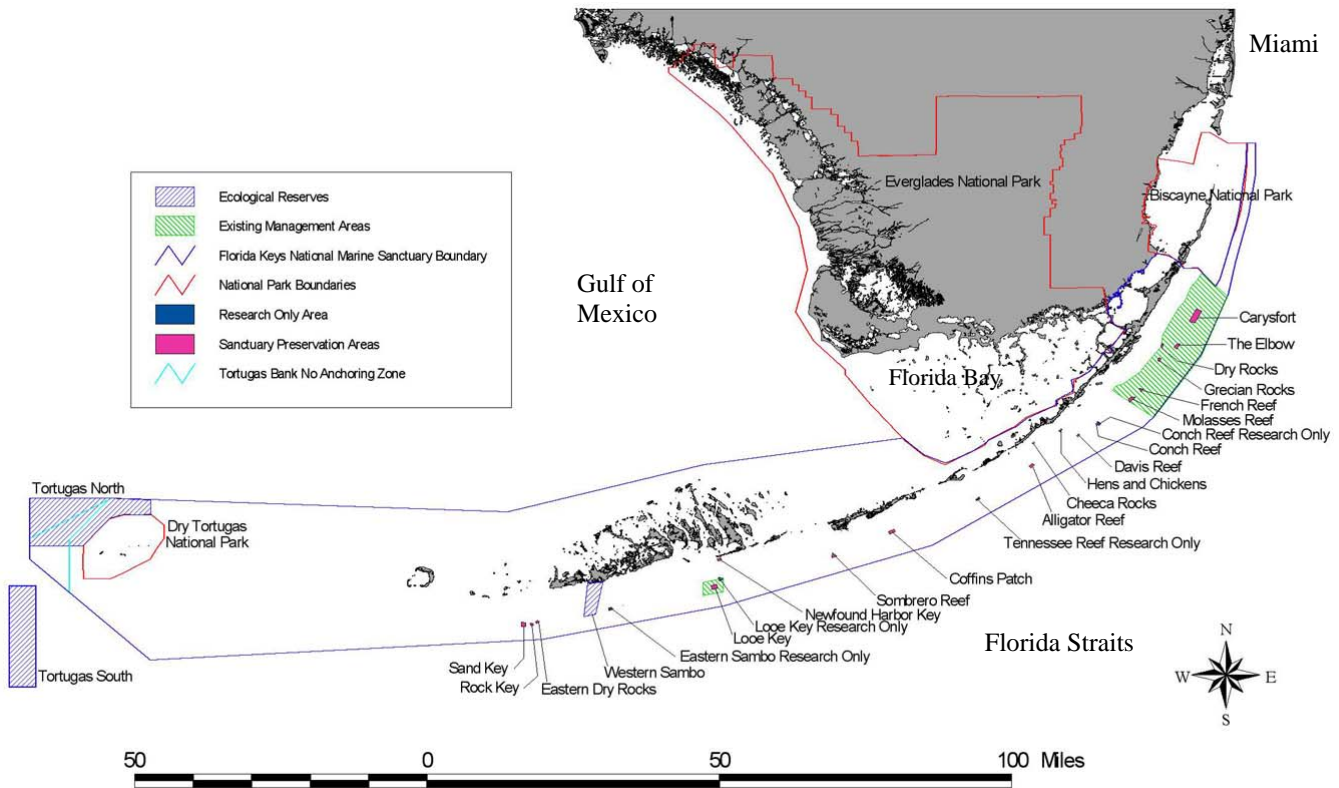


National Oceanic and Atmospheric Administration
Florida Keys National Marine Sanctuary

U.S. Environmental Protection Agency

State of Florida

*Sanctuary Science Report 2001:
An Ecosystem Report Card*



www.fknms.nos.noaa.gov/research_monitoring/welcome.html

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21 March 2003

PREFACE

We are pleased to present a somewhat different report from previous years. For the year 2001, we have added summary reports from most of the presentations at the December 2001 Florida Keys National Marine Sanctuary symposium that was held at NOAA headquarters. As was the case last year, we include brief updates on the Zone Monitoring Program with updates on the long-term monitoring projects of the Water Quality Protection Program (WQPP) to produce this *Sanctuary Science Report 2001*. These two monitoring programs are inextricably related; population and community changes that result from the Sanctuary's fully protected zones occur within the large-scale environmental patterns measured by the water quality, seagrass, and coral reef/hard-bottom community projects of the WQPP.

I thank Nancy Diersing for all the effort she contributed toward compiling and editing this report. We thank the investigators for designing projects and collecting the ecological and socioeconomic data we need to evaluate the condition of the Sanctuary's resources and responses of the ecosystem to management actions.

Brian D. Keller
Science Coordinator

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EXECUTIVE SUMMARY

The coastal ecosystem of South Florida is comprised of an assemblage of interconnected distinct marine environments. Circulation of surface waters and exchange processes, which respond to both local and remote forcings, provide linkages between the different subregions. In addition, the re-circulating current systems that connect the South Florida coastal ecosystem form an effective retention zone for locally spawned larvae.

The transport of water between the Southwest Florida Shelf and the Keys coastal zone occurs through the channels between the Keys. Measurements of the subtidal flows through these passes indicates that the net flow of water is toward the reef tract from the shelf/gulf, with most of the water moving through the Long Key and 7-Mile Bridge Channels. Late in the winter/spring dry season, intrusions of dense salty bay water that take place toward the reef tract may remain as distinct units well past the shallow reef and down to intermediate depths.

Surface drifter data demonstrate that there are three common pathways connecting the entire south Florida coastal ecosystem, specifically the waters off the Southwest coast of Florida to Florida Bay and the Keys. In the winter and spring, the primary pathway of drifters is to the southeast through channels between the Keys. In the fall, the pathway is toward the Tortugas. In the third pathway, typical during summer months, drifters move northwest off southwest Florida to join the Loop Current. After reaching Keys coastal waters, drifters in all three pathways either re-circulate in coastal eddies and wind-driven countercurrents or become entrained in the Florida Current and are moved out of the coastal ecosystem.

Results from the Water Quality Monitoring Project indicate that overall nutrient concentrations were greatest in waters on the gulf side of the Keys and lowest on the ocean side along the reef tract and in the Tortugas region. Inshore waters differed primarily from reef tract waters by having higher concentrations of nitrates. Inshore waters of the less-inhabited Upper Keys exhibited lower nitrate concentrations than the Middle and Lower Keys. Interestingly, inshore waters in the Tortugas area were similar to those of reef tract sites off uninhabited Upper Keys. Essentially, there was no inshore elevation of nitrates in the inshore waters of the Tortugas, supporting the suggestion that the source of nitrates in the Keys is due to shoreline development.

Waters on the gulf side exhibited the highest total phosphorus concentrations and turbidity. Waters on the north side of the backcountry, extending west over the northern Marquesas, exhibited the highest chlorophyll *a* concentrations. This area experiences microalgal blooms most often and is most heavily influenced by advection of Southwest Florida Shelf waters.

The increasing trends observed in total phosphorus and nitrates from 1995 to 2000 in Sanctuary waters were not evident in the 2001 data. Instead, the increasing trend was offset by a marked decline in these variables. It is important to understand, however, that six years of quarterly sampling represents only a narrow window of time relative to natural climatic fluctuations in an ecosystem.

Shallow injection is the most common form of wastewater disposal for tourist-oriented facilities in the Florida Keys. The results of wastewater-derived nutrient studies indicate that despite rapid scavenging of phosphate and extensive denitification, substantial quantities of wastewater

nutrients reached the surface waters of the Keys following shallow wastewater injection. The transport of groundwater contaminated by wastewater to surface waters is facilitated by three factors: the buoyant nature of the wastewater plume, the highly permeable nature of the limestone substrate, and penetration of the substrate by canals.

An artificial tracer study evaluated the capacity for phosphate to be adsorbed by the limestone substrate of the Keys. This study reported that radio-labelled phosphate was rapidly adsorbed onto Key Largo limestone, but the rapid uptake was followed by slower adsorption until equilibrium was reached. Once equilibrium was reached, even the addition of phosphate-free water resulted in a release of previously adsorbed phosphate from the limestone into the surrounding environment.

Seagrass monitoring is designed to identify distribution and abundance of seagrass within the Sanctuary and track changes over time through random stations and fixed sites that are concurrent with water quality monitoring stations. Information about the inter- and intra-annual variability of seagrass cover and abundance has been gained by studying these communities at fixed locations, where some striking trends have been observed. For example, seagrasses were lost completely at 3 of the 30 sites during hurricanes of the last four years. At the remaining 27 sites, the benthic communities remained relatively stable. There were no common trends across the sites in terms of seagrass cover or community composition. This absence of trends can be interpreted to mean that there were no regional trends in the health of the seagrass beds that could be detected with six years of monitoring data. However, manipulative experiments with seagrasses in South Florida suggest that the response of seagrass beds to eutrophication may be on the order of decades. In addition, the results of interactions between humans and the natural seagrass systems are not fully understood.

Seagrass communities in the Sanctuary are negatively impacted when boat operators damage or destroy the plants, especially the underground rhizome system. The result of boating impacts is often the release of unconsolidated sediments into the water column from bank tops. Research studies evaluating seagrass recovery in damaged bank tops indicate that some injuries with considerable sediment disturbance have persisted for ten years. In these physically disturbed banks where sediments have been excavated, primary restoration may be necessary to recover resource losses.

There is general consensus that multiple stressors of coral reefs are contributing to declines observed in corals within the Sanctuary. The Coral Reef Monitoring Project (CRMP) documented a decline in species richness for all habitat types from 1996 to 2001 and a general trend of decline in stony coral cover from 1996 to 2000. However, the decrease from 1999 to 2000 was not statistically significant and preliminary results from 2001 show little, if any, change in coral cover from 2000 to 2001. The significant declines in coral cover observed from 1997 to 1998 and from 1998 to 1999 were concurrent with a severe bleaching event and strong storms including Hurricane Georges in 1998. Disease data by species shows that black band disease peaked in 1998 and "white disease" peaked in coral species in 1997 and 1998. However, the "other" disease category more than doubled between 2000 and 2001 for the four stony coral species that provide 80% of all living coral cover in the Sanctuary. In general, the number of species affected by disease and the incidence of disease infection increased in stony corals from

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1996 to 2001. In the future, the CRMP plans to expand its sampling strategy in order to understand the causes of coral decline and the effects of multiple stressors.

The coral reefs and hard-bottom habitats of the Sanctuary were also sampled using a rapid assessment method. Patterns in the coverage of stony corals, algae, sponges, and the colonial zooanthid, *Palythoa mammilosa*, showed significant differences among four habitat types. Mid-channel reefs, characterized by massive reef-building corals and sponges, exhibited the highest coral cover sampled in the Sanctuary, sometimes reaching 30%. Patch reefs typically yielded the greatest species density and number of reef-building corals. Among the four habitats sampled, juvenile coral densities were generally greater on mid-channel and offshore patch reefs, and lowest in offshore fore-reef habitats, which were mostly dominated by smaller brooding coral species.

Juvenile coral populations were also monitored in sets of permanent quadrats established in fully protected zones and reference sites. Only the Western Sambo Ecological Reserve has shown higher coral recruitment compared with its reference site. Since 1998, there has been a significant increase in recruitment rates at most sites and depths, with the highest rates found at the deeper Lower Keys sites. Both brooding and broadcasting corals recruited successfully, but very few were massive framework-building species. No significant differences in juvenile coral mortality rates have been detected between fully protected zones and reference sites.

Three years of video monitoring of transects from shallow sites were examined to detect patterns in species composition of coral assemblages. At shallow depths, the three fully protected zones exhibited increases in dominance of boulder star corals, *Montastreaea* spp., the only reef framework-building species remaining at this depth since the near extirpation of staghorn coral, *Acropora cervicornis*, in recent years.

Since 1999, scientists have been examining corals at the cellular level, using an integrated Cellular Diagnostic System (CDS) designed to diagnose whether an organism is stressed and to identify likely stressors. The assay, which measures changes in cellular parameters, quantifies whether the structural integrity of the cell is compromised, the type of stress, and whether defenses have been mounted against a particular stress. Results using this bioassay technique enabled scientists to determine whether or a coral population was being stressed by a global stressor such as high sea surface temperatures or by a stressor that is local in nature such as pesticides. When used in conjunction with other technologies and monitoring methods, this biotechnology was able to identify potential stressors. Data collected on *Montastrea annularis* at four sites supported the possibility that coral cellular damage, measured in 1999, resulted from a global stressor (La Niña sea-surface temperature effects). In contrast, in 2000 patterns of these same parameters were radically different and were not correlated with sea-surface temperatures; instead, stresses on corals noted at two sites originated from local impacts. In addition, information from the CDS can be used to make a prognosis of coral health. Levels in a single biomarker allowed the prediction of whether or not a coral colony would bleach with a 96% probability a full six months prior to the observation of bleaching in the environment.

Five years of monitoring of the Sanctuary's fully protected zones indicates that some heavily exploited species exhibit differences in abundance and size between the zones and reference sites. Since protection began in 1997, there has been an increase in the percentage of legal-sized

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spiny lobsters in the Western Sambo Ecological Reserve (WSER), while the abundance of legal lobsters in its reference area is significantly lower. In addition, the mean size of lobsters has been significantly larger in the WSER in both the open and closed fishing seasons. Specifically, the mean size of males on offshore patch reefs of the WSER has increased 10 mm in the last five years. Catch rates of lobsters in traps were higher within WSER than in two adjacent non-reserve areas regardless of year or fishing season. In fact, there were more lobsters caught in WSER traps than in the two non-reserve areas combined. These data suggest that temporary refuge may be afforded to spiny lobsters by this large and spatially diverse ecological reserve. In contrast, no differences in the size of legal-sized lobsters between the smaller sized SPAs and their reference sites were detected, suggesting that the effectiveness of reserves for spiny lobsters is a function of reserve size, location, and the type of habitat protected.

Significant density increases were noted for several exploited reef fish species in fully protected zones vs. reference sites since implementation of the zones. Mean densities of gray snapper, combined grouper, and yellowtail snapper were greater in fully protected zones than in fished sites. Hogfish densities, however, remained higher in fished rather than unfished areas, perhaps because of differences in seagrass habitat. REEF's Advanced Assessment Team calculated reef fish species richness for fully protected/reference site pairs throughout the Sanctuary. In all but 4 of 16 site pairs, fish species richness was greater in the fully protected sites. Examination of the abundance trends for each of 75 species between fished and unfished sites revealed no statistical differences, yet more species increased in abundance in fully protected vs. reference sites.

During the past five years, no significant differences in populations of queen conch in fully protected vs. reference sites have been detected. Conch are distributed in well-defined aggregations that are not entirely encompassed by SPAs, with the majority of adult conch in the Lower Keys, from Looe Key south to Western Sambo Ecological Reserve. From 2000 to 2001, a large amount of recruitment of juvenile conch seems to have taken place throughout the Keys. Two separate teams continue to document very low abundances of sea urchins, especially the long-spined urchin (*Diadema antillarum*). In one study, all sampling locations yielded very low densities of *Diadema antillarum*, although several locations with large-sized urchins and clear effects of grazing were encountered.

Volunteers continue to play a key role in research conducted in the Sanctuary. Under the direction of research scientists and with the support of The Nature Conservancy, The Ocean Conservancy, REEF, and Mote Marine Laboratory, they serve many valuable functions. As part of the Marine Ecosystem Event Response Assessment Project (MEERA), volunteers reported 197 incidents in the marine environment to the MEERA project coordinator, who then evaluated the reports to see if further action was required and notified the proper experts to investigate the situation.

