lower uptake and faster elimination rates of OA toxin than *N. nodosus* in this study.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-353

Depuration and anatomical distribution of domoic acid in the scallop *Argopecten purpuratus*

G. Alvarez [1 2,*], A. Lopez-Rivera [3], E. Uribe [2], M. Araya [1], F. Alvarez [2], R. Pino [4], J. Rengel [2], J. Blanco [5]

[1]Centro de Investigación y Desarrollo Tecnológico en Algas (CIDTA), Facultad de Ciencias del Mar, [2]Departamento de Acuicultura, Facultad de Ciencias del Mar, Universidad Catolica del Norte, Coquimbo, [3]Laboratorio de Toxinas Marinas, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, [4]Doctorado en Acuicultura, Programa Cooperativo , Universidad de Chile, Universidad Católica del Norte, Pontificia Universidad Católica de Valparaíso, Coquimbo, Chile, [5]Centro de Investigacións Mariñas , Xunta de Galicia, Vilanova de Arousa, Pontevedra, Spain

Abstract: The scallop *Argopecten purpuratus* is one of the most important commercial species in Chile. Traditionally, its natural populations have been exploited by artisanal fishermen. However, due to severe overexploitation, harvesting was banned in 1986. As a consequence, the scallop aquaculture developed quickly, and 20 years later, the country has become the third largest scallop producer worldwide. In Northern Chile, several toxic outbreaks of domoic acid (DA) have been detected in the main scallop aquaculture sites. However, no information is available on depuration, or on the anatomical distribution of this toxin and its potential use to design measures to minimize the consequences of Amnesic Shellfish Poisoning outbreaks. Depuration of DA is very fast in *A. purpuratus*, and its kinetics can be adequately described by means of a first-order, one-compartment model with an estimated rate of 0.49 d^{-1}. Considering, its high depuration rate, the time in which the shellfish are unsafe for consumers, once the toxic algae populations have disappeared, is very short, and therefore the economic losses that could result by the ASP outbreaks in scallop aquaculture industry should be moderate. DA was accumulated mainly in the adductor muscle, digestive gland and the gonad with a total toxin burden of 39%, 36 % and 18%, respectively. The contribution of mantle and gill are less important with total toxin burden of 4% and 3%, respectively. The highest concentration of DA was found in the edible tissue that correspond to muscle (4.11 ± 1.25 mg kg^{-1}) and gonad (2.04 ± 1.15 mg kg^{-1}) (total weight = 15.52 ± 3.26 g^{-1}), while the non-edible tissues had a concentration of 4.47 ± 1.33 mg kg^{-1}(total weight = 9.22 ± 2.42 g^{-1}). Taking this into account, the elimination of the non-edible tissues by means selective evisceration would yield a product 10% less toxic than the whole body and, consequently, this process could not be used to improve the quality of the scallops for human consumption.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-354

Lipophilic Toxins profiles in cockle and smooth clam from the central Adriatic Sea (Croatia)

I. Ujevic [1,*], R. Roje-Busatto [2], D. Ezgeta-Balić [2]
[1]senior research, [2]research, Institute of Oceanography and Fisheries, Split, Croatia

Abstract: Searching for lipophilic toxins in seafood is of great importance for consumer protection, but it is also an indicator of changes in the marine ecosystem that has a toxin production as an outcome. The consequence of the toxic phytoplankton species occurrence is marine biotoxin accumulation in shellfish and possible derivatization of the parent form that has led to intensification of biotoxins research. Studies are usually focused on the most aquacultured species, the mussel. But, there are a number of potentially commercially important shellfish species as rough cockle *Acanthocardia tuberculata* (Linnaeus, 1758) and smooth clam *Callista chione* (Linnaeus, 1758) which are common in the Croatian Adriatic Sea. Investigation of lipophilic toxins accumulation in these two species of shellfish from Adriatic Sea has not been conducted up to now. In order to detect the potential lipophilic toxin profile of *A. tuberculata* and *C. chione* wild populations, samples were taken monthly during one year survey from the estuarine area in the central Adriatic Sea. Both investigated shellfish species had shown low levels of only two lipophilic toxins, gymnodimine and spirolide.

In *A. tuberculata* samples, the range of gymnodimine was 2.6510– 15.7702 µg kg^{-1}, while in *C. chione* tissue it was 1.1737– 6.1356 µg kg^{-1}. Ranges of spirolide in *A. tuberculata* and *C. chione* tissues were 0.9790– 5.9039 µg kg^{-1} and 0.6464– 2.1392 µg kg^{-1}, respectively.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-355

Kinetics of BMAA accumulation and detoxification by the freshwater bivalves *Anodonta anatina* and *Dreissena polymorpha*

A. Lepoutre [1,*], E. Faassen [2], A. Geffard [1], E. Lance [1]

[1]Université de Reims Champagne-Ardenne, Reims, France, [2]Wageningen University & Research, Wageningen, Netherlands

Abstract: The environmental neurotoxin β-methylamino-L-alanine (BMAA) has recently trigged an emerging interest because of its potential link with the development of neurodegenerative pathologies such as the Amyotrophic Lateral Sclerosis-Parkinsonism-Dementia complex (ALS-PDC). Although the etiological link has not been formally proven so far, the scenario of human exposure has been suggested to be the chronic ingestion of BMAA-containing food. BMAA has been detected in animals destined to human consumption, mostly marine species (fish, crustaceans, mussels and oysters), in China, USA and Europe, under a free form and associated to low-molecular-weight polypeptides in tissues. The contamination pathway of aquatic organisms could be the ingestion of phytoplankton species including some cyanobacteria, diatoms and dinoflagellates, known to produce BMAA. However, the BMAA accumulation by freshwater organisms remains unexplored, even though the occurrence of BMAA in freshwater ecosystems has been demonstrated.

Here we studied the kinetics of BMAA accumulation and detoxification by two freshwater bivalve species, filter-feeders ingesting phytoplankton, *Anodonta anatina* and *Dreissena polymorpha,* through laboratory studies supported by active caging.

Both mussel species were exposed to 1, 10 and 50 µg/L of dissolved BMAA for 3 weeks, followed by 3 weeks of depuration. At days 1, 3, 7, 14 and 21 of exposure and of depuration periods, mussels were sampled (whole individuals of *D. polymorpha* and digestive glands of *A. anatina*), and the kinetics of accumulation and detoxification of total (free and peptide-associated) BMAA were measured by LC-MS/MS.

To study if mussels accumulated BMAA *in situ*, both species have been caged during two campaigns in summer 2016 and 2017 in three stations, presenting contrasting level of cyanobacterial proliferations, of the lake «Ailette » (France). Mussels were caged at the same depth and were sampled either monthly (from July to November 2016) or weekly (in August 2017), for a quantification of total BMAA in tissues.

First available data show that mussels rapidely accumulated total BMAA within their tissues, from the first days of exposure, without a complete elimination during the depuration period. Results also showed contrasted accumulation and detoxification kinetics depending on the three exposure concentrations. Results concerning the *in situ* investigation are in the process of acquiring.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains
P-356

Lipophilic toxins in three bivalve species from Bahía de La Paz, Mexico

I. Leyva [1,*], J. E. Hernández Castro [2], C. J. Band Schmidt [2], A. Turner [3], A. O´Neill [3], E. J. Nuñez Vázquez [4], D. J. López Cortés [5], F. Hernández Sandoval [6], J. Bustillos Guzmán [5]
[1]CONACYT, IPN-CICIMAR, [2]Plancton y Ecología Marina, IPN-CICIMAR, La Paz, Mexico, [3]CEFAS, Weymouth, United Kingdom, [4]Lab. de Toxinas marinas y aminoácidos, [5]CIBNOR, La Paz, Mexico, [6]Laboratorio de Pigmentos, CIBNOR, La Paz, Mexico

Abstract: In the Gulf of California bivalves such as *Megapitaria squalida* (chocolata clam), *Atrina maura* (pen shell) and *Dosinia ponderosa* (white clam), are appreciated as delicious seafood. Natural beds of these species in Bahía de La Paz (Baja California Sur, Mexico) were monitored for lipophilic toxins from 2015 to 2017. Four individuals of each species were collected monthly, the whole organism was homogenized and processed for lipophilic toxin extraction. Tissue extracts were analyzed by LC-MSMS in CEFAS, UK. Fourteen distinct lipophilic toxins were identified including: okadaic acid (OA), dinophysis toxins, azaspiracids, pectentoxins, yessotoxins (YTX) and cyclic imines, such as gymnodimins (GYM), spirolids and pinnatoxins (PnTx). The three bivalve species had low levels of OA (< 20 µg/kg) during all the monitoring period, *M. squalida* showed the highest concentrations of this toxin, from 1.3 to 4.3 µg/kg during 2015. GYM were recurrent in both *D. ponderosa* and *M. squalida*; while homoyessotoxin (hYTX) and PnTx were more frequent in *A. maura*. The highest concentration of GYM in *D. ponderosa* was detected in January 2015 (42.6 µg/kg), *M. squalida* presented the highest concentration in September 2015 (26.2 µg/kg), while *A. maura* had the highest concentrations of hYTX (88.7 µg/kg) in April 2016. There is no information regarding blooms of dinoflagellates related with the production of GYM, hYTX and PnTx in this area. Although species such as *Gonyaulax spinifera* and *Vulcanodinium rugosum* have been reported. Species responsible of GYM production could not be identified in this study. Additionally we isolated and maintained in culture conditions dinoflagellate species which by molecular methods and morphological traits were identified as *Coolia malayensis*, *Prorocentrum mexicanum*, *Azadinium coccinum*, *Gonyaulax spinifera* and *Protoceratium reticulatum*, which could be responsible of the lipophilic toxins found in shellfish. This study is the first report where more than ten lipophilic toxins are detected in distinct species of wild bivalves in the Gulf of California. Although all toxins showed concentrations lower than the maximum level established by the international sanitary regulation, we do not know if passive accumulation of these toxins, their combination or synergic effect, has adverse effects in the mollusks or in humans, due to a long-term exposure.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-357

Experimental exposure of *Mytilus* sp. to two toxic UK Alexandrium species, implications for English monitoring programmes

A. Lewis [1 2,*], K. Dean [1], A. Turner [1], L. Percy [2], J. Lewis [2]
[1]Food Safety, Cefas, Weymouth, [2]Life Sciences, University of Westminster, London, United Kingdom

Abstract: In England there are two known producers of paralytic shellfish toxins (PST) which recur due to their ability to form cyst deposits, these are two species from the genus *Alexandrium, A. minutum* and *A. tamarense*. In Scotland *A. tamarense* has been implicated in a number of shellfish intoxications as well as affecting North Eastern England. In the rest of England, the only identified PST producer has been *A. minutum*. Where these species have undergone toxicity testing the profiles of toxins produced vary, typically the Scottish *A. tamarense* has a more complex range of toxins with higher cellular toxin quotas. The differences in the toxin profiles of *A. minutum* and *A. tamarense* can also be reflected in the contaminated shellfish, provided that the shellfish species being analysed is not capable of rapid bioconversion of those toxins present. Previous work conducted at the Centre for the Environment, Fisheries and Aquaculture Science has demonstrated that the toxin profiles in shellfish can be statistically analysed and clustered and that these clusters can be compared to source algal populations to give an indication of causative organism. By evaluating toxin profiles from shellfish tested during the routine monitoring programme it appears that *A. tamarense* has extended its range or been introduced to the Swansea bay area, in South Wales. Previous PST events tentatively attributed to *A. minutum* have also occurred in this region. Consequently, it appears that for the first time in the UK toxic strains of these two species may now share a stretch of coastline.

The experimental exposure of Welsh mussels (*Mytilus sp.*) to these two toxic species was undertaken to determine the toxin profiles of the shellfish when exposed to toxic strains of both *A. minutum* and *A. tamarense,* when these toxic species were present individually, in a mixed feed and when one species was fed sequentially after the other. Mussels were exposed to toxic strains of *A. tamarense* sourced from Northern Scotland and *A. minutum* sourced from the south of England, to best represent those strains likely to occur in this region. The intention being to determine if the characteristic toxin profiles of the feed algae gave rise to similarly characteristic toxin profiles in the exposed shellfish. The ability to infer causative species from detailed toxin data will be useful for continued monitoring efforts as even crude speciation efforts can be used to better inform predictive models, regulators and the industry. It is envisaged that moving forward this information will allow toxicity data to be used to maximise the value of the phytoplankton monitoring programme and target future research efforts.

Disclosure of Interest: None Declared

Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains

P-358

Multiple toxins determined in shellfish collected from the north Bering Sea and Chukchi Sea shelf and its northern area

C. Gao [1,*], M. Chen [2], S. Lin [2], C. Liu [1]

[1]The First Institute of Oceanography, SOA, China, Qingdao, [2]Xiamen University, Xiamen, China

Abstract: Multiple toxins were analyzed in shellfish samples collected from the north Bering Sea and the Chukchi Sea shelf and its northern area in 2014 by the liquid chromatography tandem quadrupole mass spectrometry (LC-MS/MS) analysis. The analyzed toxins consist of 15 hydrophilic toxin components (C1/C2, B1 (GTX5)/B2 (GTX6), GTX 1~4, dcGTX2/3, STX, dcSTX, NeoSTX, dcNeoSTX) and 14 lipophlic components (AZA1~3, DA, OA, DTX1~2, GYM, SPX, YTX, hYTX, 45-OH-YTX, 45-OH-hYTX, PTX1~2). From the toxin profiles in shellfish samples, four features were exhibited: 1) the complete absence of N-sulfocarbamoyl C1/C2 or B1 (GTX5)/B2 (GTX6) toxins with low toxicity in all samples, STX and dcSTX were the dominant components for hydrophilic toxins; 2) presence of YTX and SPX in all samples from the north Bering Sea and the Chukchi Sea shelf and its northern area ; 3) higher toxicity and variety of toxins in samples from high latitude locations; 4) a high concentration of PTX1(467.40μg/kg, exceeding safety limit of 160μg/kg) was detected in shellfish from the northern station (74°36’ N), that was the northernmost record of shellfish toxins to date. These results indicated that toxin contamination in shellfish in the sub-arctic north Bering Sea and the Arctic Chukchi Sea shelf and its northern area may be much more widespread and severe than we previously thought. This study highlighted the necessity to monitor potential source toxic algae including their cysts in the Arctic regions.