INTRODUCTION

Florida's coral reef tract is one of the largest bank-barrier reef systems in the world. All but the northernmost reefs lie within the boundaries of the National Oceanic and Atmospheric Administration's (NOAA) Florida Keys National Marine Sanctuary (FKNMS). The 9950-km² Sanctuary was designated in 1990 to protect and conserve nationally significant biological and cultural marine resources of the area, including critical coral reef habitats, seagrass beds, hard-bottom communities, and mangrove shorelines.

The ecologically important marine resources of the Florida Keys are being impacted by a variety of stressors, both natural and human-caused. This is evidenced in a decrease in coral cover and species diversity at most reefs and an increase in coral diseases and bleaching in recent years. Boat groundings, propeller scarring of seagrass, accumulation of debris, and improper anchoring practices have been responsible for thousands of hectares of resource damage. Serial overfishing has dramatically altered reef fish and other exploited populations, contributing to an imbalance in ecological interactions that are critical to ecosystem structure and function. Eutrophication and inadequate wastewater and stormwater treatment have degraded nearshore waters. Altered freshwater management regimes have apparently resulted in an increase in plankton blooms, sponge and seagrass die-offs, and fish kills in Florida Bay, which adjoins the Sanctuary.

The Sanctuary addresses these threats using a variety of management programs and by applying regulations that address direct and indirect impacts to coral reef resources. In addition, a network of 24 fully protected ("no-take") zones, which cover approximately 6% of the Sanctuary but protect 65% of shallow bank reef habitats and 10% of coral resources overall, were implemented in 1997 (23 zones) and 2001 (Tortugas Ecological Reserve) to preserve specific areas more completely. Recent, dramatic declines in reef resources highlight the importance of monitoring both status and trends of habitats Sanctuary-wide and changes within the fully protected zones. In addition, empirical cause-and-effect studies are critical to shed light on additional management tactics that will alleviate and improve overall ecosystem health.

To monitor changes occurring in the marine environment of the Florida Keys, the Sanctuary has implemented a comprehensive monitoring program. The objectives of the monitoring program are to establish a reference condition for biological communities and water quality conditions within the Sanctuary. A research program directed at ascertaining cause-and-effect linkages complements monitoring. In this way, research and monitoring ensure the effective implementation and evaluation of management strategies using the best available scientific information.

Monitoring is conducted by many groups, including local, state, and federal agencies, public and private universities, environmental organizations, and trained volunteers. The Sanctuary facilitates and coordinates partnerships with these groups, prioritizes activities, and disseminates relevant findings to the scientific community and to the public.

Monitoring within the Sanctuary occurs at two scales. Comprehensive, long-term monitoring is conducted through the Water Quality Protection Program (WQPP) funded by the U.S. Environmental Protection Agency (EPA), and recently, NOAA, the Florida Department of Environmental Protection, Monroe County/Tourism Development Council, and the Sanctuary

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Friends of the Florida Keys. The WQPP began in 1994 and consists of status and trends monitoring of three components: water quality, coral reefs and hard-bottom communities, and seagrasses. Sanctuary-wide status and trends monitoring is designed to detect large-scale ecosystem changes associated with Everglades restoration and other regional-scale phenomena.

The second scale is associated with the Sanctuary's 24 fully protected zones, which are monitored through the Zone Monitoring Program (ZMP). The goal of this program is to determine whether the zones are effective in protecting marine biodiversity and enhancing human values related to the Sanctuary. Measures of effectiveness include the abundance and size of fish, invertebrates, and algae; and economic and aesthetic values of the Sanctuary to its users and their compliance with regulations. The ZMP includes monitoring changes in ecosystem structure (size and number of invertebrates, fish, corals, and other organisms) and function (such as coral recruitment, herbivory, predation). Human uses and perceptions of zoned areas are also being tracked. In essence, the Zone Monitoring Program (ZMP) is "nested" within Sanctuary-wide status and trends monitoring.

This report presents results from six-seven years of status and trends monitoring under the Water Quality Protection Program and four years of data from the Zone Monitoring Program. Summaries of most of the presentations at the December 2001 FKNMS symposium at NOAA headquarters are also included.

This year's report starts with a description of circulation and exchange of South Florida coastal waters. Sanctuary-wide status and trends monitoring of water quality, seagrasses, and coral reef communities are presented next. A special program that tracks marine occurrences throughout the Sanctuary, the Marine Ecosystem Event Response and Assessment Project, is reviewed next. Individual abstracts that report results from the Zone Monitoring Program follow, grouped by topical area (coral reefs and benthic communities, fish populations, and spiny lobster and queen conch). Two reports on partnerships between the FKNMS and NOAA National Centers for Coastal Ocean Science address a Cellular Diagnostic System using corals and a study of disturbance and recovery dynamics of seagrass-coral banks. This year's annual report concludes with two studies of wastewater-derived nutrients in Florida Keys ground water. The *Sanctuary Monitoring Report 2000* is also available in downloadable format (.pdf) from the FKNMS website at www.fknms.nos.noaa.gov/research_monitoring/welcome.html. We look forward to reporting future years' results and welcome your comments.

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***Title:* Monitoring circulation and exchange of Florida Bay and South Florida coastal waters with real-time data links**

Researchers: Dr. Thomas N. Lee, Rosenstiel School of Marine and Atmospheric Science (RSMAS), University of Miami, Miami, FL; Dr. Elizabeth Johns and Dr. Peter B. Ortner, Atlantic Oceanographic and Meteorological Laboratory, National Oceanic and Atmospheric Administration, Miami, FL.

Goals: The goals of this project are to study the interaction and exchange of Florida Bay with the connecting coastal waters of the Gulf of Mexico and the Atlantic Ocean, and to provide necessary boundary conditions and validation for physical, water quality and biological models.

Methods: Observational methods include bimonthly interdisciplinary surveys over the entire South Florida coastal region including the waters of the Florida Keys National Marine Sanctuary (FKNMS), high-resolution monthly interdisciplinary surveys within Florida Bay, in situ moorings on the Southwest Florida Shelf and Florida Keys coastal zones, shipboard Acoustic Doppler Current Profiler (ADCP) transport transects in the major Keys passes, and bimonthly deployments of satellite-tracked surface drifters in the Shark River discharge plume (Fig. 1).

Findings to Date: The South Florida coastal ecosystem is made up of an interconnected set of distinct marine environments (Fig. 2). Linkages between subregions are provided by circulation and exchange processes responding to both local and remote forcing. We have analyzed new results from a combination of recent shipboard hydrographic surveys, Eulerian current measurements, and Lagrangian drifter trajectories. These results show a high degree of connectivity between these subregions and with remote upstream areas of the Gulf of Mexico (Lee et al., 2002).

The connection between the Southwest Florida Shelf and the Keys coastal zone is provided by transports through the Keys passes. Our ADCP shipboard measurements across passes of the Middle Keys show subtidal transport variations ranging from approximately +300 to -1500 m³/s, with negative values representing flow toward the reef tract and positive toward Florida Bay (Lee and Smith, 2002). Mean transports for each of the four primary Middle Keys passes are shown in Figure 3.

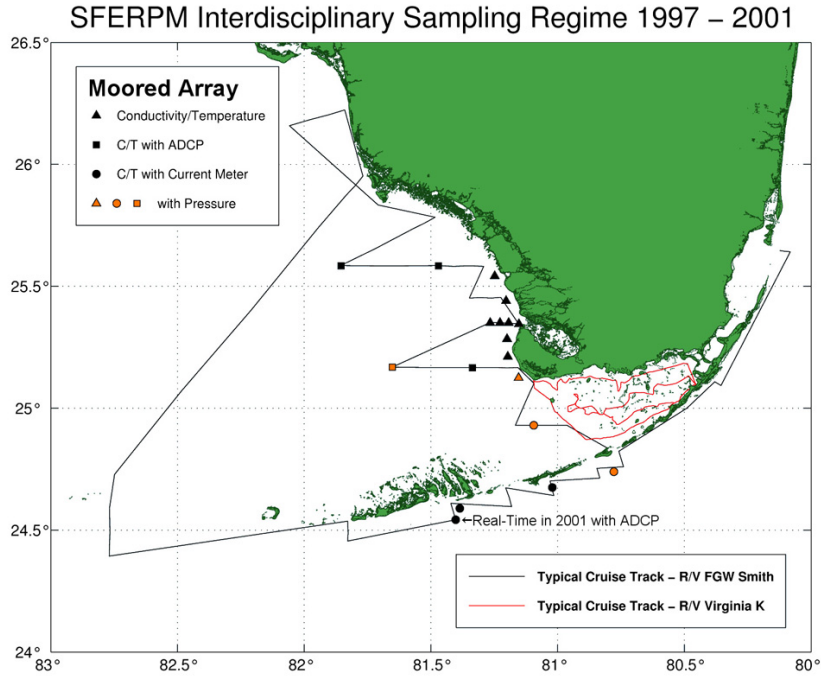


Figure 1. South Florida Ecosystem Restoration Prediction and Modeling (SFERPM) interdisciplinary sampling regime 1997-2001.

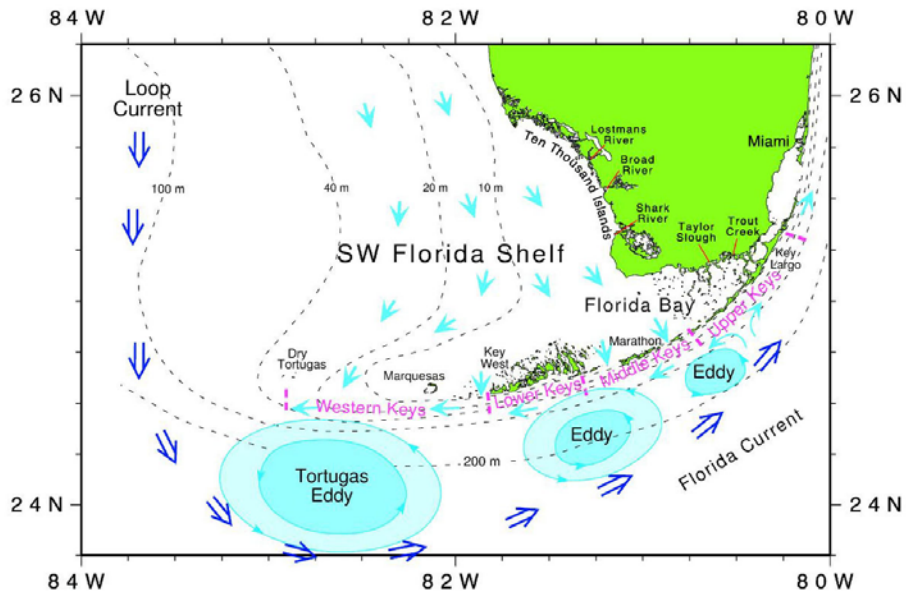


Figure 2. The interconnected distinct marine environments of the Florida Keys.

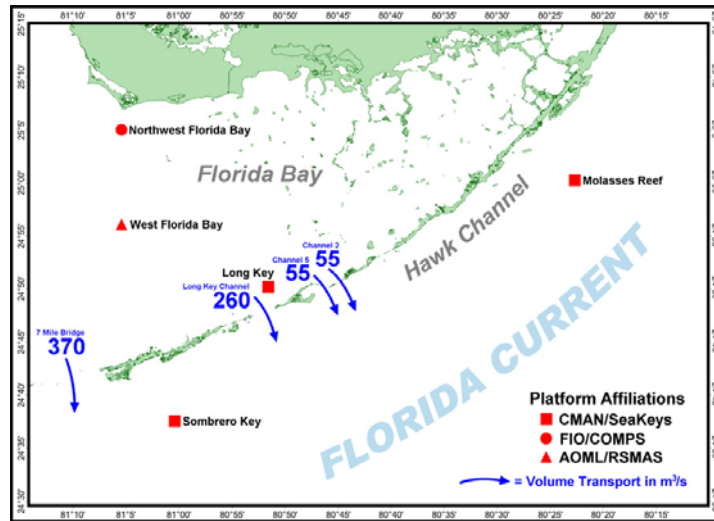


Figure 3. Mean transports for each of the four primary Middle Keys passes (Lee and Smith., 2002).

Late in the dry season (winter/spring), Florida Bay bottom water is often both salty and relatively cool so it is particularly dense. Temperatures on the reef tract are higher than Bay bottom water. This is also typically a season of strong southeastward mean flow. Since Florida Bay nutrient concentrations and turbidity are high compared with the oligotrophic conditions typical outside the Keys, these intrusions represent a potential stress to the reef tract (Porter *et al.*, 1999). We have intermittently observed intrusions during our regional CTD surveys. Figure 4 shows a representative salinity section illustrating the advection of such water across Hawk Channel and onto the reef tract. Interestingly, it remains distinct well past the shallow reef, maintaining its integrity at an intermediate depth appropriate to its density.

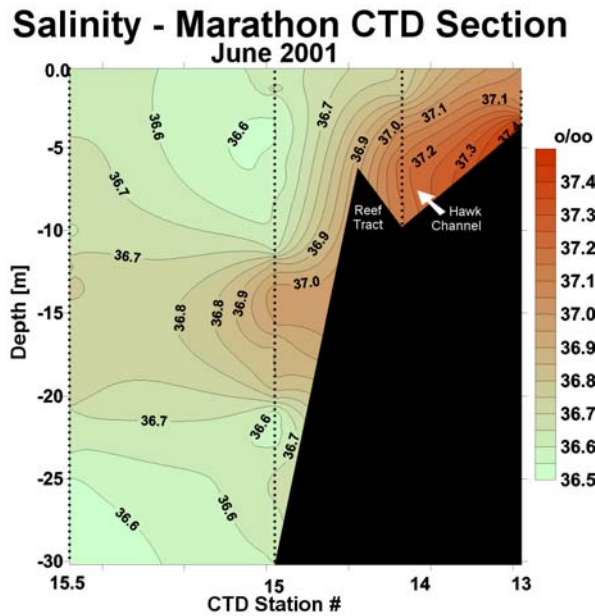


Figure 4. A salinity section illustrating the advection of water across Hawk Channel onto the reef tract. .

Trajectories of satellite-tracked surface drifters (Fig. 5) show that there are three common pathways that connect the entire South Florida coastal system. The first two pathways are either to the southeast and through the passes of the Middle Keys, which is most common during winter and spring, or southwest to the Tortugas during the fall. Advective time scales to reach the Keys coastal zone are one to two months for these routes. The third pathway, more common in the summer, is to the northwest followed by transport to the Tortugas and then eventual entrainment by the Loop Current. This exchange route takes place over a three- to six-month period. After drifters reach the Keys coastal zone they tend to either recirculate in coastal eddies and wind-driven countercurrents for periods of one to three months, or become entrained in the Florida Current and removed from the coastal system (Fig. 6). The combination of recirculating current systems that take part in linking the different subdomains of the South Florida coastal region tends to form an effective retention zone for locally spawned larvae. The varied time scales to circuit the different-sized eddies or coastal recirculations provide larval pathways and opportunities for recruitment from both local and distant sources (Criales and Lee, 1995; Lee *et al.*, 2002).