Disclosure of Interest: None Declared

INDEX OF AUTHORS

(authors are sorted by alphabetical order)

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Alfonso	Amparo	P-304, O-045
Aligizaki	Katerina	O-223, P-226
Allard	Pierre-Marie	P-167
Allen	Andrew	O-138, O-137
Allison	Edward	O-016
Allisson	Astuya	P-131
Alloisio	Susanna	P-270
Almanza	Viviana	O-055
Almazán-Becerril	Antonio	P-056
Alonso	Carlos	O-167
Alonso	Eva	O-045
Alonso Hernández	Carlos M.	O-105, P-209, P-238
Alpermann	Tilman	P-057
Al-Rashed	Wafa'A	P-208
Alsterberg	Christian Alsterberga	O-064
Alvarez	Francisco	P-353, P-124
Alvarez	Gonzalo	P-353, P-345, P-124, P-044
Álvarez	Katia	P-261
Àlvarez	Josep	P-239
Alves-De-Souza	Catharina	O-241, P-318, O-034, O-157, O-216, O-170, P-026
Al-Yamani	Faiza	P-233, O-219, P-208
Amaya	Oscar	O-167, P-134
Amnhir	Rachid	P-020
Amorim	Ana	O-106, O-128
Amzil	Zouher	O-210, O-123, O-057, O-175, O-208, P-016, P-174, P-089, O-209, O-149, P-012, P-085, O-088, O-058, P-148
Andersen	Aaron John Christian	P-141
Andersen	Nikolaj Reducha	P-135
Andersen	Per	P-082, O-116
Anderson	Clarissa	O-255
Anderson	Donald	O-023, O-126, O-142, P-325, O-070, O-102, O-116, O-013, P-057, P-330, O-103
Anderson	Ruth	O-003
Andree	Karl B	O-100, O-129
Andres	John Kristoffer	O-063, O-218
Anestis	Konstantinos	P-189, O-156, O-003
Anglès	Sílvia	O-116, P-028
Anne	Goffart	P-066
Annunziata	Rossella	O-156
Antonopoulou	Maria	O-076
Antunes	Agostinho	O-207, P-205
Anzai	Yoichi	P-342
Aquino-Cruz	Aldo	O-041
Araoz	Romulo	P-240, P-145, O-032, P-200, P-297
Arapov	Jasna	P-090
Araya	Michael	P-353, P-124
Arbeláez	Natalia	P-075
Arce	Paola	P-249

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Archer	Stephen	O-133
Ardilio	Alexis	O-169
Arévalo	Fabiola	P-268
Argyle	Phoebe	O-121
Arias	Claudia	P-252
Arif	Salah	P-247
Arimitsu	Shingo	O-124
Arin	Laura	O-116
Arneborg	Lars	O-006a, P-335, P-061
Arnich	Nathalie	O-210, P-152, O-209
Arriaga Pizano	Andrea Lourdes	P-023
Arsenakis	Minas	P-226
Artigas	Luis Felipe	P-031, O-006b, O-006a, P-030
Asakura	Hiroshi	P-237, P-342
Asencio	Lisbet	O-167
Asnaghi	Valentina	P-079, O-242, P-220
Assuncao	Patricia	P-210, P-313
Astuya	Allisson	P-194, P-138, O-187, P-261, P-316, O-216
Aubel	Mark	P-139
Audebert	Louise	O-011
Avagianos	Christos	O-076
Avello	Veronica	O-187
Ayache	Nour	O-057
Ayres	Daniel	O-240
Azanza	Rhodora	P-328, P-111, O-063, P-115, O-218
Baba	Katsuhisa	O-126
Baba	Toshinori	P-241
Babica	Pavel	P-193, P-204, P-203
Bacchiocchi	Simone	P-128
Bacher	Cédric	O-084, P-042
Bachmann	Roger W.	PL-3
Bachvaroff	Tsevetan	P-184, O-136, O-154, P-178
Backer	Lorraine	O-141
Badis	Yacine	P-249
Baek	Seung Ho	O-194, P-243, P-302, P-076
Bailey	Claire	P-147, P-160
Bailleul	Benjamin	P-289
Baird	Steve	O-150
Baker-Austin	Craig	P-291
Bakoula	Elena	P-315
Bakrač	Ana	P-090
Baldrich	Angela	P-044
Baliarsingh	S.K.	O-098
Banas	Neil	O-239
Band-Schmidt	Christine	O-041, P-356, P-002, P-023
Banga Medjo	Marie Pascale	P-096
Banks	Jonathan	P-142
Barbé	Sabrina	P-201

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Barbosa	Maria	O-186
Barhoumi	Badreddine	P-019
Baricevic	Ana	P-186, P-094, P-068, O-039
Baril	Patrick	O-049
Barkhutova	Darima	O-130
Baroukh	Caroline	O-189
Barranco	Alejandro	O-231
Barreiro	José	P-267
Barrera	Fernanda	O-135
Bartlett	Sarah	O-042
Bassi	Anna Maria	P-270
Basti	Leila	O-234, P-264
Bastianini	Mauro	P-310
Bates	Stephen S	O-166, P-049
Baumann	Kathrin	O-068
Baurain	Denis	P-125
Bayu Intan	Mariana Destila	O-036
Beach	Daniel	O-062, O-245, P-157, O-228, P-295
Bean	Tim	P-291
Becker	Emily	P-250
Beets	Kim	P-125
Belegaud	Jacques	O-209
Belin	Catherine	O-113, O-116
Bellasalaez	F.M	O-009
Belmont	Clara	P-230
Ben Gharbia	Hela	P-060, O-088, P-059
Benbrahim	Samir	P-149, P-163, O-010
Benico	Garry	P-112, P-113, O-111, P-115, P-111, P-116
Ben Mhamed	Abdelouahed	O-010
Bennett	Clayton	P-347
Bennouna	Asmae	P-149, P-163
Benoit	Evelyne	O-046
Benouareth	Djamel-Eddine	P-195
Bensouilah	Mourad	P-247
Beran	Alfred	P-286, P-054, P-103
Berard	Jean-Baptiste	O-189
Berdalet	Elisa	P-081, P-220, P-239, P-332, O-239, O-243, O-141
Bernard	Cécile	O-171, P-011, O-181, O-110, P-179, O-169
Bernard	Olivier	O-189
Berney	Cedric	O-243
Berry	Dianna	P-169
Bersano Filho	José	P-351
Berthold	David	P-100
Bertin	Thomas	P-202, P-152
Bertolotto	Rosella	P-270, O-242, P-220
Bertrand	Samuel	P-085
Besnard	Charlène	P-198
Besoain	Valentina	O-024, O-035, O-022

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Beulker	Camilla	O-132
Bialczyk	Jan	P-257
Bickman	Sarah	O-246, P-133
Bielinski	Vincent	O-138, O-137
Biessy	Laura	O-247
Bilien	Gwenael	P-108, P-249, P-238
Bill	Brian	P-273
Bille	Laura	P-206
Billien	Gwenael	P-016
Binzer	Sofie Bjørnholt	P-141, P-135, O-034, P-158
Birch	James	O-007
Biré	Ronel	O-213, P-219, P-202
Björnerås	Caroline	P-053
Bláha	Luděk	P-203
Blanco	José	P-032
Blanco	Juan	P-353, P-268, P-345, P-056, P-124
Blanda	Elisa	P-067, P-207
Blasco	Thierry	O-058
Blondel	Camille	P-030
Blossom	Hannah Eva	O-161
Bober	Beata	P-257
Bocher	Alan	P-294
Bodi	Dorina	P-146
Bodini	Sergio	O-215
Bojadzija	Gorenka	O-044
Bolch	Christopher	O-014, P-250, P-057
Borbor-Cordova	Mercy	P-329
Borchert	Jerry	O-133
Bordin	Paola	P-206
Borgonuovo	Camilla	O-156
Bormans	Myriam	O-175, O-044, O-208, O-072, O-149, P-012
Borrello	Patrizia	O-242
Borsato	Geovanna	O-107
Botana	Ana	P-162
Botana	Luis M.	O-045, P-102, P-162, P-304, P-144, O-046
Botelho	Maria João	O-232
Bottein	Marie-Yasmine Dechraoui	O-230
Bottein Dechraoui	Marie-Yasmine	O-233
Bouaita	Belkacem	P-200
Bouchez	Agnès	P-186
Bouchouicha-Smida	Donia	P-019
Bougaran	Gael	O-189, P-008
Boulais	Myrina	O-235
Bouly	Jean-Pierre	O-001
Boundy	Michael	O-203, O-247, O-229
Bourgeois	Marc	P-202
Bovee	Toine	P-196, O-082

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Bowers	Holly	O-007
Bowman	John P.	O-030
Boyer	Ariel	O-077
Boyer	Gregory	P-035, P-001, O-163
Braga	Ana	P-349
Bramich	James	O-014
Branco	Maria	O-116
Brandenburg	Karen	O-053, O-152
Brée	Françoise	P-200
Bresnan	Eileen	O-116, P-249, O-239
Briand	Enora	O-175, O-044, P-014, O-149, P-012
Briand	Jean-François	P-168
Bridle	Andrew	P-250
Brient	Luc	O-208, P-324
Brito	Carmen	P-254
Brkic	Boris	P-102
Brochard	Arthur	P-011
Broemsen	Erik L. J. E.	P-018
Brosnahan	Michael	O-006a, O-006b, O-023, P-325, P-061, O-013, O-070
Brown	Tamara	O-114
Brozman	Ondrej	P-204
Brunson	John	O-137, O-138
Bubik	Anja	O-031
Buisson	Didier	P-314
Bulot	Pierre-Yves	O-020
Burford	Michele	O-004, O-239, O-141
Burnell	Craig	O-133
Burrell	Stephen	P-317, O-188
Burris	Devin	P-033
Busse	Aly	P-033
Bustamante	Paco	O-079
Bustillos-Guzman	Jose J.	O-041, P-356, P-002, P-023
Butorovic	Nicolás	P-032
Buttigieg	Stéphane	O-011
Bužančić	Mia	P-090
C. Martins	José	P-258
Cabrini	Marina	P-310, P-286, P-054, P-103
Caceres	Julia	O-024, O-162
Cáceres-Martínez	Jorge	P-265
Caddeo	Tiziana	O-054
Caeiro	Maria Filomena	O-128
Caixach	Josep	P-228, P-272, P-232
Caldeira	Ana Teresa	P-306
Calderón	María José	O-024, O-162
Calfapietra	Anna	P-054
Camacho	Carolina	P-349
Caméan	Ana	P-258
Cameron	Tom	P-038

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Campàs	Mònica	O-100, P-228
Campbell	Katrina	P-298
Campbell	Lisa	O-025
Campos	Alexandre	P-258
Canepa	Antonio	P-062
Canepa	Camille	P-219
Canfield	Daniel E.	PL-3
Cangini	Monica	P-343
Cao	Xihua	O-160, P-307, P-275, O-251
Capellacci	Samuela	P-220, P-310, O-164, O-212
Capper	Angela	P-330
Caramelle	Lucie	P-011
Carbonell	Pamela	O-024, P-044, O-035
Cardol	Pierre	P-289
Carmela	Dell'Aversano	O-212
Carnicer	Olga	O-129, P-062, P-329, P-170
Caron	David A.	O-007, O-086
Caron	Frédérique	P-336
Carpentier	Liliane	P-008, P-016
Carrasco	Daniel	O-135
Carré	Claire	O-110
Carrier	Gregory	O-189
Carrillo-Ovalle	Leonel	P-067, P-207
Caruana	Amandine	O-057, P-014, P-174, P-089, O-149, P-012
Carvalho	Wanderson	P-262
Casabianca	Silvia	P-220, P-310, O-164, O-212
Cascales	Emma	P-255, O-022, O-035
Casellas	Claude	O-209
Casenave	Céline	P-086
Castellon	Laura	O-028
Castrec	Justine	O-235
Castro	David	P-229, P-231
Catasus	Adam	P-218
Catherine	Arnaud	O-181
Cave	Rachel	P-043, P-027
Cécile	Bernard	O-019
Cellamare	Maria	O-181, O-110
Cembella	Allan D.	YL-1, O-196, P-335, P-061, P-070, O-116, P-056, O-006a
Cerasino	Leonardo	O-108, P-315, O-172
Cerino	Federica	P-054
Chabaud	Laurent	P-240
Chahinez	Boualleg	P-066
Chaira	Karima	O-010
Chamero Lago	Donaida	O-105
Chan	Leo Lai	O-198, P-216, P-330, P-175, P-232
Chapelle	Annie	P-042, O-084, O-085
Chardon	Cecile	P-186
Charoensuk	Vichaya	O-248
Charrier	Aurélie	O-189, P-008

Chatelier	Patrice	O-011

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Chatzikonstantinou	Marianna	P-315
Chauton	Mathilde	P-278
Che	Yijia	P-337
Chekan	Jonathan	O-138, O-137
Chen	Meng	P-358
Chen	Nansheng	O-160
Chen	Ying	O-153
Chen	Zhen-Fan	O-052, O-182
Cheng	Fangjin	O-160
Chenouf	Sarra	O-018
Chiantella	Claude	O-210
Chiantore	Maria Chiara	P-079, O-242, P-332, P-220
Chiapetta	Vinicius	O-107
Chinain	Mireille	PL-4, O-123, O-202, O-205, P-223, O-224, P-297, P-192, P-219, P-232
Cho	Byung-Kwan	P-177
Cho	Hoon	P-302
Cho	Sanghyeok	P-177
Cho	Suhyung	P-177
Cho	Yuko	O-168, P-166
Chollet	Simon	O-011
Chomérat	Nicolas	P-108, P-238, P-016, P-230, O-105, O-088
Chorus	Ingrid	O-132
Chrapusta	Ewelina	P-257, O-091
Chrislain Kouadio	Ahi	O-020
Christaki	Urania	P-244
Christophoridis	Christophoros	O-076, O-092
Chu	Jeffrey	O-160
Chuang	Ann	O-004
Chun	Seong-Jun	O-194, P-243
Cigarra	Leonardo	O-135
Claquin	Pascal	O-006b, O-005
Clarke	Dave	P-132, O-008, P-176
Claudepierre	Thomas	O-209
Clausing	Rachel	P-215, P-192, O-105, O-079, O-230
Clément	Alejandro	P-254
Clément	Sofia	P-254
Closa	Daniel	O-174
Cockell	Thaís	O-107
Cohen	Jonathan	O-253, O-148
Colas	Florent	P-294
Colon	Ricardo	P-010
Comar	Alexis	P-324
Comas-González	Augusto	P-238
Combes	Audrey	P-140
Comte	Katia	P-314, P-140, P-179
Conti	Oliveri	O-206
Contreras	Victor	O-135

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Cooke	Aaron	O-190
Cormier	Patrick	O-238
Cornet	Luc	P-125
Corre	Erwan	P-171
Correa	Jorge	P-268
Correa	Nicole	P-254
Correia	Finnick	P-325
Cosgrove	Sarah	P-027
Costa	Margarida	O-048
Costa	Pedro	P-349, P-350
Couet	Douglas	P-059
Coulibaly	Kalpy Julien	P-069
Couté	Alain	O-181
Coyne	Kathryn	P-312, O-252, O-195, O-253, O-148
Crain	Sheila	O-245
Créach	Véronique	O-006b
Crescini	Roberta	P-254
Cruchet	Philippe	P-232
Cruz	Ricardo	P-022
Cuellar Martinez	Tomasa	O-167
Cui	Yingshun	O-194, P-243
Culioli	Gérald	P-168
Curley	Belinda	O-122
Currier	Robert D.	P-033
Cusack	Caroline	P-027, O-069, P-077
Dabrowski	Tomasz	P-077
D'Adamo	Sarah	O-186
Dai	Xinfeng	P-334
Dale	Andrew	P-024
Dalle	Caroline	P-179, O-169
Dammak	Lamia	P-072, P-050
Daneri	Giovanni	P-044
Dao	Ha Viet	P-137
Dao	Than-Son	O-237
Daoudi	Mouna	P-020
Darius	Hélène Taiana	O-202, P-223, O-123, P-232, P-219
Daugbjerg	Niels	O-161, O-034
Davenet-Sbirrazuoli	Valérie	P-081
David	Helena	P-224, O-128
Davidson	Keith	O-134, O-094, O-116, P-121, O-239, P-024, P-101, P-323
Davidson	Thomas	PL-3
Davis	Timothy	O-180
De Blok	Reinhoud	O-006b, P-031
De Castro Alves	L.	P-304
De Haro	Luc	O-204
De La Iglesia	Pablo	P-019
De Marco	Ario	P-286
De Medeiros	Lívia Soman	P-141

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
De Rijcke	Maarten	O-145
De Rijke	Maarten	O-116
De Schamphelaere	Karel A.C.	O-145
De Souza	Ana Paula	P-280
De Souza	Catharina Alves	O-190
De Souza Fernandes	Taynara	P-281
De Vargas	Colomban	P-063, O-243
Dean	Karl	P-357
Dearth	Nicole	P-191
Debusschere	Elisabeth	O-006b, P-031
De'Carli	Gabriela	O-107
Decencière	Beatriz	O-012, O-011
Dechraoui Bottein	Marie-Yasmine	P-215, O-224, P-230, P-192, P-134, P-219, P-163, O-167, O-079, O-105, P-149
Deeds	Jonathan	O-133, O-142, P-273
Dees	Paul	P-024
De La Fuente	Patricia	P-062
Del Campo	Javier	O-243
Del Campo	Miguel	P-296
Delagrange	Marine	P-179
Delcourt	Nicolas	O-209
Delepierre	Muriel	O-032
Delgado	Lorena	P-131
Delgado	Maximo	O-116
Dell'Aversano	Carmela	P-206, O-164, O-143, O-128
Demay	Justine	O-171
Deneudt	Klaas	P-031, O-006b
Deng	Yunyan	O-002
Derkx	François	O-011
Derminio	Dominique	O-163, P-001
Deroubaix	José-Frédéric	O-020
Derrien	Amélie	P-108, P-129, P-148
Desbourdes	Caroline	P-152
Devilly	Leon	P-132
Di Poi	Elena	P-310
Diaz	Mauricio	P-311
Díaz	Patricio A.	P-087, O-050, P-044
Díaz Asencio	Lisbet	O-105, P-230
Dietrich	Daniel	O-027
Ding	Haibing	P-025
Ding	Ling	O-062
Ding	Chi Ying Gary	P-161
Dinh	Khuong	O-237
Diniz	Bruna	O-107
Dinsmore	Donalea	O-042
Diogène	Jorge	O-129, P-062, O-100, P-228, P-170, O-116, P-232
Djediat	Chakib	P-011, O-110

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Djeha	Rosine	P-069
Doblin	Martina	P-227, P-123
Doherty	Owen	O-180
Dom	Inès	O-213
Domínguez	Paula	P-252
Dominguez Cabanelas	Iago Teles	O-186
Domit	Camila	P-344
Donnelly	David	O-248
Dorantes-Aranda	Juan José	O-030, O-216
Doroff	Angela	O-150
Dosso	Mireille	P-069
Doucette	Gregory	O-007, O-023, O-013
Douma	Mountasser	P-306, O-075
Dove	Michael	P-336
D-Q. Jiménez	Leticia	P-258
Draredja	Anis	O-088
Dreyer	Stacia	O-016
Drouet	Kévin	P-081
Dubée	Vincent	P-202
Dubois	P	O-012
Dubreil	Estelle	P-200
Duchnik	Kornelia	P-257, O-091
Ducos	Bertrand	P-179
Duffy	Conor	P-160
Dufour	Simon	P-324
Dugan	Darcy	O-150
Dupras	Jérôme	O-017
Dupuy	Danielle	P-327
Duran-Riveroll	Lorena Maria	O-196, O-041
Durieu	Benoit	P-125
Durimel	Claire	O-131
Dursun	Fuat	P-074
Duval	Audrey	P-108, P-016, P-129, P-148
Duval	Charlotte	O-171, P-011, P-202, O-110, P-179, O-169
Dyer	David	O-142
Dzifa	Denutsui	P-103
Dziga	Dariusz	O-081
Eberhart	Bich-Thuy	O-133
Ebrottié	Julie Estelle	P-069
Echenique-Subiabre	Isidora	O-131
Echterbille	Julien	P-297
Edullantes	Brisneve	P-038
Edwards	Alan	O-120
Edwards	Christine	P-006, O-044, O-068
Edwards	Martin	O-134
Egerton	Todd A.	P-327
Eikrem	Wenche	O-116, P-278, O-006a, P-061

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Eirikson	Finnur	O-048
Ekstrom	Julia	O-016
El Amrani Zerrifi	Soukaina	P-306
El Attar	Jaouad	P-163
Elias	Florence	P-025
Elisabeth	Nezan	P-249
Elleuch	Fatma	O-049
Elliott	Christopher	P-298, P-102
Ellwood	Neil Thomas William	O-040
Elmadani	Faid	O-088
El-Mougi	Dalia	O-248
Enevoldsen	Henrik Oksfeldt	O-227, O-222, O-116, O-167, O-239, P-334, P-235, O-224
Engesmo	Anette	P-278
Enguehard	Sylvain	P-232
Ennaffah	Btissam	O-010
Entfellner	Elisabeth	O-068
Erdner	Deana	O-193, P-330, O-103
Ereño	Amaia	O-231
Escalas	Arthur	O-181, O-110
Escalera	Laura	O-223, O-050
Espinosa	Luisa	P-075
Espinoza-González	Oscar	O-022, O-035, O-024
Estevez	Pablo	P-229, O-211
Estorque	Kit Joshua	O-230
Estrada	Marta	P-239
Evangelista	Sara	P-128
Ezgeta-Balić	Darija	P-354
Faassen	Elisabeth	P-355
Faassen	Els	O-082
Fabiani	Laura	O-215
Fabio	Ricci	O-164
Fabioux	Caroline	O-235
Fadel	Ali	O-183
Faeste	Christiane	P-330
Falaise	Charlotte	O-238
Falciatore	Angela	O-001
Fanara	Tracy	P-033
Farrell	Hazel	O-120, P-027
Fastner	Jutta	O-165, O-072, O-132
Fauchot	Juliette	O-005
Feilke	Kathleen	P-011
Fendri	Imen	O-049
Fensin	Elizabeth	O-241
Fernandes	Kelly	O-221
Fernandes	Luciano F.	P-238, P-351, O-125
Fernandez	Lourdes	O-116
Fernandez	Margarita	P-170
Fernandez	Raul	O-116
Fernández Zabala	Juan	O-151

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Fernandez-Herrera	Leyberth	O-041
Fernández-Tejedor	Margarita	O-129, P-062, O-100, O-223, O-116
Ferrante	Margherita	O-206
Ferrante	Maria Immacolata	O-156
Ferreira	Carlos	O-107
Ferrer	Luis	P-323
Ferriss	Bridget	O-028
Fessard	Valerie	P-197, O-032, P-219, P-199, P-198, P-201, P-200, P-195
Feuillade-Cathalifaud	Geneviève	O-090, P-091
Figueroa	Rosa	P-252
Fimbres-Martinez	Michelle	O-146, P-265
Fiore	Marli	P-125
Fischer	Alexis	P-290, O-070, O-023
Fischer	Andrew	O-014
Fleming	Lora	O-141
Flores	Cintia	P-228, P-272, P-232
Flores	Natalie	P-256
Fluch	Silvia	P-315
Fois	Nicola	O-054
Foord	Charles	O-220
Forest	Bertrand	P-294
Fornasaro	Daniela	P-054
Fortin	Nathalie	O-173
Foss	Amanda J.	P-295, P-139
Fraga	Santiago	P-224
Frame	Elizabeth	O-028
Francé	Janja	O-038, P-292
Franco	Julián	P-075
François	Cyrille	O-238
Frangež	Robert	O-031
Frangopulos	Maximo	P-029, P-097, P-026, P-065, P-122
Freissinet	Catherine	O-012, O-011
Fremy	Jean-Marc	O-209
Frick	Ondraya	O-142
Fricke	Anna	P-220
Frihi	Hocine	O-088
Fuentes	Norka	P-087
Fuenzalida	Gonzalo	O-216, P-255, P-065
Fujii	Ikuo	O-199
Fujita	Masaki	P-241
Fukuyo	Yasuwo	P-113
Fukuzawa	Ryo	P-107
Funaki	Hiroshi	P-107, O-124, O-067, P-217
Furio	Elsa	P-115
Furstenau Plec	Denis	O-012
Fusco	Laura	O-143

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Fuwa	Haruhiko	O-046
Gachon	Claire	P-249
Gago-Martinez	Ana	P-151, P-229, P-231, O-211, P-349
Gai	Frederik	P-251
Gaiani	Greta	O-188, P-147
Gaillard	Sylvain	P-008, P-016
Galindo-Sánchez	Clara	P-056, P-002
Gallardo	Juan José	P-194, P-138, O-187, P-131, P-261, P-316
Gallardo Salas	Rafael	P-132
Galli	Oscar	P-083
Gallisai	Rachele	O-174
Gámez	Tatiana	P-279
Gao	Chunlei	P-358
Gao	Guang	O-043
Gao	Han	O-192, O-159
Gaonkar	Chetan Chandrakant	O-067
Gárate-Lizárraga	Ismael	P-104
Garcés	Esther	O-174, O-116
Garcia	Ernesto	P-022
García Portela	María	O-051
García-Altares	María	O-129, P-170
García-Camacho	Francisco	P-261
Garcia-Jove	Maximo	P-077
García-Mendoza	Ernesto	P-056, O-146, P-265
Garcia-Perez	Josué	P-067, P-207
García-Portela	María	P-015, P-136
Garnier	Matthieu	O-189
Garrido Gamarro	Esther	O-224
Garvetto	Andrea	P-249
Gasparini	Stéphane	P-081, P-260
Gašparović	Blaženka	O-039
Gatti	Clémence Mahana Iti	O-123, O-205, O-202, O-224
Geffard	Alain	O-080, P-355
Geffroy	Solène	P-174, P-089
Geiser	Britta	O-132
Gellene	Alyssa	O-007
Gémin	Marin-Pierre	P-085, O-058
Geng	Hui-Xia	O-089
Georges Des Aulnois	Maxime	O-149, P-012
Gernez	Pierre	O-015
Gerry	Calvin	P-230
Gerssen	Arjen	P-196, O-082
Gerwick	William	PL-5, P-161
Ghedda	Khadija	P-274
Giacobbe	Maria Grazia	O-164, O-212, P-206
Giani	Alessandra	O-173
Giannakourou	Antonia	O-127