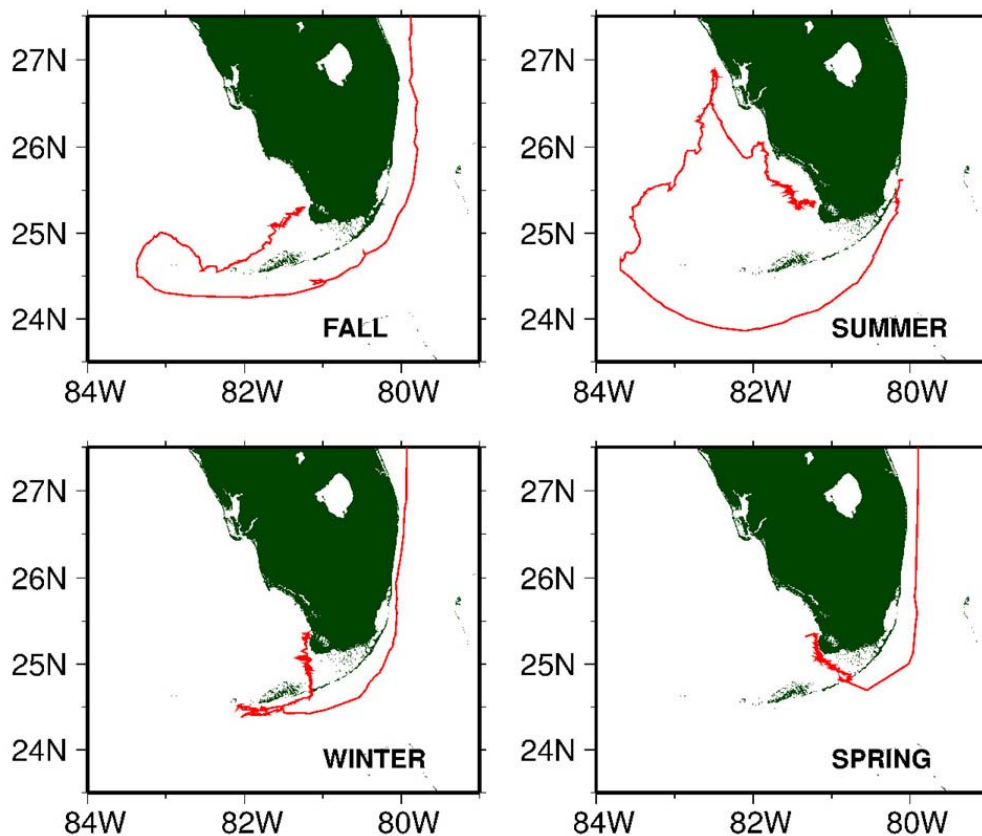


Figure 5. Trajectories of three common pathways that connect the entire South Florida ecosystem.

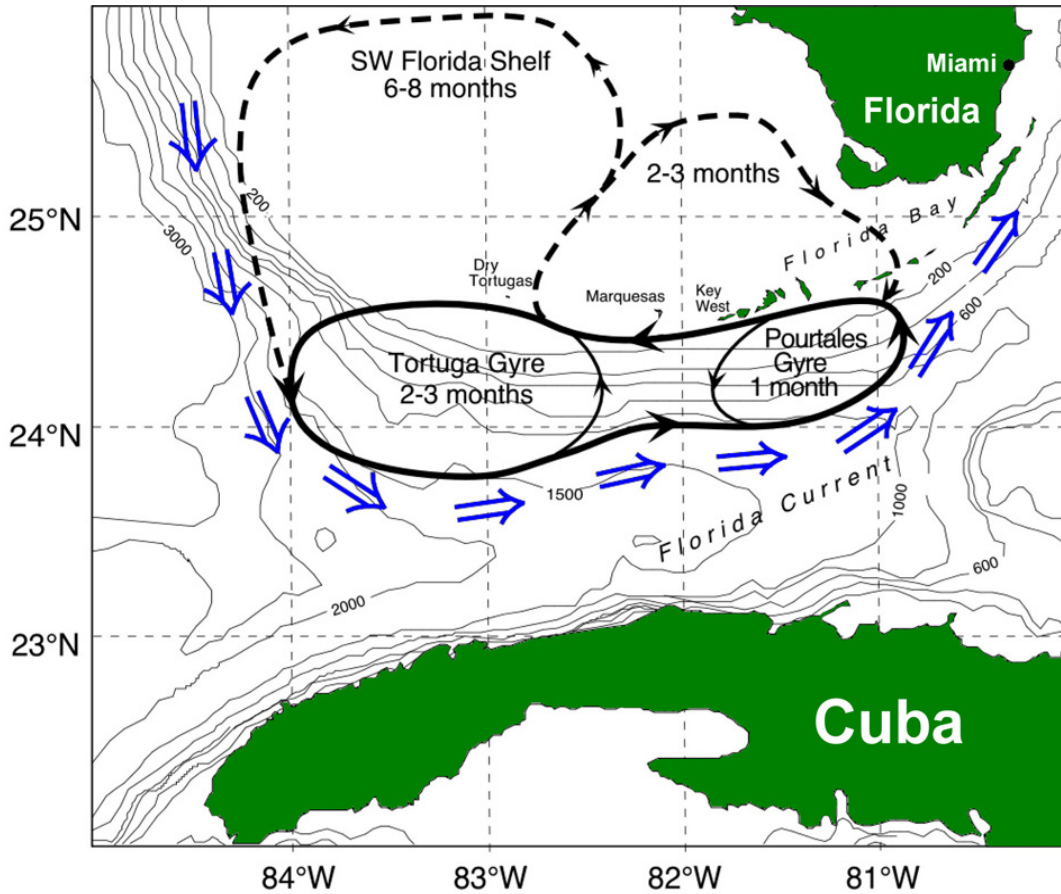


Figure 6. After drifters reach the coastal zone, they either recirculate in coastal eddies and wind-driven countercurrents or become entrained in the Florida Current and removed from the coastal system.

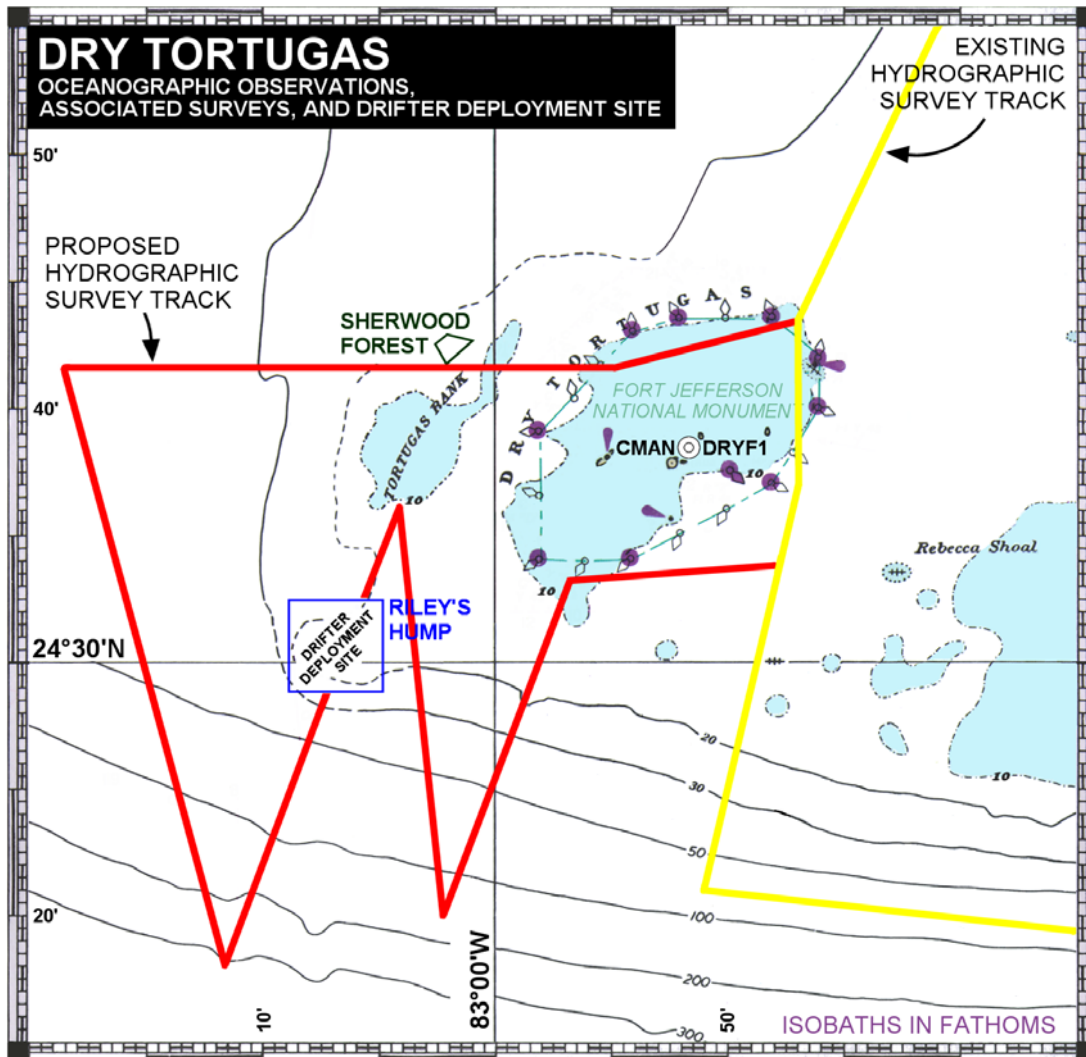


Figure 7. Bimonthly interdisciplinary hydrographic survey of the Tortugas Ecological Reserve, initiated in 2002.

Future plans: Our 2002-2003 measurement program will consist of the same basic elements: a combination of in situ moored measurements on the Southwest Florida Shelf and in the FKNMS, satellite-tracked surface drifter releases at the mouth of the Shark River, and bimonthly interdisciplinary shipboard surveys over the entire South Florida coastal system combined with detailed monthly interdisciplinary surveys of the interior of Florida Bay. The monthly surveys will be expanded in 2002-2003 to include Biscayne Bay. In addition, a new bimonthly one-day interdisciplinary hydrographic survey will be conducted in the coastal waters surrounding the Tortugas Ecological Reserve (TER), and satellite-tracked surface drifter releases will be made in the TER in the vicinity of Riley's Hump, an important larval spawning region (Fig. 7). Key observations of surface trajectories from the surface drifters, along with flow and salinity variability at the seaward edge of the reef tract at Looe Reef and flow and salinity variability in the Seven-Mile Bridge and Long Key Channels, will be transmitted to the laboratory in real time and displayed on a new web site. This real-time observational network has been designed specifically as an early warning system for the FKNMS and the Tortugas Ecological Reserve for intrusions of foreign water masses that could degrade FKNMS water quality or contain harmful algal blooms.

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Project Title: Florida Keys National Marine Sanctuary Water Quality Monitoring Project

Researchers: Ronald D. Jones and Joseph N. Boyer, Southeast Environmental Research Center (SERC), Florida International University, Miami, FL.

Goals: The Water Quality Monitoring Project for the Florida Keys National Marine Sanctuary (FKNMS) is part of the Water Quality Protection Program. The goal of this large-scale monitoring program is to assemble a holistic view of broad physical, chemical, and biological interactions occurring over the South Florida hydroscape. Water quality monitoring can be used as a tool for answering management questions and developing new scientific hypotheses, such as “Is water quality better or worse than it used to be?” This monitoring program, based on quarterly sample intervals, has revealed significant spatial trends in nutrients as described below, and we expect to see more trends in other variables as the database grows.

Methods: This project began in March 1995 and includes data collected from 25 quarterly sampling events at 154 stations within the FKNMS, including the Dry Tortugas National Park. Since initiation we have added four sampling sites and adjusted six others to increase coverage in Sanctuary Preservation Areas and Ecological Reserves. Field parameters measured at each station include salinity, temperature, dissolved oxygen (DO), turbidity, in situ chlorophyll *a* fluorescence, and light attenuation (K_d). Water chemical variables measured at each station include the dissolved nutrients nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), and soluble reactive phosphate (SRP). Total unfiltered concentrations of organic nitrogen (TON), organic carbon (TOC), phosphorus (TP), and silicate ($\text{Si}(\text{OH})_4$) are also measured. The monitored biological parameters included chlorophyll *a* (CHLA) and alkaline phosphatase activity (APA).

Findings to Date: We have found that water quality monitoring programs composed of many sampling stations situated across a diverse hydroscape are often difficult to interpret due to the “can’t see the forest for the trees” problem. This makes it difficult to see the larger, regional picture or to determine any associations among sites. In order to gain a better understanding of the spatial patterns of water quality of the FKNMS, we attempted to reduce the complicated data matrix into fewer elements, which would provide robust estimates of condition and connection. To this end we developed an objective classification analysis (OCA) procedure, which grouped stations according to similarities in water quality.

The OCA we used was a multivariate statistical protocol, which used 12 water quality variables at each site as fingerprints that were then grouped according to similarity. The result was the deconvolution of 150 stations into 8 clusters of stations with distinct water quality signatures (Fig. 1). We believe this is a more functional zonation of the FKNMS than geographical because it is driven by physical, chemical, and biological aspects of the water column.

The bulk of the stations fell into five clusters (1, 3, 5, 6, and 8), which described a gradient of water quality throughout the FKNMS. Although the differences among them were subtle, they were statistically significant. OCA allowed us to say that the overall nutrient gradient, from highest to lowest concentrations, was Cluster 8 > 5 > 6 > 3. Clusters 3, 6, and 5 were distributed widely

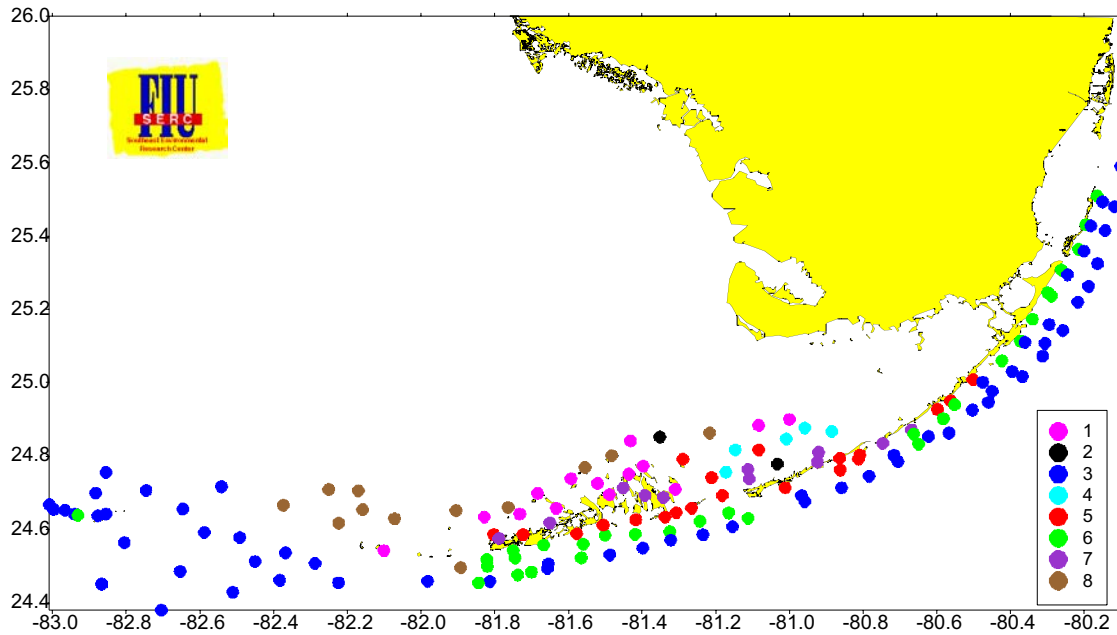


Figure 1. Sampling locations and cluster membership for all 154 sites in the FKNMS.

throughout the Atlantic side of the Keys and Tortugas while Clusters 1 and 8 were present only on the gulf side of the Keys. The stations in Cluster 3, located on the reef tract and Tortugas, had the lowest nutrient concentrations of any other group (Fig. 2). This was followed by Clusters 6 and 5, which were driven mainly by increasing NO_3^- concentrations. Inshore stations of the less-inhabited Upper Keys exhibited lowest alongshore NO_3^- levels compared to the Middle and Lower Keys. Interestingly, NO_3^- concentrations in the single Tortugas transect were similar to those of reef tract sites in the Upper Keys, i.e., there was no inshore elevation of NO_3^- in the transect off uninhabited Loggerhead Key. We suggest this source of NO_3^- in the Keys is due to shoreline development.

Cluster 1 was composed primarily those stations located within the Backcountry area. Along with Cluster 8, it was highest in TP and turbidity. Cluster 8 was made up of stations on the north side of the Backcountry extending west over the northern Marquesas and was highest in CHLA. This is the area most heavily influenced by advection of Southwest Florida Shelf waters.

Unlike last year's findings, trend analysis of the time-series data was unremarkable. The increasing trends in TP and NO_3^- for 1995-2000 have been offset by a marked decline in those variables during 2001 sampling events. It is important to understand that six years of quarterly data collection represents a narrow window of time relative to natural climatic fluctuations of the ecosystem and that significance of trends may come and go as data is added to the series. Much information has been gained by inference from this type of data collection program: major nutrient sources have been confirmed, relative differences in geographical determinants of water quality have been demonstrated, and large-scale transport via circulation pathways have been elucidated. For more information, please see our website <http://serc.fiu.edu/wqmnetwork/> to access data, reports, and integrated graphical analyses of the entire SERC water quality network (FKNMS, Florida Bay, Whitewater Bay, Biscayne Bay, Ten Thousand Islands, and Southwest Florida Shelf).

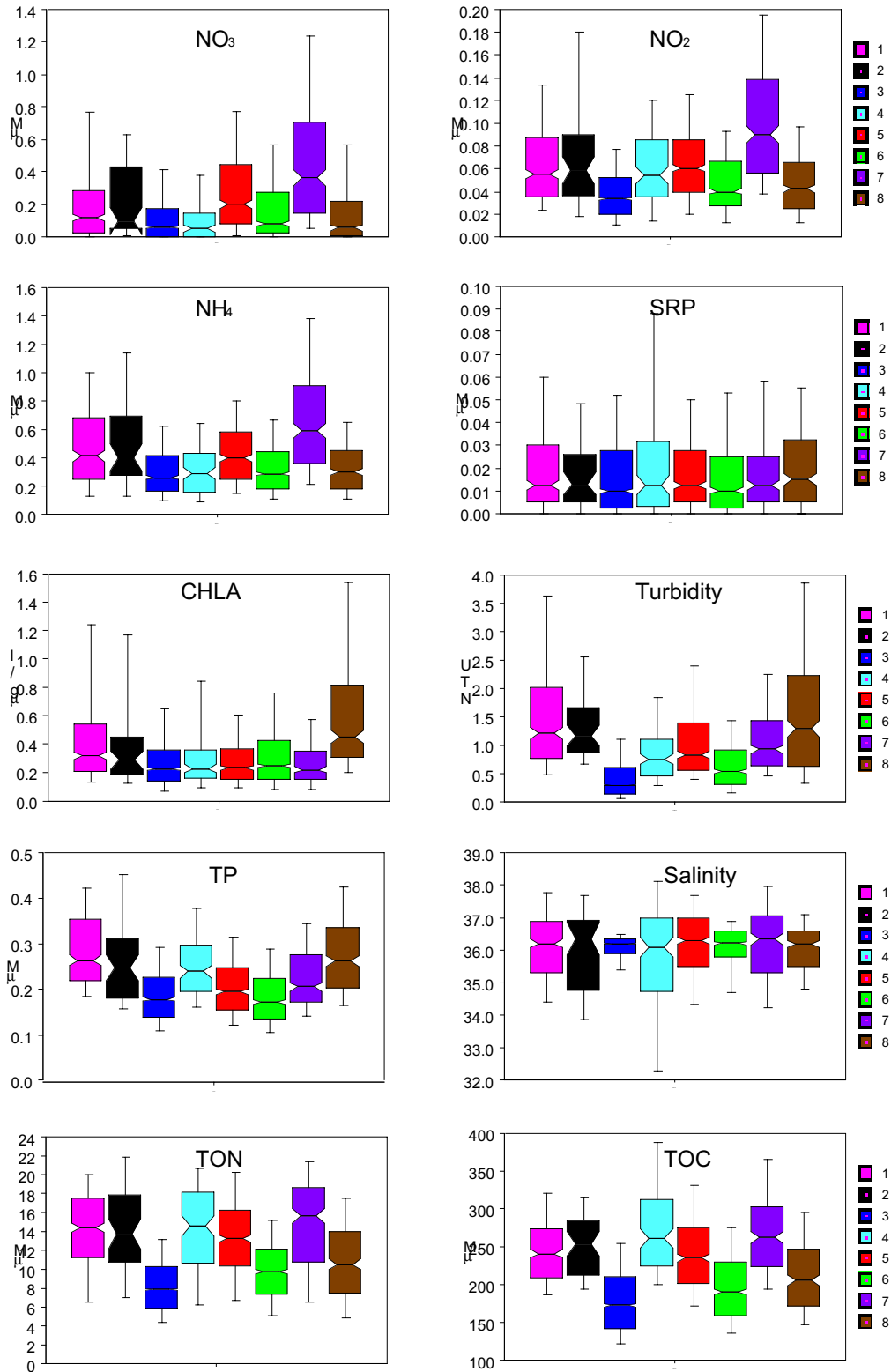


Figure 2. Median and range of variables stratified by cluster. Units are μM (nutrient and TOC concentrations), $\mu\text{g/L}$ (CHLA concentration), Normal Turbidity Units (turbidity), and Practical Salinity Units (salinity).

Project Title: Seagrass Monitoring in the Florida Keys National Marine Sanctuary FY2001

Researchers: James W. Fourqurean, Southeast Environmental Research Center and Department of Biology, Florida International University, Miami, FL; Michael J. Durako, Center for Marine Science and Department of Biology, University of North Carolina at Wilmington, Wilmington, NC; and Joseph C. Zieman, Department of Environmental Science, University of Virginia, Charlottesville, VA. **Project Managers:** Susie P. Escorcia and Leanne M. Rutten, Southeast Environmental Research Center and Department of Biology, Florida International University, Miami, FL.

Goals: The general objective of seagrass monitoring in the Florida Keys National Marine Sanctuary (FKNMS) is to measure the status and trends of seagrass communities to evaluate progress toward protecting and restoring the living marine resources of the Sanctuary. The scope and depth of this monitoring effort are without precedent or peer for seagrass ecosystems throughout the world. Specific objectives are: 1) To provide data needed to make unbiased, statistically rigorous statements about the status and temporal trends of seagrass communities in the Sanctuary as a whole and within defined strata; 2) To help define reference conditions in order to develop resource-based water quality standards; and 3) To provide a framework for testing hypothesized pollutant fate/effect relationships through process-oriented research and monitoring. In order to meet these objectives, we have developed these goals for the project:

- Define the present distribution of seagrasses within the FKNMS;
- Provide high-quality, quantitative data on the status of the seagrasses within the FKNMS;
- Quantify the importance of seagrass primary production in the FKNMS;
- Define the baseline conditions for the seagrass communities;
- Determine relationships between water quality and seagrass status;
- Detect trends in the distribution and status of the seagrass communities.

Methods: To reach these goals, four kinds of data are being collected in seagrass beds in the FKNMS:

1. Distribution and abundance of seagrasses using rapid assessment Braun-Blanquet surveys;
2. Demographics of the seagrass communities using leaf-scar counting and population demographics techniques;
3. Seagrass productivity of the dominant species of seagrass in the FKNMS (*Thalassia testudinum*) using the leaf-mark and harvest method;
4. Seagrass nutrient availability using tissue concentration assays.

These data are being collected at three different types of sites within the FKNMS. Level 1 Stations are sampled quarterly for seagrass abundance, demographics, productivity, and nutrient availability. These stations are all co-located with Water Quality Monitoring Project stations (Fig. 1). Level 2 Stations are randomly selected locations within the FKNMS, sampled annually for seagrass abundance, demographics, and nutrient availability. Each year, new locations for Level 2 stations are chosen. Level 3 Stations are also randomly selected locations within the FKNMS, sampled annually for seagrass abundance. Each year, new locations for Level 3 stations are chosen.

We are assessing both inter-annual and intra-annual trends in seagrass communities. The mix of site types is intended to monitor trends through quarterly sampling at a few permanent locations (Level 1 sites) and to annually characterize the broader seagrass population through less intensive, one-time sampling at more locations (Level 2 and 3 sites).

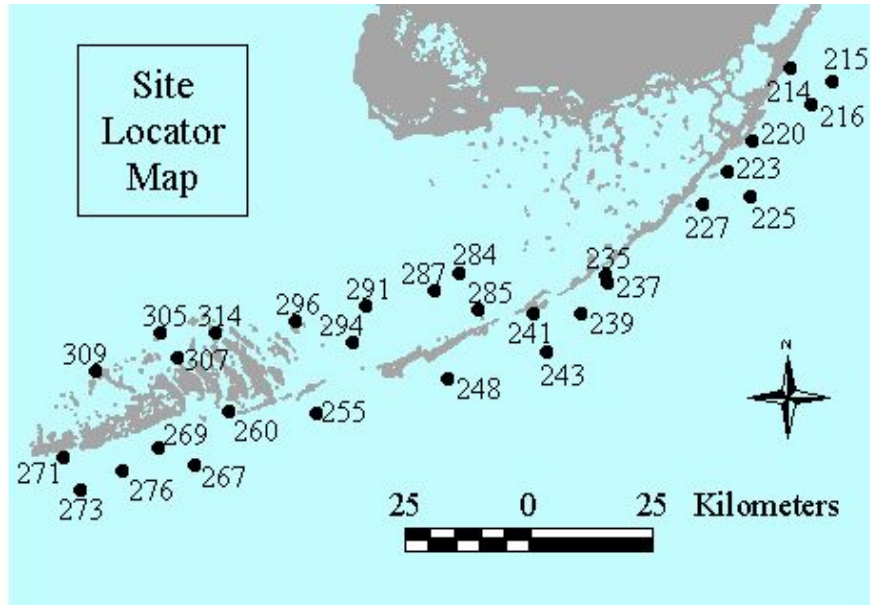


Figure 1. Location of Level 1 seagrass status and trends monitoring sites in the Florida Keys National Marine Sanctuary. Site numbers correspond to water quality monitoring locations.

In 1997, we reported data from quarterly collections from 28 permanent (Level I) stations. In cooperation with the FKNMS Zone Monitoring Program, two additional permanent Level I stations were established in the Western Sambo Ecological Reserve and Carysfort Sanctuary Preservation Area, bringing the total number of permanent monitoring stations to 30. During FY 1996 through FY 2000 summer sampling of Level 2 and 3 sites was conducted during May - August, and the number of sites visited each year is listed below (Table 1). Level 2 and Level 3 sampling was suspended in FY 2001, and will not be done in FY 2002. However, we will begin resampling the Level 2 and Level 3 stations in FY 2003. In FY 2003, we will resample the 206 stations sampled in 1996; in FY 2004, we will resample the 224 stations sampled in 1997; and so on until all stations have been resampled. This will provide over 1400 pairwise comparisons of the status of benthic communities over a seven-year interval. More intensive sampling of stations within 1 km of shoreline, the zone most likely to be affected by anthropogenic factors, was completed in FY 2001 using additional funding from the US Army Corps of Engineers as a part of the Florida Keys Carrying Capacity Study. This intensive nearshore sampling will be used to augment the Level 2 and Level 3 stations sampled during 1996-2000 to provide more accurate assessments of the benthos of the FKNMS.

Table 1. Number of Level 2 and 3 stations sampled from 1996 through 2000.

	1996	1997	1998	1999	2000
No. Level 2 Stations	65	87	82	78	70
No. Level 3 Stations	141	187	191	258	260
Total No. Stations	206	274	273	336	330

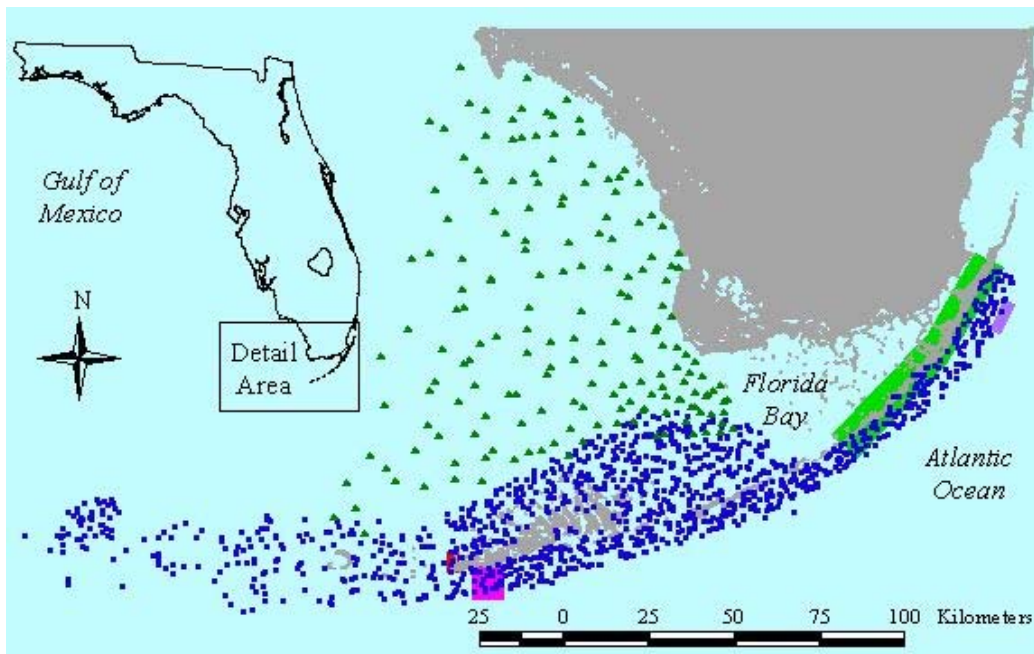


Figure 2. Location of Level 3 samples taken during FY 1996-2000. Colored polygons indicate areas of intensive surveys; green triangles indicate sampling on the Southwest Florida Shelf outside of the boundaries of the Sanctuary.

We have compiled time series data and spatial maps from the monitoring effort for the period 1996 - 2001 both on the web (<http://www.fiu.edu/~seagrass>) and on a CD. These formats allow for easy navigation through the data using Internet browsing software, either by site for time series graphs or by taxa for distribution data. Limited numbers of the CD are available; please make a request for a copy to Jim Fourqurean at fourqure@fiu.edu.

Findings to Date: Our surveys have provided clear documentation of the distribution and importance of seagrasses in the FKNMS. The seagrass bed that carpets 80% of the FKNMS is part of the largest documented contiguous seagrass bed on earth. These extensive meadows are vital for the ecological health of the FKNMS and the marine ecosystems of all of South Florida. Maps of spatial distributions can be found on the web or CD.

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Synoptic surveys completed to date clearly describe the spatial extent of the seagrass beds, but these surveys were not designed to elucidate trends at this point because sites were chosen randomly each year. Rather, the original EMAP protocols call for revisiting the exact sites in a second round of sampling. We propose that this second round of sampling be postponed until FY 2003 because possible changes in the seagrass communities are expected to occur over this longer time scale. Second-round data will allow for the direct comparison of the status of the seagrass communities at over 1000 sampling points. Further, the original sampling design was adequate to address questions at the scale of the FKNMS as a whole, but it did not place enough emphasis on the very-nearshore (within 500 m of the waterline) regions where anthropogenic effects are likely to be concentrated. We have begun to address this nearshore region with a cooperative project funded by the US Army Corps of Engineers as part of the Florida Keys Carrying Capacity Study. In the future, the monitoring effort will need to be adjusted to put more emphasis on this region.