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Giard	Marine	O-205
Giddings	Sabrina D.	O-228
Gin	Yew-Hoong Karina	O-093
Giraldez	Jorge	P-151, O-211
Girault-Sotias	Pierre-Emmanuel	P-297
Girisha	Sk	O-064
Giussani	Valentina	P-270, O-242, P-220
Gjeci	Nikolla	P-220
Glaizal	Mathieu	O-204
Glaze	Tom	P-139
Glibert	Patricia	O-185
Glukhov	Evgenia	P-161
Go	Jiyoji	O-234
Gobler	Christopher	P-169, O-139, O-254, O-160, O-239, O-178, P-185, O-180
Godhe	Anna	P-215, O-006a, P-053, O-064, P-061, O-071
Goh	Kau Kiat Kelvin	P-161
Gollnisch	Raphael	P-172
Gomes	Cidália	O-207, P-205
Gomes	Lenora	P-281
Gomez	Fernando	P-031, P-031
Gómez Batista	Miguel	O-105
Gontarek	Steve	O-094
González	Humberto	P-029
González Acosta	Bárbara	P-023
González Vilas	Luis	O-009
González-Gil	Sonsoles	O-050
González-Jartín	Jesús M.	P-304
Gordillo	Mariam	O-116
Gorney	Rebecca	O-163
Got	Patrice	O-110
Goto	Akitoshi	P-341
Gottschling	Marc	P-108
Goutal	Nicole	P-086
Gouveia	Neide	P-228
Grasso	Christopher	O-195
Grattan	Lynn	O-028
Grbec	Branka	P-090
Greenfield	Dianne	P-191
Grégori	Gérald	O-006b
Gregory	Clynton	P-043, P-027
Griffith	Andrew	P-169, O-180
Grobler	Kolette	P-251
Gröger	Matthias	P-335
Grosjean	Philippe	P-030
Gu	Haifeng	O-127, O-083, P-048, O-112, O-119
Gu	Jiarui	P-088
Gu	Song	P-173
Guallar	Carles	P-042

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Guan	Chunjiang	P-334
Guðfinnsson	Hafsteinn	O-116
Guellati	Fatma Zohra	P-247
Guérin	Thierry	P-202, P-152, O-213
Gueroun	Sonia	P-060
Guerrini	Franca	P-245, P-343
Gugger	Muriel	O-109, O-171, P-179, O-169
Guidi-Guilvard	Laurence	P-260
Guilherme	Sofia	P-349
Guillebault	Delphine	P-292, P-293
Guillou	Catherine	P-240
Guillou	Laure	PL-6, P-174, P-289, O-243, P-171
Guitton	Yann	P-167
Gustad	Eli	P-278
Gutiérrez Aguirre	Ion	O-031
Guzmán	L.	P-065
Guzmán	Leonardo	O-024, P-044, O-162, O-022, O-216, O-035, O-087, P-032
Gwinn	Jessica	O-065
Gyingiri Achel	Daniel	P-103
Habibi	Hamid	P-259
Hachani	Mohamed Amine	P-220
Hagino	Kyoko	O-124, O-067
Hagiwara	Etsuko	O-250, P-046
Haigh	Nicky	P-334
Haigh	Nicola	P-266, O-114, O-166
Hakanen	Päivi	P-052
Halafihi	Tuikolongahau	O-121
Hall	Nathan	O-241
Hallegraeff	Gustaaf	O-030, O-238, O-216, P-331, O-222
Hällfors	Heidi	P-028
Hamaguchi	Masami	P-253
Hamilton Vailea	Amy	O-133
Hamlaoui	Sahima	O-181
Hamza	Asma	P-050, P-072
Han	Myung-Soo	O-066, P-273
Han	Xiao-Tian	P-073
Hansen	Per Juel	P-141, P-135, O-161, P-189, O-003, O-034
Haq	Sadeff	O-136
Hardardóttir	Sara	O-033, P-082, O-140, P-188
Hardison	Rance	P-033, O-214
Harke	Mathew	P-169
Harke	Matthew	O-139
Harmel	Tristan	O-015
Harrington	Neil	P-273
Hartung	Thomas	PL-1
Harwood	Tim	O-202, O-247, O-121, O-122, P-198, O-203, O-229
Hashimoto	Kana	P-107, O-124, P-217

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Hastie	Lee	P-323
Hatch	Marco	P-322
Hatfield	Robert	P-291
Hattehrath-Lehmann	Theresa	O-180
Havenhand	Jonathan	O-064
Hawes	Ian	O-247
Hayashi	Aiko	O-030, O-238
Hayashi	Kendra	O-007, O-077, P-290
He	Jie	O-017
He	Liyan	O-160, P-307, P-275, O-251
He	Yan-Bin	P-181
Hébert	Pierre-Alexandre	O-006b
Heck	Kenneth	P-330
Hégaret	Hélène	O-210, P-008, O-235, O-060
Heinze	Lutz	P-143
Helenes-Escamilla	Javier	O-146
Henderson	Rita	O-097
Henderson	Ruth	P-323
Henrichs	Darren	O-025
Henry	Kévin	P-223
Henry	Ted	O-221
Hense	Inga	P-092
Herard	Fanny	P-293
Hernandez	Carlos Alonso	P-330
Hernández	Cristina	O-024
Hernández Albernas	Joan	O-105
Hernandez Butista	Rene	O-048
Hernández Castro	Jesús Ernestina	P-356
Hernández Guerrero	Claudia	P-023
Hernández Sandoval	Francisco	P-356
Hernández-Leyva	Olidia	P-238
Hernandez-Sandoval	Francisco E.	O-041, P-002, P-023
Hernández-Urcera	Jorge	P-015
Hervé	Fabienne	O-057, P-238, P-050, P-014, P-174, P-089, P-085
Hess	Philipp	O-032, O-088, O-123, O-202, O-175, O-147, O-224, P-008, P-016, P-050, P-102, P-136, P-167, P-219, P-223, P-238, P-337
Hessel-Pras	Stefanie	P-201, P-200
Hian	Meghan	P-211
Higuchi	Keita	O-234
Hii	Kieng Soon	P-048, P-049, O-059
Hildebrandt	Jan-Peter	P-204
Hiller	Angelika	P-143
Hilscherová	Klára	P-193, P-263
Himelbloom	Brian	O-021
Hinners	Jana	P-092
Hirae	Sou	P-021
Hirama	Masahiro	O-199

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Hiremath	Shivakumar	P-215
Hiskia	Anastasia	O-212
Hiskia	Anastasia	O-076, O-092
Hmimina	Gabriel	O-012, O-011
Hocine	Frihi	P-066
Hoffmann	Anja	O-132
Hogeveen	Kevin	P-195, P-199, P-198
Holderied	Kris	O-150
Holland	Aleicia	O-060
Holland	Chris	O-241
Holland	William C.	P-033, P-327
Hollanda	Stephanie	P-230
Hondolero	Dominic	O-150
Hong	Yi	O-012, P-086, O-011
Honsell	Giorgio	P-013, O-143
Hoppenrath	Mona	P-123
Hort	Vincent	P-152
Hotta	Ayano	P-342
Hou	Dao-Yi	P-173
Houghton	Erin	O-042
Houliez	Emilie	P-014
Hoyer	Mark V.	PL-3
Hu	Yang	O-078
Hu	Zhangxi	O-002
Hua	Chenfeng	O-192
Huang	Dorothy Yu	O-248
Hubbard	Katherine	O-007
Hubert	Clarisse	P-016
Huet	Sylvie	P-198
Huggett	Megan	P-330
Hugoni	Mylène	O-110
Huguet	Antoine	P-197, P-199, P-200
Huisman	Jef	P-303, P-305, O-072, O-073
Hulot	Florence	O-011
Humbert	Jean-François	O-012, O-011, O-020, O-019, O-131, P-247, P-069
Hunter	Rosa	P-322
Hurtaud-Pessel	Dominique	P-200
Husak	Thomas	P-254
Huysman	Steve	O-145
Hwang	Seungwoo	P-243
Hyndes	Glenn	P-330
Hynes	Paula	P-132, P-176
Ibelings	Bastiaan	O-165, O-072
Ichihashi	Kazuko	P-301
Igarashi	Tomoji	O-244, O-200
Iglesias	Eva	P-097
Igor	Gabriela	P-044
Ihara	Chiho	O-124

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Iliadou	Marina	P-226
Imai	Ichiro	O-250
Imran Khan	Sara	O-097
Ine Van Nieuwenhove	Ine	P-295
Inglez	Jefferson	P-352
Iriarte	Jose	P-029, P-097
Iriarte	Luis	O-162, O-024
Iriarte	Jose Luis	P-122, P-026
Ishikawa	Akira	P-003
Ismael	Amany	P-040, O-223
Isoherranen	Nina	O-028
Ito	Mami	P-237
Ivančić	Ingrid	O-039
Ivanova	Lada	P-330
Iwataki	Mitsunori	P-137, O-112, P-111, P-112, P-113, O-036, O-111, P-114, P-117, P-115, P-116
J Oliveira	Paulo	O-201
Jaffrezic	Enora	P-331
Jaffrézic	Florence	P-192
Jais	Jean-Philippe	P-192
James	Adèle	O-238
Jang	Bora	P-064
Jankowiak	Jennifer	O-139, O-178, P-185
Janssen	Colin R	O-145
Jardillier	Ludwig	P-244
Jardine	Sunny	O-016
Jasser	Iwona	P-098
Jaubert	Marianne	O-001
Jauzein	Cécile	P-081, P-005, O-058, P-220, O-193
Jean	David	O-116
Jenkinson	Ian	P-025
Jenne	Rika	P-321
Jeon	Boo Seong	P-119
Jeon	Che Ok	P-242
Jeong	Hae Jin	P-039, P-118
Jeong	Yujin	P-177
Jeppesen	Erik	PL-3
Jerney	Jacqueline	O-152, O-126, O-053, P-052, O-176
Jex	Aaron	O-004
Jézéquel	Didier	O-110
Ji	Ying	P-337
Jiang	Peng	P-269, O-026
Jiménez-Alesanco	Ana	O-174
Johansson	Emma	P-053
Johansson	Johannes	O-006a
Johansson	Malin	O-006a
LAST NAME	FIRST NAME	ABSTRACT NUMBERS

John	Uwe	O-152, O-140, O-053, O-033, P-335, P-061, P-189, O-003, P-057, P-188
Johnson	Devan	O-114
Jolley	Dianne F	O-060
Jonas	Ibo	O-020
Jones	William	P-191
Jonsson	Per R	O-071
Jørgensen	Kevin	P-141
Jose Dorantes-Aranda	Juan	O-154
Joy West	Jennifer	P-335
Juel Hansen	Per	P-158
Justine	Evrard	P-294
Kaas	Raymond	O-189
Kadiri	Medina	P-319
Kahla	Oumayma	P-019
Kaitala	Seppo	P-282
Kakumu	Akinori	O-250
Kaloudis	Triantafyllos	O-076, O-092, O-212
Kalpy Coulibaly	Julien	O-020
Kam	Oleh	O-020
Kaminski	Ariel	O-091
Kanamori	Makoto	P-339
Kang	Chang-Min	P-299
Kang	Heejun	O-074
Kang	Yoonja	O-180
Kanwit	Kohl	O-133
Kapp	Katrin	P-143
Kappas	Ilias	P-226
Karasiewicz	Stéphane	O-084
Karlson	Bengt	O-116, O-006b, O-006a, P-335, P-061, P-325, O-239
Karray	Fatma	P-019
Karunasagar	Iddya	P-215
Karunasagar	Indrani	P-215, O-064
Kashnitskiy	Aleksander	P-078
Katakis	Ioanis	O-100
Katakura	Seiji	O-115
Kato	Tsuyoshi	O-244
Kawakami	Yoko	O-115
Kawano	Shuichi	P-241
Kayal	Ehsan	P-171
Keafer	Bruce	O-013
Kéfi-Daly Yahia	Ons	O-088, P-060, P-059
Keith	Inti	O-129, P-170, P-062
Kelly	Jonathan	P-132
Kempnich	Michael	O-197
Kendrick	Preston	O-028
LAST NAME	**FIRST NAME**	**ABSTRACT NUMBERS**
Kermagoret	Charlène	O-017
Kerrin	Elliott	O-245, P-157, O-228

Keupers	Ingrid	P-321
Khomutovska	Natalia	P-098
Kibler	Steve	O-150
Kibler	Steven	O-021
Kielosto	Sami	P-028
Kilbourn	Eric	P-001
Kilcoyne	Jane	O-008, P-317, O-188, O-228, P-298
Kim	Daekyung	P-127
Kim	Guebuem	P-095
Kim	Hyung Seop	P-064
Kim	Jin Ho	O-066, P-302, P-076
Kim	Joo-Hwan	O-066, P-273
Kim	Jungmin	P-299
Kim	Minkyung	P-242
Kim	Miran	P-118
Kim	Seog-Ku	O-074
Kim	Sunju	P-246
Kim	Woel-Ae	P-334
Kim	Young Ok	P-076
Kim	Youngmin	O-074
Kim Tiam	Sandra	O-171, P-011, P-179, O-169
Kimoto	Katsunori	P-301
Kinniburgh	David	O-248
Kirilovsky	Diana	P-011, P-179
Kirkpatrick	Barbara	P-033
Kishbaugh	Scott	O-163
Kleiven	Mona	P-278
Klijnstra	Mirjam	O-082, P-196
Klinger	Terrie	O-016
Klun	Katja	P-292
Ko	So-Ra	P-243, P-177
Kobayashi	Atsushi	P-023b
Kobos	Justyna	O-116
Koehler	Henry	O-008
Kogaluru Shivakumaraswamy	Santhosh	P-215
Kohli	Gurjeet Singh	O-154, O-155, O-122, P-189
Koike	Kazuhiko	P-166, P-285, P-021, O-250, P-046
Kojima	Takashi	P-237
Komova	Anastasia	O-130
Kon	Nyuk Fong	O-059
Konan	Felix	P-069
Koné	Mamadou	P-069
Koné	Mariatou	O-020
Kong	Fan-Zhou	O-052, O-182, P-269

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Kong	Xiang	O-214
Konoki	Keiichi	O-168, P-166
Kopp	Benjamin	P-201

Laliberte	Gina	O-042
Lam	Paul K.S.	P-088, P-093, P-225, P-175
Lam	Veronica T.T.	P-225, P-088
Lamb	Philip	P-287
Lambert	Christophe	O-060
Lampen	Alfonso	P-201, P-200
Lance	Emilie	O-208, O-080, P-355, O-209
Lanceleur	Rachelle	P-219, P-195
Lankford	Shelley	O-133
Laplace-Treyture	Christophe	P-293
Lara	Cecilie	P-251
Lara	Yannick	P-125
Larsen	Jacob	O-227
Larsen	Thomas Ostenfeld	P-141, P-135, O-034, P-158, P-189, O-003
Larsson	Michaela	P-123, P-227
Lassudrie	Malwenn	P-336
Latimier	Marie	P-167, O-147
Lau	Winnie Lik Sing	P-048, O-112
Laughinghouse Iv	Haywood Dail	P-125, O-055, P-100
Laurent	Sébastien	P-294
Lauridsen	Torben L.	PL-3
Lavenu	Laura	P-230
Lavery	Paul	P-330
Lavier-Aydat	Louis-Josselin	P-120
Lavigne	Andrea	P-109
Lavison-Bompard	Gwenaëlle	P-152, O-213
Lavrova	Olga	P-078
Law	Ing Kuo	P-048, O-112
Lawton	Linda	P-006, O-044
Laza-Martínez	Aitor	P-224, P-120, O-128
Le	Minh-Hoang	P-086
Le Gac	Mickaël	O-147, P-089
Le Goïc	Nelly	O-235
Le Hégarat	Ludovic	P-201, P-200
Le Manach	Séverine	O-171, O-169
Le Medec	Yves	P-324
Le Tortorec	Anniina H.	O-184
Le Tortorec	Eric	O-184
Leao	Jose Manuel	P-229, O-211
Leaw	Chui Pin	P-048, O-112, O-119, O-059, P-049, P-115
Leboulanger	Christophe	O-110
Lechat	Marc-Marie	P-174
L'Ecuyer-Sauvageau	Chloé	O-017
Lee	Bokjin	O-074
Lee	Bora	O-144

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Lee	Eunju	P-177
Lee	Li Keat	O-119
Lee	Minji	P-243, P-302, P-076
Lee	Sangbum	P-064, P-153

Lee	Sang-Jun	P-299
Lee	Wai Hin	O-198, P-175, P-216
Lee	Yunho	P-242
Lees	David	P-291
Lefebvre	Alain	O-117, O-006b, P-030, P-031
Lefebvre	Kathi	O-028
Lefran	Angéline	O-069
Legall	Patrick	O-210
Legrand	Catherine	P-053, O-176
Lehman	Peggy	P-288
Lehtinen	Sirpa	O-006b, O-006a, P-028
Leite	Isabel	P-352, O-125
Lemaire	Bruno J.	O-183, O-011, P-086, O-012
Lemée	Rodolphe	P-081, P-220, P-005, O-058, P-260, O-141, P-332
Lemoine	Maud	O-113, O-116
Leong	Sandric Chee Yew	O-112, P-115, P-099
Leon-Grooters	Mariel	O-073
Lepoutre	Alexandra	O-208, O-080, P-355
Leroux	Justin	O-017
Lesley	Rhodes	O-247
Letourneur	Franck	P-192
Leung	Priscilla T.Y.	P-088, P-093, P-225
Lewis	Adam	P-291, P-357
Lewis	Bryant	O-133
Lewis	Jane	P-357, O-070, P-101
Leynse	Alex	P-236
Leyva-Valencia	Ignacio	O-041, P-002, P-356
Lhaute	Korian	P-219
Li	Aifeng	P-337, O-062, O-078
Li	Chenlu	O-012, P-086
Li	Dong-Xu	P-182
Li	Hong-Ye	P-340, P-173
Li	Jing	P-093, P-308
Li	Ling	P-175
Li	Ming	O-185
Li	Wenxi	O-093
Li	Xin	P-088, P-093
Li	Zhun	P-105, P-106
Lian	Ziru	P-130
Liang	Zhao Xun	P-161
Liefer	Justin	O-103
Ligia Sousa	Maria	O-048
Lim	Hong Chang	O-066, O-112, P-049, P-115
Lim	Po Teen	O-066, P-048, O-112, O-119, P-049, O-059, P-115, O-239
LAST NAME	**FIRST NAME**	**ABSTRACT NUMBERS**
Lim	Stephanie	O-023
Lim	Weolae	P-095
Lim	Young Kyun	P-302, P-076
Lim	Zhen Fei	O-119
Lima	Carlos Henrique	P-280

Lin	Chin-Hsien (Michelle)	O-185
Lin	Lin	O-153, P-180, P-181, P-183, P-182
Lin	Senjie	P-175
Lin	Shuya	P-358
Lindehoff	Elin	O-184, O-176
Lirdwitayaprasit	Thaithaworn	P-115
Litaker	R. Wayne	O-123, O-021, P-033, P-327, O-105, O-150, P-057, O-119
Litchman	Elena	PL-2
Liu	Chenlin	P-358
Liu	Feng	P-269, P-041
Liu	Jie-Sheng	P-340, P-173
Liu	Jiu-Ling	P-181
Liu	Shuya	O-251
Liu	Si-Qi	P-181
Liu	Tian-Qi	P-183
Liu	Yuyang	O-002
Livingston	Virginia	O-142
Lizon	Fabrice	O-006b, O-006a, P-031
Llanos	Alejandra	P-194, O-187, P-316, P-261
Lochhead	Michael	O-246, P-133
Loeffler	Christopher	P-146
Long	Marc	O-060
Longo	Sébastien	P-223, P-297
Lopes	Vanessa	P-350
López	Loreto	O-022
Lopez-Cortes	David J.	O-041, P-023, P-356
López-Puerto	Laura	O-174
Lopez-Rivera	Americo M	O-135, P-353
López-Rosales	Lorenzo	P-261
Lotliker	Aneesh	O-098
Louchart	Arnaud	O-006b, O-006a, P-030, P-031
Loudiki	Mohammed	P-306, O-075
Loulad	Safia	O-010
Lourencini	Amanda	P-071
Lourencini	Samara	P-071
Loures	Patricia	P-015
Louvard	Clarisse	O-181
Louw	Deon	P-251
Louzao	M. Carmen	P-144, O-046, O-045
Løvberg	Kjersti E.	O-228, P-295, P-150
Low-Decarie	Etienne	P-038
Lowther	James	P-291
Lozano-Leon	Antonio	P-151
Lu	Chung-Kuang	P-216, P-232

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Lu	Mi-Yu	P-340
Lu	Yanan	P-158
Lugliè	Antonella	O-054
Lukomska	Ewa	O-189
Lum	Wai Mun	P-112, P-113, P-114, O-111, P-115, P-116

Lundholm	Nina	P-082, O-140, O-033, P-251, P-019, P-188
Lunghi	Mathias	O-210
Luo	Hao	P-175
Luo	Zhaohe	O-127, P-048
Lurling	Miguel	O-165
Luz	Ligia	P-351
Lv	Xuning	P-275
Maamour	Naima	P-020
Mabin	Christopher	O-014
Macarthur	Melanie	P-147
Macey	Brett	O-220
Mackenzie	Lincoln	P-051, P-142, O-037, O-126, O-239, P-331
Macleod	Eilidh	P-121
Macleod	Jill	O-133
Madesis	Panagiotis	P-315
Maeda	Akiko	P-301
Maeda	Hiroto	O-067
Maeda	Mizuo	O-099
Mafra	Luiz	P-351
Mafra Jr.	Luiz L.	P-238, P-344, P-352, O-125
Magalhães	Valéria	P-259
Maguire	Julie	P-323
Mahfoudhi	Mabrouka	P-050
Maignien	Thomas	O-209
Maillot	Fanny	P-219
Maimouni	Soufiane	O-010
Mairet	Francis	O-189
Maister	Jennifer Mary	O-063, O-218
Mak	Yim Ling	O-198, P-175, P-216, P-093
Makino	Ikuyo	P-023b
Maksylewicz	Anna	O-081
Maldonado	Edio	P-296
Malki	Mohamed	P-274
Mallia	Vittoria	P-156
Malo	Florent	P-008, P-014, P-016, P-174, P-089, O-149
Maman	Luz	O-116, P-323
Man	Xiao	O-004
Manes	C-L	P-292, P-293
Manganelli	Maura	O-206
Mangialajo	Luisa	P-220, P-332
Mann	Stephane	P-314
Manning	Schonna	P-279, P-035
Mansfield	Lisa	O-220
Mantzouki	Evanthia	O-165

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Mao	Xiao-Tong	P-173
Marçal	Raquel	P-349
Marchand	Laetitia	P-014
Marcinek	David	O-028
Mardones	Jorge I.	O-226, O-216, P-044

Marhraoui	Mohamed	P-020
Marić Pfannkuchen	Daniela	O-039, P-094, P-068, P-186
Marie	Benjamin	O-171, P-011, P-202, P-179, O-169
Marie Hjort	Ditte	O-140, P-188
Marin	Roman	O-007
Marini	Mauro	P-310
Mark	Olokotum	O-019
Markussen	Bo	O-161
Maroszek	Magdalena	O-081
Marques	Ana	P-349
Marques	António	P-349, P-228
Marro	Sophie	O-058, P-260
Marsden	Islay	O-121
Martens	Stefan	O-108, P-315
Marti	Alexis	O-190
Martin	Clementine	P-148
Martin	Jennifer L	O-166
Martin-Atienza	Beatriz	P-036
Martinez	Ana	P-110
Martinez	Camila	P-309, P-311
Martínez	Rodrigo	O-024,O-162
Martinez-Vicente	Victor	O-015
Martin-Jézéquel	Véronique	O-057
Martins	Fabiola Chrystian	P-034, P-071
Martins	Leandro	P-352
Martorell	Miquel	P-194
Maskrey	Benjamin	P-155, P-214, P-215
Masseret	Estelle	O-036, O-088
Matarazzo	Matheus	P-017
Mateus	Marcos	P-323
Matsushima	Ryoji	P-338, P-339, P-137, P-264
Matsuura	Keiichi	P-342
Matsuyama	Yukihiko	P-301, P-127
Mattei	César	O-209
Matthijs	Hans C. P.	P-305
Matweyou	Julie	O-021
Maunula	Petri	P-282
Maxwell	Declan	P-006
Mazariegos-Villarreal	Alejandra	P-104
Mazur-Marzec	Hanna	P-053
Mazur-Marzec	Hanne	O-116
Mazzega	Elisa	P-286

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
McCarron	Pearse	P-337, O-062, O-133, O-245, P-157, P-147, O-228, P-330, O-103
McCarthy	Annaclare	P-132, P-176
McCoy	Amy	O-188
McDermott	Georgina	P-043
McGaraghan	Anna	P-290

McGirr	Stephen	O-008, P-176
McGrath	Sara	O-142
McKenzie	Cynthia	O-225, O-166, O-116
McKinney	April	O-116
McKinnie	Shaun	O-138, O-137
McMahon	Kathryn	P-330
Medhioub	Walid	P-050, P-102
Medić	Nikola	O-003
Medlin	Linda	P-284, P-102
Mehic	Sanjin	O-197
Méjean	Annick	P-289, P-140, P-314
Melchiorre	Nunzia	P-270, O-242
Melliti Ben Garali	Sondes	P-019
Melnikova	Anna	O-130
Melo	Claudia	P-194
Mendez	Silvia	O-226, P-110, P-083
Mendoza	Christopher	P-154
Mendoza	Jenelyn	O-063, O-218
Mendoza-Flores	Armando	P-002
Mendoza-Lara	Ana C.	O-041
Mengmeng	Tong	O-192
Mengs	Gerardo	P-102, P-284
Menschel	Eduardo	P-029
Merabet	Rym	P-195
Meriluoto	Jussi	O-081
Meroni	Lorenzo	P-079
Mertens	Kenneth	P-108, P-129
Mesfioui	Abdelhalim	P-149
Meteignier	Claire	P-016
Metian	Marc	O-079
Michalak	Anna	OL
Micheli	Laura	O-215
Micoulaud	Adèle	P-289
Mikulski	Christina	O-007
Milandri	Anna	P-343, P-206
Miles	Christopher O.	O-228, P-295, P-150, P-156, P-330, O-103, P-317
Miles	Zachary	O-137
Miller	Molly	P-330
Miller	Peter	P-323
Miller	Todd	P-256, O-042, P-159
Milligan	Steve	O-116
Millot	Alexis	O-011

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Mills	Toby	P-190
Milz	Beatriz	P-017
Mimura	Katsuya	P-107
Miranda	Lilibeth	P-058
Mitroi	Veronica	O-020, O-019
Miyachi	Kanako	P-107
Moestrup	Øjvind	P-251

Mohamed	Laabir	P-066, O-010
Mohamed Anis	Draredja	P-066
Mohlin	Malin	O-006a
Molgo	Jordi	P-240
Molgó	Jordi	O-046
Möller	Klas	O-006b
Moltke Lyngsgaard	Maren	P-082
Mondeguer	Florence	O-175, P-167, O-147
Montero	Paulina	P-044
Montresor	Marina	O-051, O-223, O-156, O-239, O-243
Moore	Bradley	O-138, O-137
Moore	Kathleen	O-016
Moore	Stephanie	O-016
Moorthi	Stefanie D.	O-086
Moosbrugger	Katharina	O-068
Mora	C.	P-026
Moraes	Adriana	P-259
Moraes	Ágatha	O-107
Morais	Gisele	P-352
Moreira	Cristiana	O-207, P-205
Moreira	Sylvia	P-293
Moreira Gonzalez	Angel Ramon	P-238, P-344
Mori	Akinori	O-126
Moroño	Ángeles	P-268
Morozova	Tatiana	P-334
Morquecho	Lourdes	P-104
Mortelmans	Jonas	P-031
Mortensen	Rebecca	P-191
Mortreux	Serge	O-210
Moscetta	Emilia	O-215
Moscetta	Pompeo	O-215
Mouat	Beth	P-024
Mouget	Jean-Luc	O-238
Mouhri	Khadija	O-075, P-306
Mouton	Anna	O-220
Mozetič	Patricija	O-038, P-292
Mrabet	Charaf	P-059
Muawanah	Tajri	O-036, P-213
Mueller	Nancy	O-163
Mullor	Jose	P-315
Munday	Rex	O-203
LAST NAME	**FIRST NAME**	**ABSTRACT NUMBERS**
Mundhenk	Lars	O-132
Muñoz	Francisca	P-254
Muñoz	Verónica	O-024, O-162
Muñoz-Sobarzo	Lilian	P-316
Murray	J. Sam	O-203, O-229, O-122
Murray	Shauna	O-120, O-066, P-287, P-336, P-187, O-154, P-227, O-155, O-122, P-123, P-057
Mustapa	Nurin Izzati	O-119