Our permanent monitoring sites have provided valuable data on the inter- and intra-annual variability of seagrass cover and abundance. Time series of species composition, seagrass productivity, nutrient availability and physical parameters can be found for each permanent monitoring site on the web site or the CD. There have been some striking trends in the seagrass communities at these permanent sites: seagrasses were lost completely at 3 of the 30 sites during hurricanes over the last four years. At the remaining 27 sites, the benthic communities are relatively stable. There are no common trends across the sites in seagrass cover or community composition. This can be interpreted to mean that there are no regional trends in the health of the seagrass beds represented by the permanent monitoring sites that can be detected with the six years of monitoring data. However, manipulative experiments in seagrass beds in South Florida demonstrate that the time course of the response of seagrass beds to eutrophication is on the order of decades, and we do not understand completely the interaction man has with the natural dynamics of these systems. These 30 sites should continue to be monitored on a quarterly basis.

Detailed analyses of the monitoring data have led to 12 peer-reviewed publications that can be viewed at: <http://serc.fiu.edu/seagrass/publications.htm>. These publications address aspects of the functioning, status and trends of benthic communities as well as lay the groundwork for forecasting future anthropogenic impacts on this ecosystem.

Project Title: U.S. EPA / FKNMS Coral Reef Monitoring Project

Researchers: Jennifer Wheaton, Walter C. Jaap, Keith Hackett, Matthew Lybolt, M.K. Callahan, Jim Kidney, and Selena Kupfner, Florida Fish and Wildlife Conservation Commission/Florida Marine Research Institute (FWC/FMRI), St. Petersburg, FL; James W. Porter and Vladimir Kosmynin, University of Georgia, Athens, GA; and Chris Tsokos and George Yanev, University of South Florida, St. Petersburg, FL.

Goal: The Coral Reef Monitoring Project (CRMP) is part of the Water Quality Protection Program for the Florida Keys National Marine Sanctuary (FKNMS). The goal of this project is to utilize broad spatial coverage, repeated sampling, and statistically valid findings to document status and trends of coral communities within the Sanctuary. As coral reef monitoring is integrated with the seagrass and water quality projects, the results can be used to focus research on determining causality and to fine tune and evaluate management decisions.

Methods: Sampling site locations were chosen in 1994 using a stratified random sampling procedure (US EPA EMAP). Forty reef sites were selected within the FKNMS and permanent station markers were installed in 1995. Annual sampling began in 1996 and has continued through 2001. Three additional sites were installed and sampled in the Dry Tortugas beginning in 1999. The project's 43 sampling sites include 7 hard-bottom, 11 patch, and 12 offshore shallow and 13 offshore deep reef sites.

Field sampling consists of station species inventories and video transects (three video transects per station) conducted at four stations at each site. Station Species Inventory (SSI) consists of counts of stony coral species (Milleporina and Scleractinia) present in each station to provide data on stony coral species richness (S). Two observers conduct simultaneous timed (15 min) inventories within the 22 x 2 m stations and enter the data on underwater data sheets. Each observer records all stony coral taxa and fire corals and enumerates long-spined urchins (*Diadema antillarum*) within the station boundaries. After recording the data, observers compare (5 min) data underwater and confirm species recorded by only one observer. Data sheets are verified aboard the vessel and forwarded to FMRI for data entry and processing. This method facilitates data collection with broad spatial coverage at optimal expenditure of time and labor. During the species inventory any species within a station that exhibits specific signs of either bleaching or disease (black band, white complex, and other) is documented on the data sheet.

Videography: All sampling through 1999 was filmed with a Sony CCD-VX3 using full automatic settings. Beginning in 2000, the project upgraded to digital video filming all sites with a SONY TRV 900. To ensure quality images, artificial lights are used when necessary. A convergent laser light system aids the videographer in maintaining the camera at a uniform distance above the reef surface (40 cm). The videographer films a clapperboard prior to beginning each transect. This provides a complete record of date and location of each segment recorded. Filming is conducted at a constant swim speed of about 4 meters per minute yielding approximately 9,000 video frames per transect. Images for all transects are framegrabbed, and written to and archived on CD-ROM.

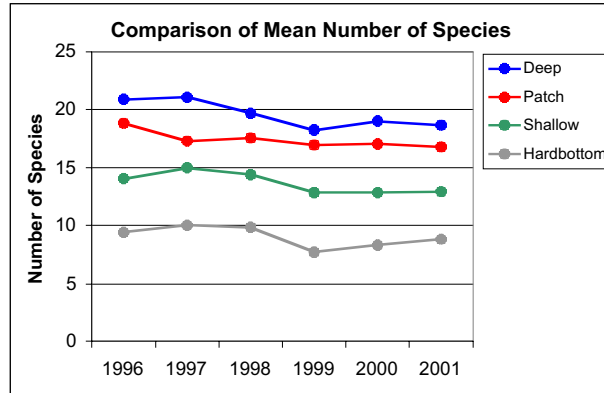


Figure 1. Species richness by habitat type, 111 stations, 1996-2001.

Findings to Date: The results reported here are based on hypothesis testing and statistical analysis to determine significance, and are defined by the following regions: Upper Keys (north Key Largo to Conch Reef), Middle Keys (Alligator Reef to Molasses Keys), Lower Keys (Looe Key to Smith Shoal), and Tortugas (Dry Tortugas to Tortugas Banks). SSI data from 1996 until 2001 for 111 stations that remained after station reduction are presented. Benthic cover analyses are for 1996-2000. Tortugas data are presented separately because sampling began in 1999.

The project documented a decline in stony coral species richness for all habitat types (Fig. 1). The offshore deep and patch reef stations had the greatest numbers of stony coral taxa; hard-bottom stations had the least. Between 1996 and 2001, in the Upper Keys, there was a loss of stony coral species at 26 of 34 stations (76.5%); 5 stations gained species, and at 3 stations, presence of stony coral species was unchanged. In the Middle Keys, 17 of 31 stations (54.8%) lost stony coral species; 9 stations gained species, and 5 stations were unchanged. In the Lower Keys, 27 of 46 stations (58.7%) lost stony coral species; 14 stations gained species, and 5 stations were unchanged (Fig. 2).

By habitat type (Table 1), 19 (65.5%) of 29 patch reef stations had stony coral species losses; 4 stations gained, and 6 stations were unchanged. For shallow reef stations, 23 of 39 (59.0%) showed stony coral species losses; 13 stations gained, and 3 were unchanged. Eighteen of 26 (69.2%) deep reef stations had stony coral species losses, 5 stations gained, and 3 stations were unchanged. Ten of 17 (58.8%) hard-bottom stations had stony coral species losses; 6 hard-bottom stations gained, and 1 station was unchanged. Looe Key Shallow Station 2 had a maximum of five stony coral species gained. Grecian Rocks Station 4 had the greatest loss in stony coral species richness at a single station, where 12 species were lost.

Table 1. Number of stations with change in stony coral species richness, by habitat type, 1996-2001.

Habitat Type	Patch			Shallow			Deep			Hardbottom			Total		
	Number of Stations With:			Number of Stations With:			Number of Stations With:			Number of Stations With:			Number of Stations With:		
Comparison	No Change	Gain	Loss	No Change	Gain	Loss	No Change	Gain	Loss	No Change	Gain	Loss	No Change	Gain	Loss
1996 vs 1997	5	3	21	6	24	9	4	11	11	2	10	5	17	48	46
1997 vs 1998	7	13	9	8	9	22	4	4	18	5	5	7	24	31	56
1998 vs 1999	7	8	14	6	6	27	5	4	17	2	0	15	20	18	73
1999 vs 2000	8	12	9	8	14	17	7	14	5	4	8	5	27	48	36
2000 vs 2001	5	11	13	9	17	13	3	9	14	1	9	7	18	46	47
1996 vs 2001	6	4	19	3	13	23	3	5	18	1	6	10	13	28	70

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Figure 3 represents the distribution of change for CRMP stations along with a normal distribution of change expected in a stable environment. The CRMP data is skewed to the left, indicating a decline in species richness. Sanctuary-wide from 1996 to 2001, stony coral species richness declined at 70 stations (63.1%); 28 stations (25.2%) had a gain and 13 stations (11.7%) were unchanged.

In the Dry Tortugas from 1999 to 2001, 5 stations (41.7%) showed a decrease in the number of stony coral species identified; 6 stations (50.0%) gained species and 1 station (8.3%) was unchanged (Fig. 4).

From 1996 to 2001, statistical analyses revealed significant decreases in the number of stations where *Acropora cervicornis*, *Millepora alcicornis*, *Mycetophyllia lamarckiana* were documented ($\alpha = 0.10$ and $1 - \beta = 0.75$). *Siderastrea siderea* was the only species with a significant gain.

In general, stony corals in CRMP stations experienced an increase in disease infections from 1996 to 2001 (Fig. 5). A significant increase in the number of stations with corals affected by “white” and “other” disease was reported between 1996 and 2001 ($\alpha = 0.10$ and $1 - \beta = 0.75$). The number of stations with corals affected by black band disease was statistically unchanged. Overall, there were increases in the number of stations containing diseased coral, the number species with disease, and the different types of diseases that were observed.

Further analysis of the disease data by species shows the number of stations with all selected species infected by black band disease peaked in 1998. Black band infections declined thereafter (Fig. 6). In 1997 and 1998, four of the selected species experienced peaks in “white” disease, but have since declined (Fig. 7). The apparent reduction in “white” disease for *Acropora cervicornis* and *A. palmata* is likely attributable to 97% and 88% reductions in percent cover, respectively. Three species had a maximum occurrence of white disease in 2001. The number of stations where “white” disease infected *Agaricia agaricites* in 2001 nearly tripled the previous high.

In the “other” disease category, the number of stations infected peaked in 2001 for all species depicted (Fig. 8). Four species (*M. annularis*, *M. cavernosa*, *P. astreoides*, and *S. siderea*) provide 80% of all living coral in the survey. The number of stations where “other” disease infected each of these four species more than doubled between 2000 and 2001.

Sanctuary-wide, there was a general trend of decline in stony coral cover. The decline in mean percent coral cover from 1997 to 1998 and from 1998 to 1999 was significant with a p-value of 0.03 or less for the Wilcoxon rank-sum test. The change observed from 1996 to 1997 and from 1999 to 2000 was not statistically significant. Preliminary results from 2001 data showed that stony coral cover did not change significantly between 2000 and 2001 (Addendum Figure). Detailed results of these data will be presented in a future report.

The percent stony coral cover by habitat type was determined for each year, 1996 through 2000. When analyzed by habitat type, the greatest mean percent stony coral cover was consistently observed at patch reef stations. Though deep sites have the greatest number of species present, percent stony coral cover is consistently lower than at their shallow counterparts. Percent cover in hard-bottom habitat is the lowest recorded for all habitat types. Sixteen of the 28 hard-bottom

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stations had insufficient stony coral cover for hypothesis testing ($< 0.3\%$ stony coral cover). Different methods are required to detect change at stations with minimal stony coral cover. Analyses of different parameters would better characterize change in these habitats. Regionally, there was a greater relative change in mean stony coral cover in the Upper Keys. Additionally, a greater percentage of Upper Keys stations showed significant loss of coral cover compared to Lower and Middle Keys stations.

For the 144 stations with sufficient stony coral cover for analysis, 92 stations (63.9%) had a significant decrease in stony coral cover, 47 stations (32.6%) showed no significant change, and only 5 stations (3.5%) had significant gains. In the Upper Keys, 29 stations (72.5%) experienced significant loss of coral cover, 10 (25.0%) had no significant change, and only 1 station experienced a significant gain in coral cover. In the Middle Keys, 19 stations (47.5%) experienced significant coral cover losses, 20 stations (50.0%) had no significant change, and only 1 station gained significant coral cover. In the Lower Keys, 44 stations (68.8%) lost a significant amount of coral cover, 17 stations (26.6%) had no significant change, and 3 stations (4.7%) showed a significant gain in coral cover. In the Dry Tortugas, for 1999-2000, 8 stations (66.7%) had no significant change in stony coral cover and 4 stations (33.3%) had a significant loss.

By habitat type, 20 patch reef stations (50.0%) had a significant loss in coral cover. Cover at 18 stations (45.0%) was statistically unchanged and 2 stations (5.0%) had a significant gain in coral cover. For offshore shallow sites, 40 stations (83.3%) had a significant loss of coral cover, 8 stations (16.7%) had no significant change in cover, and no station exhibited a significant gain. In the offshore deep habitat, a significant loss in coral cover was documented at 26 stations (59.1%), 17 stations (38.6%) had no significant change in cover, and only 1 showed a significant gain in coral cover. For the hard-bottom habitat stations, hypothesis testing was only applicable for 12 of 28 stations because of sparse stony coral cover. Overall, there were significant losses in coral cover for 71.7% of offshore reef stations, both shallow and deep.

Percent cover data for functional groups were analyzed for the geographic regions from 1996 to 2000. Functional groups included: stony corals, octocorals, zoanthids, sponges, macroalgae, seagrass, and substrate (rock, rubble and sediments). Zoanthid and seagrass percent cover values were too low to represent graphically. In all three geographic regions, stony coral, sponge and octocoral cover decreased whereas macroalgae and substrate cover increased over the five years. Macroalgal percent cover exhibited higher variability than all other functional groups.

Percent cover of functional groups from 1996 to 2000 was also analyzed by habitat type. Generally, functional group cover trends were consistent with those observed at the regional level. Macroalgal percent cover increased dramatically at deep stations, but declined slightly in the other three habitat types. Sponge percent cover declined more in deep and shallow stations than in patch reef and hard-bottom stations. Zoanthid cover decreased sharply at deep and hard-bottom stations, but increased slightly at shallow and patch reef stations.

An understanding of the overall trend in stony coral cover can be gained through further analysis of change in percent cover of the most common species. The six species with the greatest mean percent coral cover Sanctuary-wide in 1996 were *Montastraea annularis* (3.39%), *M. cavernosa*

(1.36%), *Acropora palmata* (0.90%), *Siderastrea siderea* (0.87%), *Millepora complanata* (0.80%), and *Porites astreoides* (0.55%). *M. annularis* represented approximately 33% of the coral cover at CRMP stations. The relative percent cover of *M. annularis* and *M. cavernosa* increased although their mean cover decreased (*M. annularis* 3.39% in 1996 to 2.41% in 2000 and *M. cavernosa* 1.36% in 1996 to 1.22% in 2000). The most striking changes were declines in coral cover for *Acropora palmata*, *A. cervicornis*, and *Millepora complanata*. At shallow stations, the mean percent cover of *A. palmata* dropped from 3.01% (1996) to 0.35% (2000), representing an 88% loss. Sanctuary-wide, percent cover of *A. cervicornis* dropped from 0.20% (1996) to a barely detectable 0.006% (2000), a 97% reduction. *M. complanata* declined from a mean percent cover of 2.65% (1996) to 0.12% (2000) for all shallow stations, a loss of 95%. Only two species exhibited an increase in percent cover from 1996 to 2000. *Siderastrea siderea* increased slightly from a mean of 0.87% in 1996 to 0.89% in 2000, representing an increase of 2.5%. *Porites astreoides* showed slight increases in mean as well as relative coral cover. The three other species, *Agaricia agaricites*, *Colpophyllia natans*, and *M. alcicornis*, all experienced less dramatic declines in mean percent coral cover.

Discussion and Conclusions: The Coral Reef Monitoring Project (CRMP) constitutes the first successful, long-term monitoring project that has documented status and trends of coral reefs throughout the 9,950 square-kilometer FKNMS. This data set has been, and will continue to be an indispensable asset for sound resource management decisions. Between 1996 and 2000, the project reported a 37% reduction in stony coral cover Sanctuary-wide. Hypothesis testing revealed that 63.9% of project stations suffered a significant loss in stony coral cover while only 3.5% showed a significant increase. By region, the Upper Keys experienced the greatest decline with significant loss in coral cover at 72.5% of stations, followed by the Lower Keys with loss at 68.8% and the Middle Keys with a loss at 47.5% of stations. With regard to species richness, 63% of all stations lost one or more stony coral species between 1996 and 2001, 25% gained species, and 12% were unchanged. These documented declines in coral cover and species richness were concurrent with an increase in disease infection. The number of stations where disease was documented increased from 26 in 1996 to 131 in 2000 and the number of species affected increased from 11 to 36.