Mynott	Sebastian	P-102
Nagae	Mika	O-200
Nagai	Kiyohito	O-234
Nagai	Satoshi	O-115, O-126, P-264, P-057
Naganuma	Daisuke	P-348
Nagasaki	Keizo	O-067, O-158
Nagasoe	Sou	P-301
Naito	Kanako	P-004
Nakajima	Yoshihiro	P-021, O-250
Nakano	Atsumi	O-250
Nakashima	Akie	P-342
Nakayama	Natsuko	P-253
Nakayama	Takahiro	P-023b
Nalca	Aysegul	O-142
Namsaraev	Zorigto	O-130, P-271
Nanjappa	Deepak	O-195, O-253
Naouli	Jaouad	P-149
Nascimento	Silvia	O-107, P-262, O-201
Nasr	Yousef	P-164
Natali	Vanessa	P-054
Naustvoll	Lars Johan	O-116, P-335
Nedoluzhko	Artem	O-130
Neilan	Brett	O-004, P-190
Nelson	Harry	P-288
Neurath	Hartmud	O-132
Neves	Jorge	P-102
Neves	Raquel	O-107, P-262
Neves	Raquel	O-201
Nézan	Elisabeth	P-108
Nguyen	Minh Tue	P-341
Nguyen	Minh-Tuyet	O-032
Ni	Wenfei	O-185
Nicolas	Marina	P-152, O-213
Nicolau	Elodie	O-189
Nielsen	Torkel Gissel	O-033, O-140, P-188
Niero	Cristina	P-017
Nieva	Joyce	O-249, P-084
Nimptsch	Jorge	O-237
Ninčević Gladan	Živana	P-090
Nishi	Noriko	O-115
LAST NAME	**FIRST NAME**	**ABSTRACT NUMBERS**
Nishikawa	Toshio	O-168
Nishimura	Tomohiro	P-107, O-124, O-067, P-217
Nishitani	Goh	P-003
Nishiyama	Yoshitaka	P-009, P-007
Nochit	Phatchada	P-221, P-222
Noh	Won	P-299
Noirmain	Fanny	P-145
Norambuena	Luis	P-255, P-044, O-216
Norbis	Walter	P-083

Nordi	Cristina	P-017
Norlin	Andreas	O-161
Norman	Karma	O-016
Novak	Thana	O-039
Novellino	Antonio	P-270
Novoa	Franco	P-261
Nuel	Gregory	P-192
Nunes Ludolf Gomes	Lenora	P-280
Nuñez-Vazquez	Erick J.	O-041, P-356
Nzeneri	Samuelson	O-085
O´Neill	Alison	P-356
O’Rourke	Eleanor	P-077
Ocana	Asuncion	O-116
Ocelka	Tomas	P-102
O'Connor	Wayne	P-336
Oda	Tatsuya	P-127
Ogawa	Kenta	O-250
Oh	Hee-Mock	O-194, P-243, P-177
Oh	Hye-Cheol	O-074
Ohara	Shizuka	P-285, O-250, P-046
Oikawa	Hiroshi	P-338, P-339, P-137, P-166, O-126
Oja	Johanna	P-028
Okamura	Kazumaro	P-301
Okano	Sho	O-234
Okello	William	O-019
Okolodkov	Yuri B.	O-196, O-129
Okubo	Hirohide	P-237
Okumura	Yutaka	P-339
Okunishi	Suguru	O-067
Olesen	Anna	P-082
Oliveira	Fernanda	O-107
Olivos-Ortiz	Aramis	O-146
Olson	Robert	O-013, O-023, P-325
Omondi Oduor	Steve	P-346
Omoruyi	Osasere	P-319
Omura	Takuo	P-166
Ondrusek	Michael E.	P-327
O'Neill	Alison	P-155
Onodera	Ken-Ichi	O-168

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Orive	Emma	P-224
Orlova	Tatiana	P-334
Oshima	Yasukatsu	P-166
Oshiro	Naomasa	O-199, P-237, O-200, P-342
Osmond	Steven	O-220
O'Sullivan	Ciara	O-100
Otis	Fitz	P-033
Ottaviani	Ennio	O-242, P-220
Ouattara	Aminata	O-020
Ouelad Abdellah	Mohamed Karim	P-020

Ozasa	Kazunari	O-099
Ozawa	Mayu	P-137
Pacheco	H.	P-026, P-065
Pacheco	Hernán	P-029, O-162, O-087, P-255
Pacheco	Mário	P-349
Padedda	Bachisio Mario	O-054
Paerl	Hans	O-043
Pagano	Aldo	P-270
Pagou	Kalliopi	O-127
Pajares-Moreno	Silvia	O-196
Palenzuela	Jesús M T.	O-009
Palleschi	Giuseppe	O-215
Pallier	Virginie	O-090
Pallier	Virgnie	P-091
Palm	Linda	P-103
Palmieri	Luisa	P-315
Pančić	Marina	O-033
Pang	Li Mei	P-161
Panksep	Kristel	O-165
Paredes	Javier	P-309, P-311, P-248
Paredes-Banda	Patricia	P-056
Park	Bum Soo	O-066, P-076
Park	Jeongha	P-118
Park	Jong Woo	P-064, P-080, P-095
Park	Myung Gil	P-119, P-246, P-118, O-144
Park	Seonghwan	P-299
Park	Taegyu	P-095, P-334
Park	Woojun	P-242
Parker	Laura	P-336
Parrow	Matthew W.	P-018
Parsons	Michael	P-212, P-218, O-102, P-211, P-330, O-103
Pasella	Marisa	O-040
Patterson	William	O-065
Pavaux	Anne-Sophie	P-260
Pazos	Yolanda	O-116, P-277, O-009
Peacock	Melissa	P-322
Pedreros	Pablo	O-055
Pelayo-Zárate	María Del Carmen	O-196
LAST NAME	**FIRST NAME**	**ABSTRACT NUMBERS**
Pelin	Marco	O-143
Peltekis	Alexandra	P-289
Penna	Antonella	P-220, P-310, P-206, O-164, O-212, O-128
Pequeño	Ana	P-229, P-231
Peralez	Manon	O-233
Percy	Linda	P-357
Pereira	Vitória	P-349
Perez	Olga	O-173
Pérez Agúndez	José A.	O-018
Perez Calderon	Ruth	O-245
Pérez-Santos	Iván	P-044

Perkins	Marygail	O-163
Perri	Katherine	P-035
Perrière-Rumebe	Myriam	P-016
Perrin	Aurélien	O-011
Petroupoulos	Konstantinos	O-215
Pezzolesi	Laura	O-177, P-245, P-343, P-013
Pfannkuchen	Martin	P-186, P-094, O-039, P-068
Pham	Thanh-Luu	O-237
Piccioni	Francesco	P-086
Pichardo	Ana	P-267
Pichierri	Salvatore	P-245
Pichon	Chantal	O-049
Pichon	Valérie	O-209, P-140
Piel	Tim	P-303, P-305
Piersanti	Arianna	P-128
Pigozzi	Silvia	P-206
Piñeiro-Redondo	Y.	P-304
Pinilla	Elias	O-024
Pino	Roberto	P-353
Pinto	Ernani	O-221, O-034
Pinto	Marco	P-029, P-097
Pinto Torres	M. A.	P-122, P-026
Pípal	Marek	P-263
Pistocchi	Rossella	O-177, P-245, P-343, P-013
Pitcher	Grant	O-220
Pizarro	Gemita	P-044, O-226, O-050, P-097, O-035, O-087, P-032, P-255, P-026, P-065, P-122
Place	Allen	O-136, P-184, P-327, O-154, P-178, P-018
Planquette	Hélène	O-060
Plec	Denis	P-086
Plus	Martin	O-085
Poder	Thomas	O-017
Poelman	Marnix	O-116
Poisson-Caillault	Emilie	O-006b, O-117
Poli	Mark	O-142, O-143
Polikarpov	Igor	P-233, O-219
Pompei	Marinella	P-310, P-343

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Ponce-Rivas	Elizabeth	P-056
Porchetta	Alessandro	O-215
Porntepkasemsan	Boonsom	P-221, P-222
Portier	Liza	P-196
Portillo	Eduardo	P-313, P-210
Pouil	Simon	O-079
Powell Schuster	Kim	O-150
Prato	Maurizio	O-143
Preiss-Weigert	Angelika	P-143, P-146
Priebojová	Jana	P-263
Pringault	Olivier	P-019, P-059
Procházková	Tereza	P-263

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Reis Costa	Pedro	O-061
Reñé	Albert	O-054
Rengefors	Karin	P-172, P-053
Rengel	José	P-345, P-353
Retho	Michael	P-016
Reubrecht	Sébastien	O-175
Réveillon	Damien	O-208, P-148, P-168, P-085
Reverté	Laia	P-228
Rey	Verónica	P-162
Reyes-Salinas	Amada	P-104
Rhimane	Hassan	O-010
Rho	Jung Rae	P-064, P-153
Rhodes	Lesley	P-283, O-203, O-121, O-155, O-122, P-331
Riadi	Hassane	P-020
Rial	Pilar	P-136, P-015
Ribeiro	Matheus	P-017
Ribeiro	Tiago	O-048
Ribera D'Alcalà	Maurizio	O-051
Richard	Marion	O-210
Richlen	Mindy L.	P-330, O-103, O-102
Rijal Leblad	Benlahcen	P-020, O-088
Rijkeboer	Machteld	O-006b, P-031
Rimet	Frédéric	P-186
Rindi	Fabio	O-177
Riobo	Pilar	P-110
Rios	Gisela	P-131
Ríos	Catalina	P-087
Rise	Frode	P-330
Rivas	David	P-265
Rivas	J.	P-304
Rivas	Maria Paz	P-316
Rivas-Camargo	David	O-146
Rivera	Alejandra	P-194, O-187, P-131, P-138
Rivera	Peter Paolo	O-217
Rivera Belmar	Andrea	P-320
Roberston	Alison	P-218
Robert	Elise	O-208
Robertson	Alison	P-214, P-215, O-028, P-236, P-347, P-330, O-065, O-103, O-102
Rodríguez	Francisco	P-224, P-015, P-044, P-136, P-110, P-065, P-252, P-109, O-051
Rodríguez	Inés	P-304
Rohrlack	Thomas	P-346
Rojas	Diego	P-296
Roje-Busatto	Romana	P-354
Rolland	Jean-Luc	O-236
Romagnoli	Tiziana	P-245, O-040
Romanis	Caitlin	P-190
Romano	Filomena	O-156

Romero	Marc Lawrence	P-154
Rongo	Teina	O-122
Rosa	Filipa	O-048
Rosa	Kaianan	P-344, P-352
Rosa	Rui	P-350
Rose	Georg	P-270
Rostan	Julie	P-260
Roué	Mélanie	O-123, O-202
Rourke	Wade	P-266
Roux	Pauline	O-149, P-005
Rouxel	Catherine	O-189
Rovillon	Georges-Augustin	O-210, P-014, P-016, P-174, P-089, P-148, P-238
Ruano	Miguel	P-261
Rubilar	Ignacio	O-135
Rubio	Fernando	P-139
Rudnitskaya	Alisa	O-232
Ruiz	Manuel	P-323
Ruiz	Pablo Flores	P-315
Ruiz	Sara	O-142
Ruiz	Mary Carmen	P-022
Ruíz	Gerardo	P-134
Ruiz Fernández	Ana Carolina	O-167
Ruiz-De La Torre	Mary Carmen	P-036
Ruiz-Olvera	Patricia	O-142
Rundberget	Thomas	O-228
Ruohola	Jani	P-282
Russo	Monia Teresa	O-156
Ruvindy	Rendy	P-287
Ryan	John	O-007
Ryu	Byung-Gon	O-191, P-300
S.R. Gomes	Maria Teresa	O-232
Saad	M	O-012
Sabour	Brahim	P-102
Saburova	Maria	P-233, O-219
Saez	Stephanie	P-254

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Sagebiel	Daniel	O-132
Sagou	Reqia	O-010
Sagristà	Núria	P-228
Sahraoui	Inès	P-019
Saint-Jean	Bruno	O-189
Sainz	M. J.	P-304
Saito	Maki	O-244
Sakamoto	Setsuko	O-126, P-004, P-046, P-334
Sakka Hlaili	Asma	P-019
Salas	Rafael	P-176, P-317, O-188, O-227, P-147, O-069
Saldivia	Marcela	P-254
Salgado	Pablo	O-087, P-065
Salgueiro	Fabiano	O-107
Salmaso	Nico	O-108, P-315, O-172