These documented trends are alarming; however, they are reasonably consistent with trends documented by researchers elsewhere in the Caribbean basin. There is general consensus that multiple stressors acting at local, regional, and global scales have negative impacts on coral reefs. It is clear that multiple stressors are contributing to coral decline in Florida.

Future Plans: The Comprehensive Everglades Restoration Plan (CERP) aims to re-establish the historical flow of water through South Florida and into Florida Bay. This project will inevitably alter biological communities and water quality in Florida Bay. Downstream of Florida Bay, the Florida Keys reef tract provides the last opportunity to quantify CERP-induced changes. As water quality is impacted by changes in the volume of water delivered to Florida bay, reefs may decline in channel areas based on similar experiences in other locations (Tomascik and Sanders, 1985; Richmond, 1993; Furnas and Mitchell, 2001; Geister, 2001). Therefore, continued monitoring is crucial in order to document status and trends of coral reefs in FKNMS. In addition to the ongoing monitoring, the CRMP will expand its sampling strategy in order to better

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understand causes of coral decline and effects of multiple stressors under the new name, Coral Reef Evaluation and Monitoring Project (CREMP).

We propose to continue annual non-consumptive sampling at 40 established sites from Key Largo to Tortugas Banks to document status and trends in the coral reef ecosystem. Inventories of stony coral species richness and presence of disease and bleaching will be conducted. Underwater video will be analyzed to determine percent cover of stony coral and other benthic components (Octocorallia, Zoanthidea, macroalgae, etc.). Hypothesis testing and multivariate change analyses will be performed to quantify significant changes in these indicators.

In addition we will collect a more comprehensive suite of indicators at 11 of the established 40 sites. By following the fate of individual coral colonies, the CREMP will better understand coral community dynamics and mortality rates associated with individual stressors. For example, the density of bioeroding sponges of the genus *Cliona* is an indicator of organic enrichment in the water column. Human enteroviruses are used to distinguish the source of nutrient input (human vs. other). Temperature is implicated in bleaching and disease pathogenicity. Sedimentation is associated with coastal development and is a limiting factor for larval recruitment.

The comprehensive monitoring data set on stony coral cover, species richness, bleaching, disease, bioeroders, temperature, fate tracking, human enteroviruses, recruitment, and sedimentation will assist in development of landscape-seascape program models to characterize physical, chemical, and biological stressors. Not only will these data assist managers in determining if the fully protected Tortugas Ecological Reserve and Sanctuary Preservation Areas (SPAs) are functioning to protect sensitive resources, it will also provide definitive feedback on the downstream effects of the CERP.

A report of the Florida Fish and Wildlife Conservation Commission and the University of Georgia pursuant in part to U. S. Environmental Protection Agency Award No. X994649-94-6.

A digital version of this document is available in the "Marine Biology" section of <http://www.floridamarine.org>.

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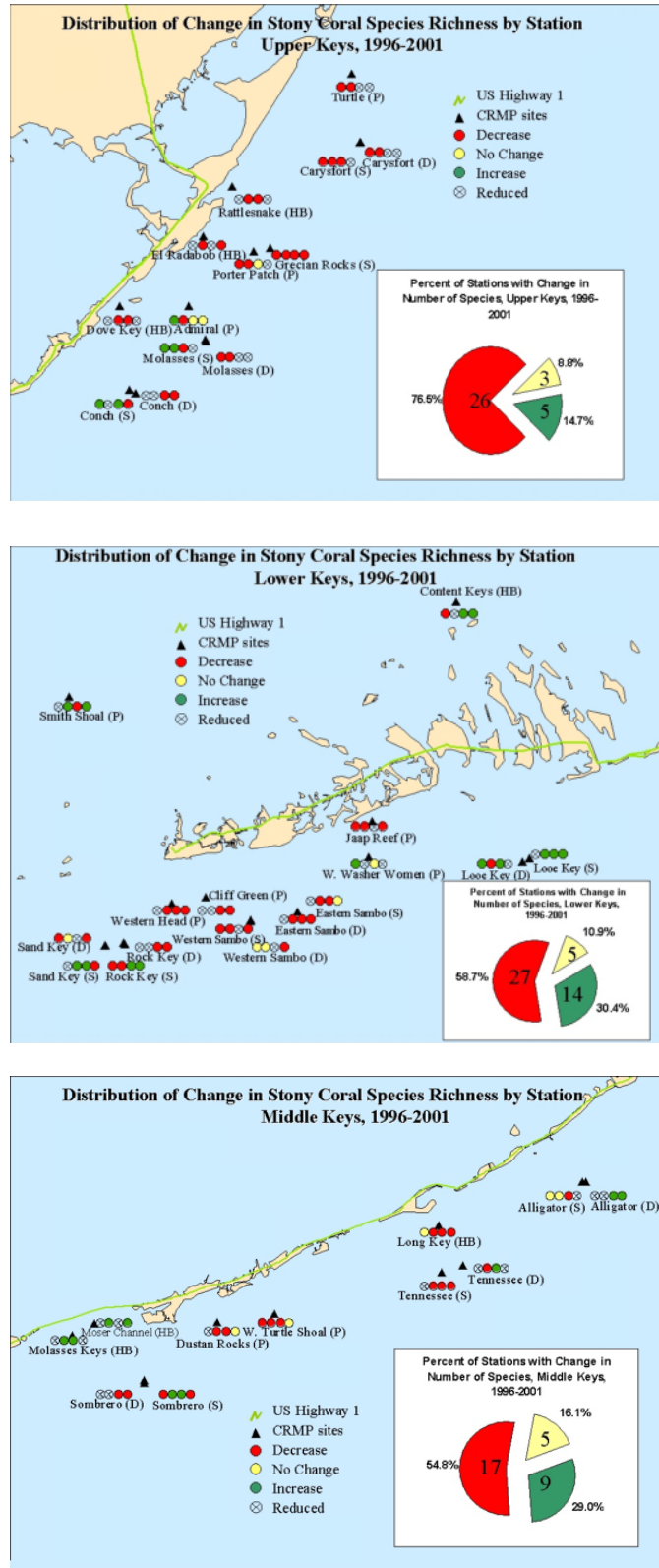


Figure 2. Distribution of change in stony coral cover, by region.

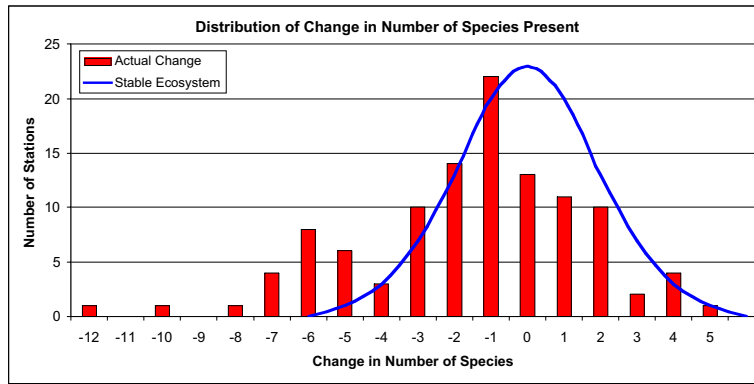


Figure 3. Distribution of change for stations compared with normal distribution of change expected in a stable environment.

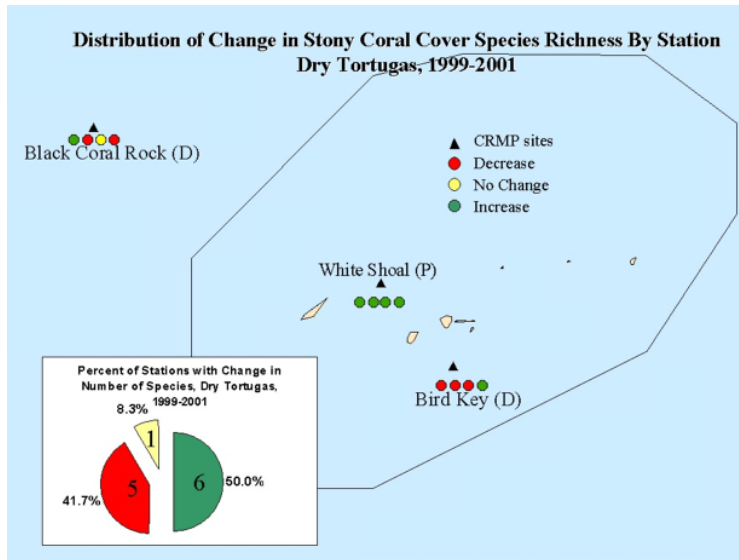


Figure 4. Trends in the distribution of change in stony coral species richness by station, Dry Tortugas, 1996-2001.

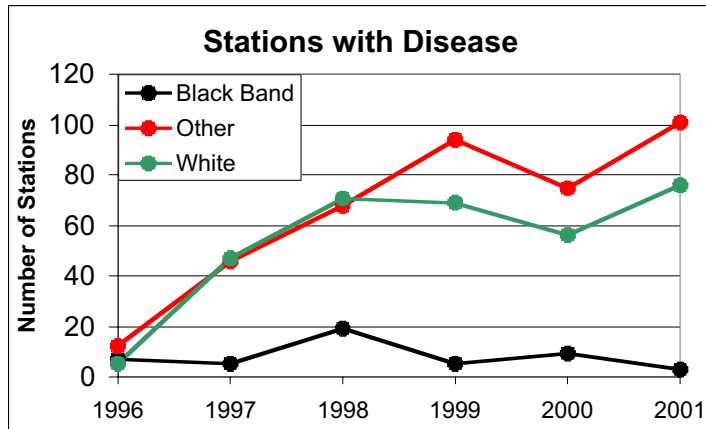


Figure 5. Trends in the number of stations with disease, 1996-2001.

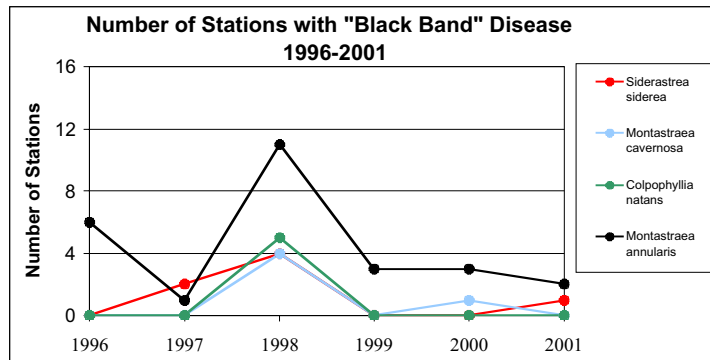


Figure 6. Trends in the number of stations with black band disease, 1996-2001.

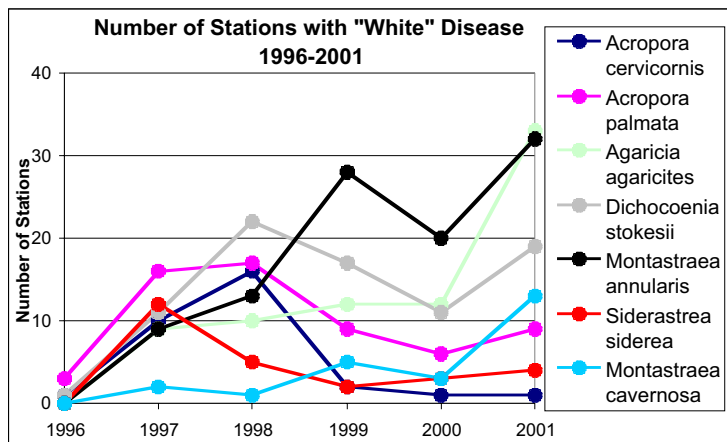


Figure 7. Trends in the number of stations with "white" disease, 1996-2001.

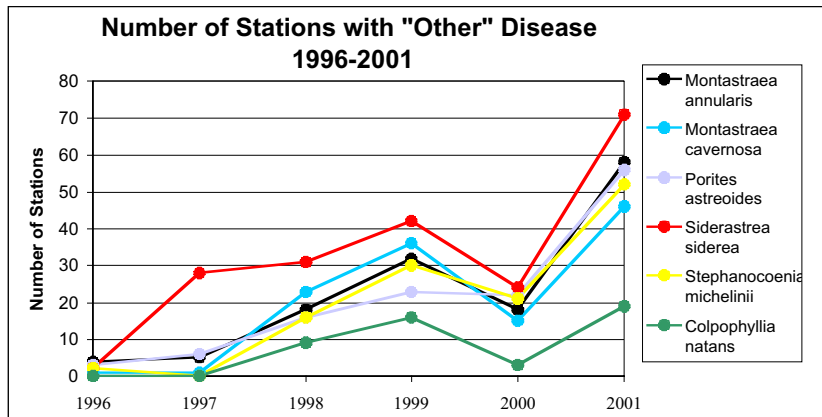
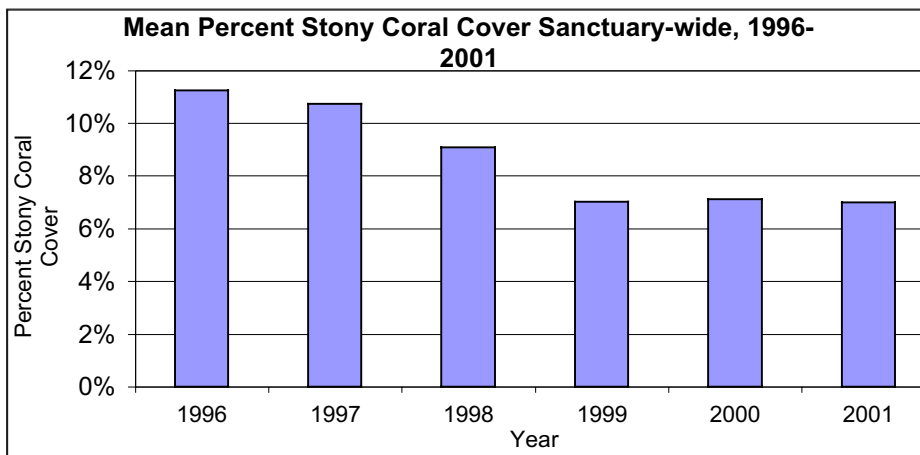


Figure 8. Trends in the number of stations with “other” disease, 1996-2001.



Addendum Figure. Mean percent stony coral cover Sanctuary-wide, 160 stations 1996-2001 (preliminary analysis).

Project Title: Marine Ecosystem Event Response and Assessment (MEERA) Project

Researchers: Erich Mueller and Erich Bartels, Mote Marine Laboratory Center for Tropical Research, Summerland Key, FL.

Goals: Initiated in late summer, 1997, as the Rapid Biotic Assessment (RBAT) Project, this project was originally designed to provide an early warning and assessment program for biotic events on reefs of the Florida Keys National Marine Sanctuary (FKNMS). In December, 1999, the project was renamed the Marine Ecosystem Event Response and Assessment (MEERA) Project to more accurately portray the overall scope and objectives of the project, which include any event that impacts the marine environment.

Methods: The Marine Observer Network continues to be the most important component of the MEERA project, whereby anyone can call, e-mail, fax, or file a report on-line to submit observations to the Project Coordinator for evaluation. Public outreach efforts have expanded to reach as large and diverse an audience as possible, including the following:

Fishing Guides	FWC/Florida Marine Research Institute	The Nature Conservancy
Charter Captains	Florida Keys National Marine Sanctuary	Seacamp
Dive Operators	Sanctuary Law Enforcement	U.S. Coast Guard
Commercial Fishermen	National Marine Fisheries Service	U.S. Fish and Wildlife Service
Tropical Fish Collectors	The Ocean Conservancy (TOC)	All Keys Residents

Findings to Date: A total of 197 reports were received in 2001 from sources including a variety of researchers, State and Federal personnel, and residents, as well as fishermen and divers (Table 1). Due to multiple observations included in some reports, a total of 278 observations were logged that included mainly reports of sea turtle strandings, algal blooms, coral disease and bleaching, and fish disease or fish kills (Table 2). Other reports included various invasive species, discolored water, vessel groundings, and various unusual observations.

Response efforts included the collection, analysis, and shipping of samples; photo-documentation of reports or events; and providing assistance or logistical support for other researchers and organizations. Efforts utilized a combination of volunteers, cooperative agency work, and Mote Marine Laboratory staff and equipment. These efforts included the following:

- Responded to several marine mammal-stranding reports to recover specimens and provide Stranding Network personnel a location to conduct necropsies.
- Received training for and became member of Florida Sea Turtle Stranding and Salvage Network (FWC) to respond to, document, and report dead or injured sea turtles.
- Coordinated volunteers to collect water samples during potential algal bloom events to assist FWC and Mote Marine Laboratory's Red Tide Monitoring Projects.
- Investigated several possible invasive species reports in several local canals, and worked with residents to prove invasive species had not established local populations.
- Assisted U.S. Fish and Wildlife Service investigating numerous dead and sick pelicans in the Marquesas by collecting water samples to eliminate "red-tide" as possible cause.
- Worked with TOC-RECON instructor to train volunteer divers to conduct coral reef health surveys, with an emphasis on the identification of coral disease and bleaching.

- Attended FWC’s Fish Kill Training workshop, and investigated several local fish kills affecting canals on Cudjoe Key and Big Pine Key to determine cause and provide information to FWC Aquatic Health Network.
- Provided “ground-truthing” for NOAA’s remote sensing of coral bleaching by passing on observations during periods of probable bleaching events.
- Documented coral disease and coral bleaching reports and providing logistical support and collaborative efforts on a variety of related research projects (Table 3).

Future Plans: As the project continues to see more than a 300% increase in reports submitted, receiving more reports in 2001 than in the previous four years combined, there is a clear indication that Marine Observer participation is increasing, and that there is a significant need for increasing response efforts in the future. Several goals have been identified as necessary to increase the MEERA Project’s effectiveness:

- Find a source of continued funding to continue expanding the Marine Observer Network and initiate aggressive response efforts incorporating increased community participation.
- Continue to improve communication with State and Federal agencies, and other researchers to maximize MEERA’s involvement and assistance with response efforts.
- Further develop the MEERA website (www.mote.org/~emueller/MEERAphtml) to allow researchers, resource managers, and the public to access recent reports, submit reports online, view past events, and link to related sites.
- Increase public awareness by providing weekly news articles on recent events, and produce a quarterly newsletter to summarize the project’s accomplishments and goals.

Table 1. Reports by Source

Report Source	# of Reports
Researcher	113
Resident	44
Web/Media	23
Fish/Dive Industry	13
Law Enforcement	2
Tourist	2
Total	197

Table 2. Observations by Type

Event Type	# of Reports
Turtle/Mammal Strandings	98
Algal Blooms/Discolored Water	51
Coral Disease/Bleaching/Mortality	48
Fish Disease	19
Fish Kill	15
Others	47
Total	278

Table 3. Related Research Efforts Focused on Coral Disease and Bleaching in 2001

Project	Objectives
Coral Disease Workshop	Provide training for coral disease monitoring and collection methods
CIS-NET Project	Study effects of increasing UV radiation on coral bleaching
US EPA Coral Disease Surveys	Monitor coral disease and bleaching in Florida Keys and Bahamas
NOAA CCEMBR White Plague	Assessed biomarker samples in infected corals
Sustainable Seas Expedition	Utilize submersible and ROV technology to monitor deep reefs
Cornell Univ. Sea Fan Studies	Conduct laboratory and field studies investigating sea fan diseases
EPA Special Studies	Study effects of reef fish feeding on coral disease distribution
University of Vienna	Conduct reef surveys to examine effects of algal growth
Nature Conservancy Programs	Coordinate Sea Stewards and RECON coral reef monitoring efforts

Project Title: Coral Reef Ecosystem Process Studies in the Fully Protected Zones of the Florida Keys National Marine Sanctuary.

Researchers: Richard B. Aronson, Dauphin Island Sea Lab, Dauphin Island, AL; Margaret W. Miller, NOAA/National Marine Fisheries Service, Miami, FL; Struan R. Smith, Bermuda Biological Station for Research, Inc., St. George's GE01, Bermuda; and Thaddeus Murdoch, Dauphin Island Sea Lab, Dauphin Island, AL, John C. Ogden (Project Director); Florida Institute of Oceanography, St. Petersburg, FL;

Goal: The primary purpose of this continuing study of ecological processes and ecosystem function is to evaluate the relationships among coral cover, coral recruitment and juvenile mortality, herbivory and algal dynamics at three fully protected ("no-take") zones and three reference sites in the Florida Keys National Marine Sanctuary (FKNMS). The fully protected zones (FPZs) are South Carysfort (Carysfort Sanctuary Preservation Area) in the Upper Keys, and Eastern Sambo Research-only Area and Western Sambo Ecological Reserve in the Lower Keys. The reference sites are Maitland, located near the *M/V Maitland* ship-grounding site in the Upper Keys, and Middle Sambo Reef and Pelican Shoal in the Lower Keys.

Background: In this report several extraordinary funding circumstances must be taken into account. The original contract for this research program, executed in 1997, covered the pilot work (mainly site selection) and the first year of fieldwork in 1998. Subsequently, the 1999 fieldwork was funded from the FY1998 budget and the 2000 fieldwork was funded from the FY1999 budget. In 2000 a transition in Sanctuary science program management took place. As our program did not appear in the FY2000 budget, funds were inadvertently not allocated for our 2001 field program. FKNMS cobbled together a minimal budget that amounted to only 42% of our budget the previous year. Furthermore, contract problems and federal transitions drastically delayed the transfer of funds until late November 2001. Our teams went into the field in 2001 with no funding in hand, and only a letter from the FKNMS confirming minimal support.

Findings to Date: The three areas of research are described separately below.

Video Monitoring: The study sites were videographically monitored in late September-early October 2001 to assess the cover of components of the sessile biota (corals, sponges, gorgonians, etc.). Ten randomize video transects were made at both the shallow and deep sites. Coral cover, coral species richness, and sponge cover remained consistent over time within sites from 1998 to 2001 (Fig. 1). The cover of encrusting octocorals (*Erythropodium caribbaeorum* and *Briareum asbestinum*) increased at all the deep sites from 2000 to 2001. The four years of transect data from the shallow sites (1998-2001) were ordinated by multidimensional scaling (MDS) to search for patterns in species composition of the coral assemblages at the sites. There was little change in coral cover within sites, with Western Sambo exhibiting considerably higher coral cover than the others. At the shallow depth range, two FPZs (Western Sambo and Eastern Sambo) have shown recent increases in dominance of *Montastraea* spp., the only reef framework builders remaining at these depths since the near-extirpation of *Acropora cervicornis* over the last two decades. Middle Sambo, one of the reference sites, is also moving toward increased dominance of *Montastraea*. Pelican Shoal and Maitland, the other two reference sites, were moving away from dominance by *Montastraea* in 2001. In a separate MDS ordination, the deep sites showed a

different pattern. They clustered by sector of the reef tract, with the sites in the Lower Keys set apart from the sites in the Upper Keys, reflecting the lower cover and diversity of corals at the latter sites.

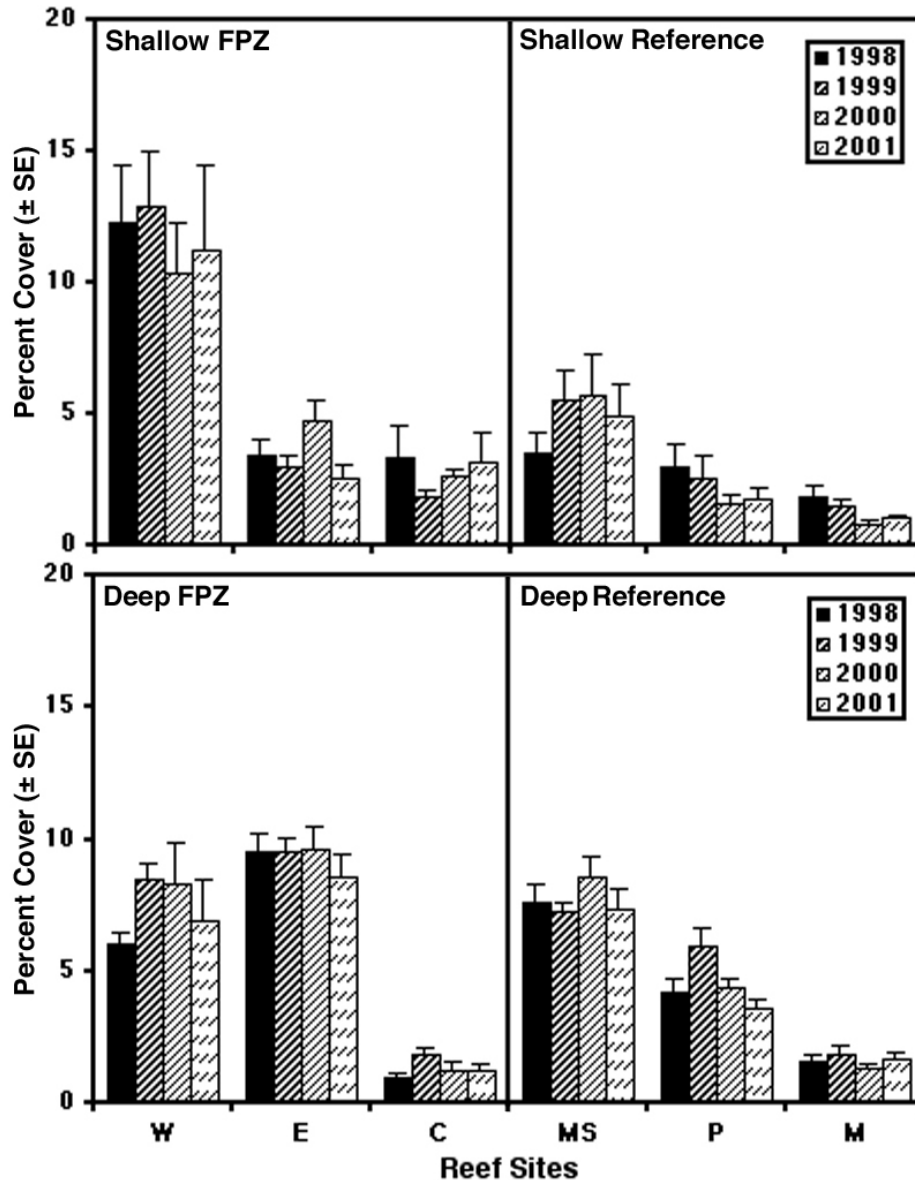


Figure 1. Percent coral cover (\pm SE) at the shallow (8-11 m) and deep sites (14-18 m) from 1998 to 2001. FPZ sites: W (Western Sambo), E (Eastern Sambo), C (South Carysfort). Reference sites: MS (Middle Sambo), P (Pelican Shoal), M (Maitland Reef). N= ten transects per site.

Juvenile Corals: The recruitment and mortality of juvenile corals were monitored in sets of 34 permanent quadrats established within the FPZ and reference sites from 2000 to 2001 at two depths (6-9 m, 16-18 m). This is the third period of annual changes observed in the juvenile coral

populations since the project began in 1998. Only the Western Sambo FPZ has shown higher coral recruitment compared to the companion site at Middle Sambo. There has been a significant increase in recruitment rates at most of the sites and depths since 1998-99 with the highest rates consistently found at the deeper Lower Keys sites (Fig. 2). Both brooding corals (agariciids, poritids) and broadcast spawning corals (*Siderastrea siderea*, *Montastraea cavernosa*) corals have recruited successfully, but very few of the massive framework-building species, e.g. *Montastraea "annularis"*. No differences have been detected in juvenile coral mortality rates between the FPZ and reference sites since 1998, although mortality rates at the deeper sites are significantly higher than the shallow sites, since the storm effects in 1998 (Fig. 3).

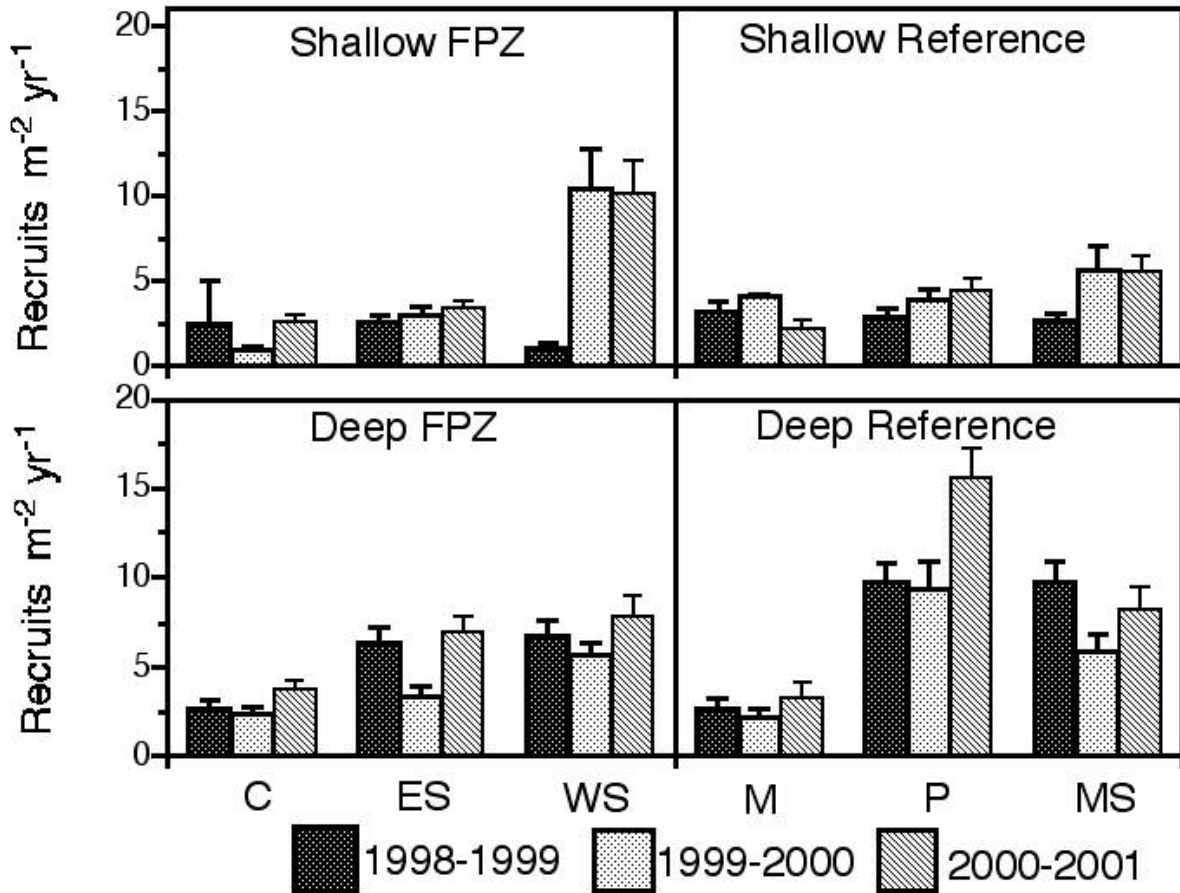


Figure 2. Patterns of juvenile coral recruitment in the Fully Protected Zones (FPZs) and adjacent reference areas from 1998 to 2001. Thirty-four permanent quadrats were censused visually on an annual basis at each depth at each site. C= Carysfort, ES= Eastern Sambo, WS = Western Sambo, M = Maitland, P= Pelican Shoal, MS= Middle Sambo. Error bars =1 SE.