Sdiri	Khalil	P-192
Séchet	Véronique	P-008, O-015, P-016, P-085, P-136
Sechi	Nicola	O-054
Sedmak	Bojan	O-031
Sefbom	Josefin	O-071
Seger	Andreas	O-238
Seguel	Miriam	O-135, P-309, P-311, P-248, P-044
Sehnal	Luděk	P-263
Seisdedo-Losa	Mabel	P-238
Selander	Erik	O-033
Selwood	Andrew	O-229, P-126, O-203
Semyalo	Ronald	O-019
Seoane	Sergio	P-120
Seong	Kyeong Ah	P-039
Seppälä	Jukka	O-006b, O-006a, P-028, P-282
Sepúlveda	Joaquín	P-296
Serfor-Armah	Yaw	P-103
Servent	Denis	P-145, P-240, P-297, O-032, O-046
Seuront	Laurent	P-025
Shah	Soham	P-259
Shapiro	Jesse	O-173
Sharaf	Najwa	O-183
Sharko	Fedor	O-130
Shea	Damian	O-214
Shikata	Tomoyuki	P-009, P-007, P-004
Shin	Bora	P-242
Shin	Hyeon Ho	P-105, P-106
Shin	Hyeonseok	P-177
Shiomoto	Akihiro	O-115
Shivanagouda Hosagoudar	Shankargouda	P-215
Shum	Sara	O-028
Siano	Raffaele	P-167, O-147, P-081, O-116, P-063, P-249, O-243
Sianturi	Oksto Ridho	O-036

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Sibat	Manoella	O-123, O-202, O-175, O-147, P-016,P-148, P-167
Sibat-Dubois	Manoella	P-136
Sidabutar	Tumpak	O-036
Siemering	Beatrix	O-069, P-077
Sildever	Sirje	O-115, O-126
Silke	Joseph	O-008, P-176, O-116, P-077, P-323, P-160, P-298, O-069
Silva	Alexandra	O-116
Silva	Clarissa	O-107
Silva	Edson	P-262
Silva	Janette	P-316
Silva	Lisangela	P-100
Silva	Macarena	P-131
Silva	Marisa	P-162
Silva	Patricia	P-262
Silva	Rodrigo	O-107

Simis	Stefan G. H.	O-184
Simiyu	Benard	P-346
Simon	Nicolas	O-204
Simon	Stéphanie	P-145
Simons	Véronique	P-125
Simons	Victoria	O-253
Siracusa	Melania	P-128
Sison-Mangus	Marilou	O-197
Sitoki	Lewis	P-346
Six	Gonzague	O-012, O-011
Skejić	Sanda	P-090
Slim	Kamal	O-183
Sloan	Catherine	P-273
Slodysko	Kristen	O-163
Smith	G. Jason	O-007
Smith	Juliette	O-023
Smith	Kirsty	O-247, P-283, P-142, O-037, O-121, O-155, O-122
Smith	Laura	O-148
Smith	Marie	O-220
Smith	Matthew	P-159
Smith	Tyler	O-065, O-102
Smith	Tyler B.	P-330, O-103
Smith	Zacharias	O-163
Smodlaka Tankovic	Mirta	P-094, P-068, P-186, O-039
Smutná	Marie	P-263
Sobrinho	Bruna	P-351
Soler Onís	Emilio	O-106, O-151
Soman De Medeiros	Livia	P-158
Son	Moonho	P-095
Søndergaard	Martin	PL-3
Song	Jialiang	O-078
Song	Simon	O-099
Song	Xiuxian	O-160, P-307, P-275, O-251, P-308
LAST NAME	**FIRST NAME**	**ABSTRACT NUMBERS**
Sorin	Jean-Luc	O-011
Sosa	Silvio	O-143
Sosik	Heidi	O-023, O-013
Souard	Florence	P-167
Soudani	Kamel	O-012, O-011
Soudant	Dominique	O-113, O-084
Soudant	Philippe	O-235, O-060
Souilmi	Fatima	P-274
Sourisseau	Marc	P-063, O-085, P-323
Spada	Emanuela	O-242
Sreekumar	Jeyan	P-326
Stanković	David	O-038
Stefanelli	Mara	O-206
Steiner	Konstanze	P-142
Steinhovden	Kristine	P-278
Stemmann	Lars	O-006b, O-006a

Stenico	Estella	P-125
Steynen	Julie	P-283
Stockwell	Jason	P-256
Straka	Maja	P-090
Strangman	Wendy	O-190, O-170
Stuetz	Richard	O-097
Stumpf	Richard P.	P-033, P-327
Stutts	Whitney	O-133, P-273
Suárez	Pamela	P-267
Suarez-Isla	Benjamin A	O-135
Subrt	Miroslav	P-298
Südfeld	Christian	O-186
Sugimoto	Keita	O-168
Sugita	Noriko	P-342
Suikkanen	Sanna	P-052, O-176, P-028, P-276
Sukarji	Nur Hanisah	O-093
Sun	Yanni	O-198
Sundqvist	Lisa	O-071
Sunensen	Inés	O-226
Sunesen	Ines	P-109
Suska-Malawska	Malgorzata	P-098
Suzuki	Takafumi	P-342
Suzuki	Toshiyuki	P-338, P-339, P-137, O-124, P-113, P-144, P-264
Svahn	Emma	O-176
Svenssen	Daniel Killerup	P-141, P-135, O-034
Švihálková Šindlerová	Lenka	P-193, P-203
Svircev	Zorica	O-081
Swan	Sarah	O-134, P-121
Sychrová	Eliška	P-263
Sylvers	Peter	O-254
Szymczak	Jeremy	P-171

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
T Rodrigues	Elsa	O-201
T. Costa	Sara	O-232
T. Lah	Tamara	O-031
Tabbada	Rhett Simon	P-154, O-230
Tachihara	Katsunori	P-237
Taddei	Lucilla	O-001
Takahashi	Kazuya	O-112, P-137, P-111, P-112, P-113, O-036, O-111, P-114, P-117, P-115, P-116
Takano	Yoshihito	O-158
Takayama	Haruyoshi	P-114
Taleb	Hamid	P-020
Tambosco	Kevin	O-012, O-131, P-247, O-011
Tammert	Helen	O-165
Tamminen	Timo	P-028, P-282
Tan	Lik Tong	P-161
Tan	Suh Nih	P-049
Tan	Toh Hii	O-119

Tanabe	Shinsuke	P-341
Tanaka	Kouki	P-107, O-124, P-217
Tang	Xaingming	O-043
Tang	Yingzhong	O-002, O-160
Tanii	Yuta	P-107, P-217
Tardivel	Morgan	P-294
Tardivo Kubis	Jonás	P-109
Targett	Timothy	O-253
Tarifeño	Estefanía	P-138
Tartaglione	Luciana	P-206, O-164, O-212, O-143
Tas	Seyfettin	O-223
Taylor	Steve	P-326
Tazart	Zakaria	P-306, O-075
Te	Shu Harn	O-093
Tebaa	Lamiaa	P-306, O-075
Tebben	Jan	O-249
Tedetti	Marc	P-019, P-059
Teiguel	Karenina	P-254
Teng	Sing Tung	O-066, O-112, P-049
Tenorio	Cecil	P-124
Ternon	Eva	O-252, O-233
Terrat	Yves	O-173
Terre-Terrillon	Aouregan	P-108, P-129, P-148
Teske	Kinga	O-132
Testai	Emanuela	O-206
Tester	Patricia	O-119, O-021, O-224, O-105, O-150, P-235, O-118, O-123
Tett	Paul	O-134
Teucilide Rivera	Alec	P-314
Tezini Minoti	Ricardo	P-280, P-281
Thebault	Anne	O-210
Thoha	Hikmah	O-036, P-115
LAST NAME	**FIRST NAME**	**ABSTRACT NUMBERS**
Thomas	Krista	O-245, P-157
Thomas	Olivier P.	O-233
Thornsteindottir	Margret	O-048
Thuret Benoist	Helene	O-090, P-091
Thyssen	Melilotus	O-006b
Tibirica	Carlos Eduardo	O-125
Tillmann	Urban	O-127, P-047, P-108, O-249, O-083, P-084, P-317, O-188, P-147, P-273
Tocornal	María Angélica	O-024
Tocornal Ríos	María Angélica	P-320
Todd	Jonathan	O-217
Toebe	Kerstin	P-047, O-083, P-335
Tokunaga	Takahisa	P-301
Toldrà	Anna	O-100
Tomaru	Yuji	O-158
Tomas	C.	P-026
Tomas	Carmelo	P-318, O-190, O-170
Tomlinson	Michelle C.	O-025, P-033, P-327

Tong	Mengmeng	O-159
Torboli	Valentina	P-054
Tores	Frédéric	P-192
Toro	Carolina	P-255, P-029, O-087, P-026, P-065
Torrents	Romain	O-204
Torres	Gadys	O-226, P-329
Torres	Rodrigo	P-097
Totti	Cecilia	O-177, P-245, O-040, P-220
Touibi	Nour	O-110
Touzet	Nicolas	O-008
Tra	Fulbert	O-020
Trainer	Vera	O-239, O-098, P-334, P-273
Tran Khac	Viet	O-012, O-011
Tranchet	Mélanie	O-205
Tremblay	Réjean	O-238
Tri Retno	Dyah Larasati	P-213
Triantis	Theodoros T.M.	O-076, O-092
Tritscher	Angelika	O-224
Tromas	Nicolas	O-173
Troussellier	Marc	O-181, O-110, O-019
Tsuchiya	Shigeki	P-166
Tsumuraya	Takeshi	O-199
Tsyrenova	Dulma	O-130
Tubaro	Aurelia	O-143, P-102
Tucci	Andrea	P-100
Tudó	Àngels	P-228
Tuladhar	Anupama	O-029
Tunin-Ley	Alina	P-168, P-230
Turk Dermastia	Timotej	O-038
Turki	Souad	P-220
LAST NAME	**FIRST NAME**	**ABSTRACT NUMBERS**
Turner	Andrew	P-291, P-356, O-063, O-064, P-155, P-206, P-357, P-024, O-212, P-214, P-215
Turner	Lucy	P-215, O-064
Turquet	Jean	P-168, P-230, P-219
Tušek Žnidarič	Magda	O-038
Tuya Cortes	Fernando	O-106
Uchida	Hajime	P-338, P-339, P-137, O-124
Ueki	Shoko	P-055
Ueyama	Nozomi	O-168
Uhlig	Silvio	P-150, P-156, P-295, P-330
Ujevic	Ivana	P-354
Ulloa	Viviana	P-138
Ung	André	O-202, P-223, P-232
Untung	Sugiharto	P-213
Ünlü	Selma	P-074
Urban	Ed	O-239
Urbatzka	Ralph	O-048
Urbina	Fabiola	P-296
Uribe	Eduardo	P-353, P-345, P-124

Urrutia	Roberto	O-055
Ussar	Siegfried	O-048
Usup	Gires	O-119, O-239
Uvarov	Ivan	P-078
V. Turkina	Maria	P-258
Valbi	Eleonora	O-164
Vale	Carlos	O-232
Vale	Carmen	O-045
Valestrand	Louise	P-278
Valle-Pombrol	Aimee	P-238
Van Acker	Emmanuel	O-145
Van Asten	Mark	P-287, O-096
Van De Waal	Dedmer	O-056, O-152, O-053
Van Der Molen	Lynndal	O-220
Van Herk	Maria	P-305, O-073
Van Nieuwenhove	Ine	P-150
Van Teulingen	Corne	O-073
Vandegehuchte	Michiel	O-145
Vandersea	Mark	O-105, O-150, O-123
Vanhaecke	Lynn	O-145
Vanucci	Silvana	P-343
Vanysacker	Louise	P-321
Varela	Daniel	P-309, P-311, P-248
Varga	Elisabeth	P-141, P-135, P-189, O-003, O-034
Vargas Osorio	Z.	P-304
Vargas-Montero	Maribelle	P-067, P-207, P-110, P-234
Varriale	Fabio	P-206, O-212
Vasconcelos	Vitor	P-258, O-207, P-205, O-048, P-162, P-102
Vásquez-Yeoman	Rebeca	P-265

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Vassalli	Massimo	O-242, P-220
Vasselon	Valentin	P-186
Veen	Arnold	P-031, O-006b
Vega	Karina	P-138
Velo-Suárez	Lourdes	O-050
Venetsanopoulou	Amalia	O-127
Venturini	Enzo	P-324
Venugopal	Mn	O-064
Verdickt	Liesbeth	P-321
Verma	Arjun	O-154, O-155, O-122
Vernoux	Jean-Paul	O-209
Viallon	Jerôme	P-219
Viallon	Jérôme	O-123, O-202, P-223, P-232
Vichi	Susanna	O-206
Vidyarathna	Nayani	O-148
Vieytes	Mercedes R.	P-144, O-045, P-304
Vila	Magda	O-116, P-220, P-239
Vilariño	Natalia	O-045, P-144
Vilariño	Oscar	P-151, P-229, P-231
Villa	Elisa	P-292

Villeriot	Nicolas	O-110
Vinçon-Leite	Brigitte	O-183, P-086, O-011, O-012
Virtanen	Elina	O-184
Visser	Petra	O-165, P-303, O-073, P-305, O-072
Vissio	Anne	P-332
Vitale	Laura	O-156
Viure	Laia	O-116, P-239
Vo	Thi-My-Chi	O-237
Vogel	Jeremy	P-309
Vogelbein	Wolfgang K.	P-327
Volland	Hervé	P-145
Volpe	Giulia	O-215
Vulturius	Gregor	P-335
Waaler	Thor	O-228
Wacquet	Guillaume	O-006b, P-030
Wai	Tak-Cheung	P-088, P-225
Walsh	Debbie	P-132, O-227
Wang	Da-Zhi	O-153, P-180, P-181, P-182, P-183
Wang	Jiangtao	O-179, P-130
Wang	Lei	O-127
Wang	Rui	O-179
Wang	Shuqin	O-078
Wang	Wenling	O-214
Wang	Wentao	P-275
Wang	Yanfei	P-312, O-252, O-195
Wang	Zitong	P-308
Warner	Mark	O-148
Wasmund	Norbert	P-037
LAST NAME	**FIRST NAME**	**ABSTRACT NUMBERS**
Watanabe	Ryuichi	P-338, P-339, P-137, P-144
Weber	Mike	P-102
Weenink	Erik	P-305, O-073
Wei	Bofan	O-163
Wei	Xiao-Meng	P-340
Weisbrod	Barbara	O-027
Wells	Mark	O-098
Wencheng	Li	P-127
Werner	Vera	P-100
White	Emily	P-303
Whyte	Callum	O-094, O-134, P-121, P-024
Wickliffe	Lisa	P-235
Wiegand	Claudia	O-044, O-237
Wietkamp	Stephan	P-047, O-083
Wijffels	René	O-186
Wikfors	Gary	O-218
Wilhelm	Steven	O-043
Williams	Ernest	P-184, O-136, P-178
Willis	Anusuya	O-004
Wilmotte	Annick	P-125, O-055
Wilson	Cara	O-098

Winarti	Andayani	P-213
Wingert	Charles	P-318, O-170
Winterton	Cathy	P-101
Wohlrab	Sylke	O-152, O-140, O-053, O-033, P-189, P-188
Wolfender	Jean-Luc	P-167
Wollman	Francis-André	P-289
Wolny	Jennifer L.	P-327
Won	June	O-099
Wood	Susie	O-247, O-027
Woodhouse	Jason	O-004
Woods	April	O-007
Wright	Bruce	O-021
Wright	Elliott J.	P-295, P-337
Wright	Jeffrey L.C.	O-170
Wu	Jiajun	O-198, P-175, P-216, P-232
Wu	Peng-Fei	P-182
Wu	Zaixing	P-307, P-275
Wu	Zhen	P-175
Wyatt	Timothy	O-050, P-025
Wysocki	Julie	P-145
Xia	Xin	O-214
Xie	Zhang-Xian	O-153, P-181, P-182
Xu	Tianshuo	P-303
Xu	Xin	O-160
Xu	Yixiao	O-103
Xue	Qiaona	O-179
Yaegashi	Yuji	O-168

LAST NAME	FIRST NAME	ABSTRACT NUMBERS
Yahagi	Yuji	P-023b
Yakes	Betsy	O-028
Yamada	Takuma	P-342
Yamaguchi	Haruo	P-217
Yamaguchi	Mineo	O-126
Yamamoto	Shigeyoshi	P-342
Yamasaki	Yasuhiro	P-241, P-127
Yamatogi	Toshifumi	O-067, P-021
Yan	Meng	P-088, P-093, P-225
Yan	Tian	P-269, P-073
Yanagida	Ippei	P-107, O-124, P-217
Yang	Wei-Dong	P-340, P-173
Yano	Ryoko	P-285, O-250, P-046
Yao	Kouamé Eric	P-069
Yasumoto	Takeshi	P-237, O-244, O-200
Yépez-Rendón	Josselyn	P-062
Yéprémian	Claude	O-169, O-181
Yih	Wonho	P-064, P-039, P-153
Yilmaz	Mete	P-164, P-126
Yilmaz	Pelin	O-243
Yim	Shereen	P-058
Yin	Kedong	O-239

Yip	Ki Chun	O-198, P-216
Yiu	Sam K.F.	P-088, P-225
Ylöstalo	Pasi	P-028, P-282
Yñiguez	Aletta	O-063, O-218, P-328
Yoneyama	Hiroyuki	O-250, P-046
Yong	Hwa Ling	O-119
Yoo	Yeong Du	P-064, P-039
Yoo	Yung Du	P-153
York	Robert	P-318, O-170, P-122, O-190, P-026
Yoshida	Hidetsugu	P-339
Yoshii	Shouta	P-217
Yoshioka	Takuya	P-217
Yotsu-Yamashita	Mari	O-168, P-166
Yu	Liying	P-175
Yu	Ren-Cheng	O-089, O-052, O-182, P-269
Yu	Zhiming	O-160, P-307, P-275, O-251, P-308
Yuan	Chun-Juan	P-180
Yuan	Yongquan	O-160, P-307, P-275
Yuasa	Koki	P-009, P-007
Yunes	Joao	P-100
Yurdun	Türkan	P-074
Zabala	Juan	O-106
Zamaydi	Arash	O-101
Zammit	Anthony	O-120
Zamora	Claudia	O-035
Zamyadi	Arash	O-097
LAST NAME	**FIRST NAME**	**ABSTRACT NUMBERS**
Zébazé Togouet	Serge Hubert	P-096
Zenteno	Katherine	O-216
Zenteno-Savin	Tania	O-041
Zhang	Fan	O-185
Zhang	Hao	O-153, P-181, P-182, P-183
Zhang	Peipei	P-308
Zhang	Qing-Chun	O-052, O-182, P-269
Zhang	Shu-Fei	P-181
Zhang	Shu-Feng	O-153, P-180, P-182
Zhang	Xu	O-248
Zhang	Yanwu	O-007
Zhang	Yong	P-181
Zhang	Yue	P-308
Zhao	Wei-Hong	P-073
Zhao	Yue	O-182
Zhou	Mingjiang	P-269
Zhou	Ming-Jiang	O-089, O-052, O-182
Zhou	You-Ping	P-183
Zhou	Zheng-Xi	O-182
Zhu	Jianan	O-251
Zingone	Adriana	O-222, O-223
Zmerli Triki	Habiba	P-059, O-088
Zuccarello	Pietro	O-206

Zumaya-Higuera	Miriam	O-041
Zuñiga	Andrea	P-311
Žužek	C. Monika	O-031
Zykwinska	Agata	P-014

INDEX PER TOPIC

(topics are sorted by alphabetical order)

TOPICS	COUNT OF ABSTRACTS	ABSTRACT NUMBERS
Accumulation and Biotransformation of algal, bacterial and cyanobacterial toxins in aquatic trophic chains	34	O-061, O-062, O-063, O-064, O-065, O-077, O-078, O-079, O-080, O-081, O-082, P-336, P-337, P-338, P-339, P-340, P-341, P-342, P-343, P-344, P-345, P-346, P-347, P-348, P-349, P-350, P-351, P-352, P-353, P-354, P-355, P-356, P-357, P-358
Biological oceanography and limnology of HABs	25	O-006a, O-006b, O-007, O-008, O-009, O-010, O-022, O-023, O-024, O-025, O-026, O-027, P-024, P-025, P-026, P-027, P-028, P-029, P-030, P-031, P-032, P-033, P-034, P-035, P-036
Biotechnology or adding value to toxic and harmful algae	12	O-186, O-187, O-188, O-189, O-190, O-191, P-313, P-314, P-315, P-316, P-317, P-318
Ciguatera and related benthic HAB organisms and toxins	48	O-102, O-103, O-105, O-106, O-107, O-118, O-119, O-120, O-121, O-122, O-123, O-199, O-200, O-201, O-202, O-203, O-205, P-103, P-207, P-208, P-209, P-210, P-211, P-212, P-213, P-214, P-215, P-216, P-217, P-218, P-219, P-220, P-221, P-222, P-223, P-224, P-225, P-226, P-227, P-228, P-229, P-230, P-231, P-232, P-233, P-234, P-235, P-236, P-237
Ecology – from the ecological niche to population dynamics and biogeography	63	O-033, O-034, O-035, O-036, O-037, O-038, O-050, O-051, O-052, O-053, O-054, O-055, O-066, O-067, O-068, O-069, O-070, O-071, O-083, O-084, O-085, O-086, O-087, O-088, O-089, O-194, P-037, P-038, P-039, P-040, P-041, P-042, P-043, P-044, P-045, P-046, P-047, P-048, P-049, P-050, P-051, P-052, P-053, P-054, P-055, P-056, P-057, P-058, P-059, P-060, P-061, P-062, P-063, P-064, P-065, P-066, P-067, P-068, P-069, P-070, P-071, P-072, P-073
Ecology - harmful algae and global change	48	O-146, O-147, O-148, O-149, O-150, O-151, O-162, O-163, O-164, O-165, O-166, O-167, O-180, O-181, O-182, O-183, O-184, O-185, P-074, P-075, P-076, P-077, P-078, P-079, P-080, P-081, P-082, P-083, P-084, P-085, P-086, P-087, P-088, P-089, P-090, P-091, P-092, P-093, P-094, P-095, P-096, P-097, P-098, P-099, P-100, P-101, P-102, P-103
Eco-physiology & cellular biology of harmful algae and cyanobacteria	40	O-001, O-002, O-003, O-004, O-005, O-039, O-040, O-041, O-042, O-043, O-044, O-056, O-057, O-058, O-059, O-060, P-001, P-002, P-003, P-004, P-005, P-006, P-007, P-008, P-009, P-010, P-011, P-012, P-013, P-014, P-015, P-016, P-017, P-018, P-019, P-020, P-021, P-022, P-023, P-023b
Epidemiology, reports of human outbreak cases	3	O-204, P-319, P-320

TOPICS	COUNT OF ABSTRACTS	ABSTRACT NUMBERS
HABs causing aerosol and/or direct contact toxicity	7	O-141, O-142, O-143, O-144, O-145, P-238, P-239
HumanToxicology	18	O-028, O-029, O-030, O-031, O-032, O-209, P-193, P-194, P-195, P-196, P-197, P-198, P-199, P-200, P-201, P-202, P-203, P-204
Impact of microalgae/cyanobacteria on aquatic organisms (incl. fish kills and shellfish mortalities)	23	O-216, O-217, O-218, O-219, O-220, O-221, O-234, O-235, O-236, O-237, O-238, P-254, P-255, P-256, P-257, P-258, P-259, P-260, P-261, P-262, P-263, P-264, P-265
Integrated ecosystem assessment and foresight planning (scenario building, HABs and multi-use offshore platforms, HABs and desalination…)	1	P-323
Medical applications of algae, cyanobacteria and their toxins	6	O-045, O-046, O-047, O-048, O-049, P-240
Microbial interactions (allelopathy, parasites…)	30	O-157, O-158, O-159, O-160, O-161, O-174, O-175, O-176, O-177, O-178, O-179, O-192, O-193, O-195, O-196, O-197, O-198, P-241, P-242, P-243, P-244, P-245, P-246, P-247, P-248, P-249, P-250, P-251, P-252, P-253
Mitigation of HABs	31	O-072, O-073, O-074, O-075, O-076, O-090, O-091, O-092, O-093, O-094, O-250, O-251, O-252, O-253, O-254, O-255, P-299, P-300, P-301, P-302, P-303, P-304, P-305, P-306, P-307, P-308, P-309, P-310, P-311, P-312
Networking activities around HABs : GlobalHAB, Global HAB Status report, ICES-WGs and other initiatives	19	O-222, O-223, O-224, O-225, O-226, O-227, O-239, O-240, O-241, O-242, O-243, P-328, P-329, P-330, P-331, P-332, P-333, P-334, P-335
New major events & exploitation of longtime series (monitoring & case studies)	28	O-113, O-114, O-115, O-116, O-117, O-130, O-131, O-132, O-133, O-134, O-135, P-266, P-267, P-268, P-269, P-270, P-271, P-272, P-273, P-274, P-275, P-276, P-277, P-278, P-279, P-280, P-281, P-282
New tools (omics, lab-on-a-chip, ecotron…)	22	O-096, O-097, O-098, O-099, O-100, O-101, P-283, P-284, P-285, P-286, P-287, P-288, P-289, P-290, P-291, P-292, P-293, P-294, P-295, P-296, P-297, P-298
"Omic" technologies: genomics, transcriptomics, proteomics & metabolomics of HABs	44	O-136, O-137, O-138, O-139, O-140, O-152, O-153, O-154, O-155, O-156, O-168, O-169, O-170, O-171, O-172, O-173, P-165, P-166, P-167, P-168, P-169, P-170, P-171, P-172, P-173, P-174, P-175, P-176, P-177, P-178, P-179, P-180, P-181, P-182, P-183, P-184, P-185, P-186, P-187, P-188, P-189, P-190, P-191, P-192

TOPICS	COUNT OF ABSTRACTS	ABSTRACT NUMBERS
Optical sensors and drone systems for the monitoring of harmful blooms	9	O-011, O-012, O-013, O-014, O-015, P-324, P-325, P-326, P-327
Risk assessment for algal and cyanobacterial toxins	6	O-206, O-207, O-208, O-210, P-205, P-206
Socio-economic impacts of HABs	8	O-016, O-017, O-018, O-019, O-020, O-021, P-321, P-322
Taxonomy	34	O-108, O-109, O-110, O-111, O-112, O-124, O-125, O-126, O-127, O-128, O-129, P-104, P-105, P-106, P-107, P-108, P-109, P-110, P-111, P-112, P-113, P-114, P-115, P-116, P-117, P-118, P-119, P-120, P-121, P-122, P-123, P-124, P-125, P-126
Toxin analysis – Novel detection methods	55	O-211, O-212, O-213, O-214, O-215, O-228, O-229, O-230, O-231, O-232, O-233, O-244, O-245, O-246, O-247, O-248, O-249, P-127, P-128, P-129, P-130, P-131, P-132, P-133, P-134, P-135, P-136, P-137, P-138, P-139, P-140, P-141, P-142, P-143, P-144, P-145, P-146, P-147, P-148, P-149, P-150, P-151, P-152, P-153, P-154, P-155, P-156, P-157, P-158, P-159, P-160, P-161, P-162, P-163, P-164

SPONSORS ACKNOWLEDGMENTS

The Organizing Committee wishes to warmly thank the following institutions for their support and contribution:

INSTITUTIONAL SUPPORTS

AGENCE FRANÇAISE
POUR LA BIODIVERSITÉ
ÉTABLISSEMENT PUBLIC DE L'ÉTAT

FROM RESEARCH TO INDUSTRY
cea

institut universitaire
mer et littoral
FR CNRS 3473

MUSÉUM NATIONAL D'HISTOIRE NATURELLE

International Science Council
SCOR
Scientific Committee on Oceanic Research

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
NOAA
U.S. DEPARTMENT OF COMMERCE

UNIVERSITE
BRETAGNE
LOIRE

Organized in cooperation with the:

IAEA
International Atomic Energy Agency