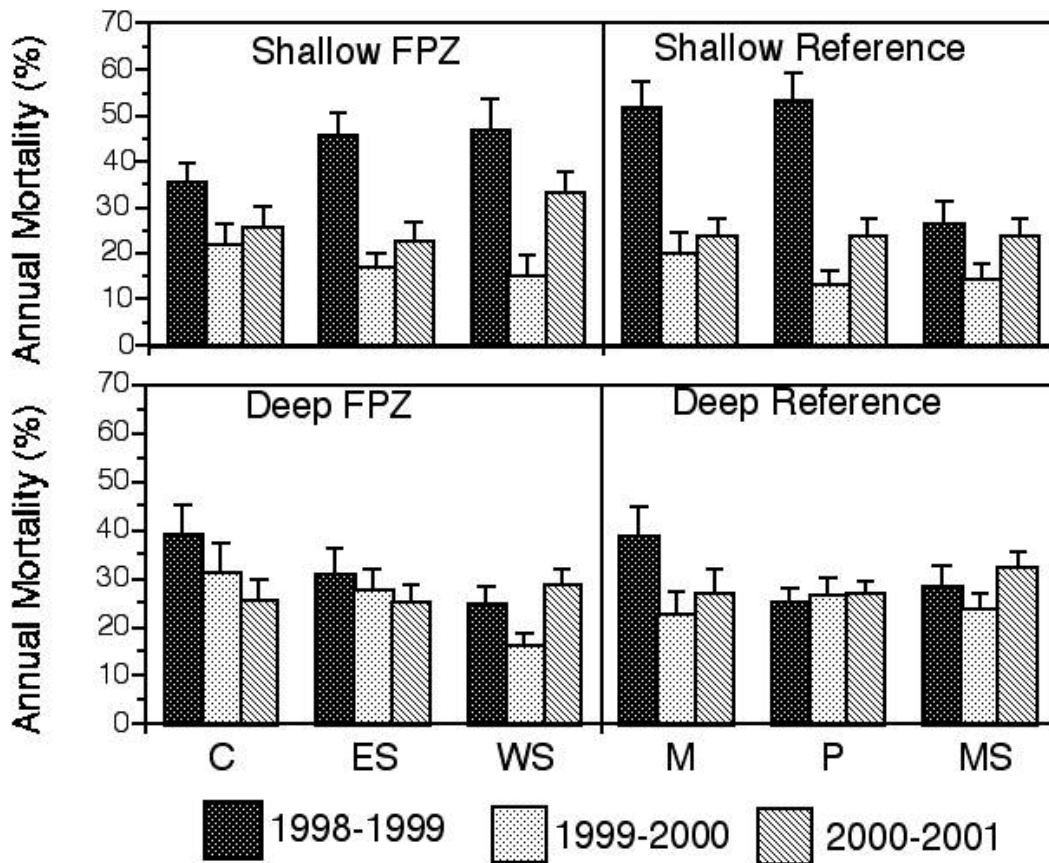


Figure 3. Patterns of juvenile coral mortality in the Fully Protected Zones (FPZs) and adjacent reference areas in the permanent quadrats from 1998 to 2001. Site labels as listed in Figure 2. Error bars =1 SE.

Macroalgal Biomass and Herbivory: Macroalgal biomass was quantified by hand harvesting haphazardly placed 40x40 cm quadrats. On the shallow fore reef (8-10 m) macroalgal biomass has been generally higher at the reserve sites than at adjacent reference sites throughout the study (Fig. 4). The exception to this pattern is due to higher macroalgal biomass at Pelican Shoal in comparison to the Eastern Sambo reserve. However, recent summer samples have shown increases in biomass at Eastern Sambo (to levels higher than Pelican Shoal).

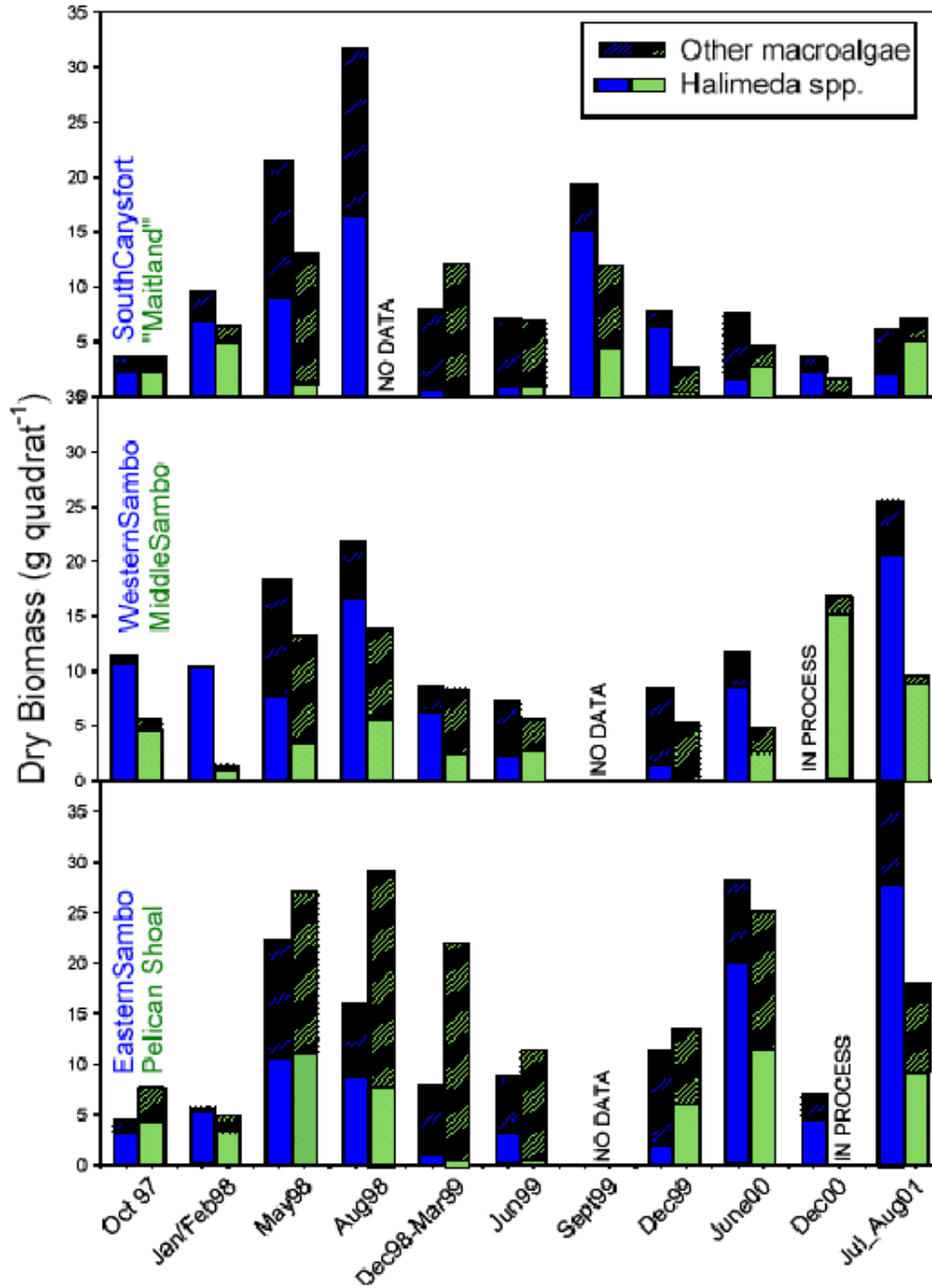


Figure 4. Mean total dry biomass of macroalgae (*Halimeda* spp. and “other” components indicated) at three reserve /reference site pairs (blue/green, respectively) at 8-10m depth. N=10.

Herbivory assays: Replicate pieces of pre-weighed *Thalassia* and various algae were affixed to lengths of rope and exposed to herbivores at the shallow sites for 2 hours. Some temporal trends were observed in the *Thalassia* assays (indicating consumption by parrotfishes) which decreased at Eastern Sambo FPZ and increased at Middle Sambo (reference) (Fig. 5). Increased herbivory

at Middle Sambo was also evident in fleshy red algal assays (indicating consumption by surgeonfishes), but no trend was observed in fleshy red algal consumption at Eastern Sambo. So far, such site-specific patterns fall short of coherent support for predicted changes in ecological function (i.e., decreased herbivory and, hence, increased macroalgal abundance) of reserves.

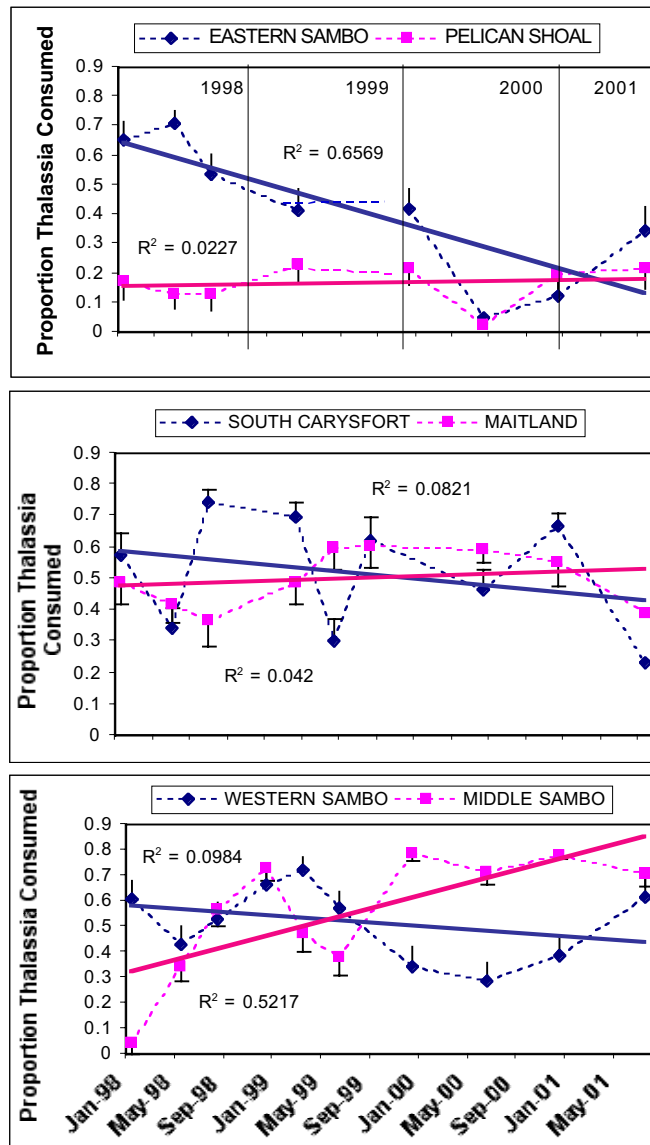


Figure 5. Mean proportion of *Thalassia* blades (\pm 1SE) removed in 2-hour afternoon exposure at each reef on each occasion. Solid lines, equations and R² values from linear regression.

We explored possible correlations of macroalgal abundance and herbivory with coral recruitment as both factors have been demonstrated to control recruitment in other reef systems. The

recruitment rates were not reduced by higher macroalgal biomass (Fig. 6). Higher herbivory rates did not increase coral recruitment by removing competitive algae (Fig. 7). Possible explanations may be low rates of coral larval supply as well as adequate substrate availability for recruitment, due to low algal and coral cover.

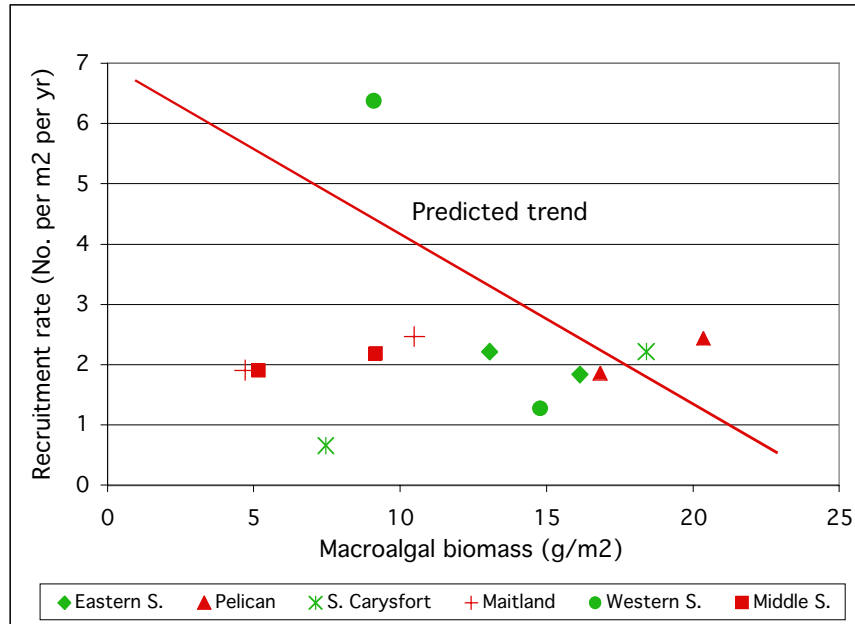


Figure 6. Correlation of annual coral recruitment rate and macroalgal biomass at shallow sites from 1998-2000. Biomass data are annual three-point moving averages, based on 2-3 samples per year at each site. Red symbols indicate reference areas, green indicate FPZs.

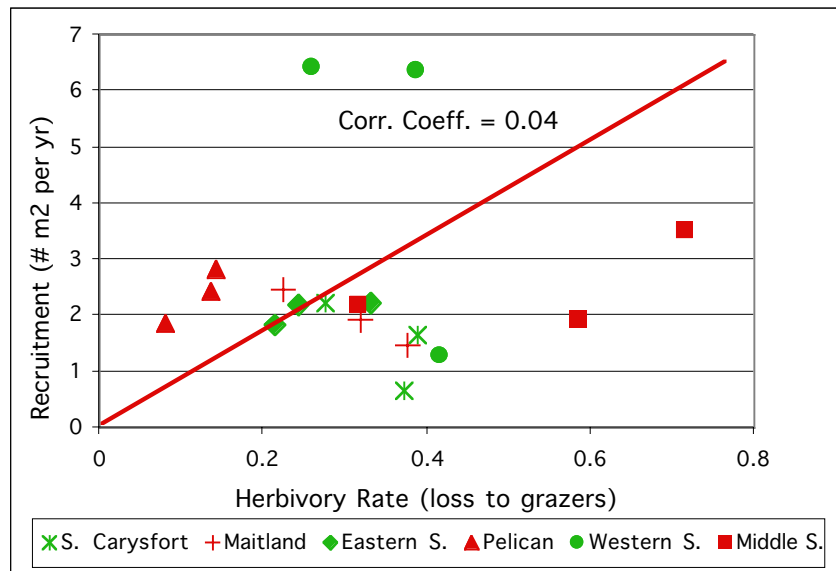


Figure 7. Correlation of annual coral recruitment rate and herbivory, using three-point moving averages of mean proportion of algae and *Thalassia* biomass removal from 2-3 assays per year, at the shallow sites from 1998-2001. Red symbols indicate reference areas, green indicate FPZs.

Project Title: Rapid assessment and monitoring of coral reef habitats in the Florida Keys National Marine Sanctuary: Annual Summary Report: Summer 2001 Zone Monitoring

Researchers: Steven L. Miller, National Undersea Research Center (NURC), University of North Carolina at Wilmington (UNCW), Key Largo, FL.; Mark Chiappone and Helga Dienes, Center for Marine Science Research, UNCW, Key Largo, FL.; and Dione W. Sanson, Center for Marine Science Research, UNCW, Key Largo, FL and Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL 33149

Goals: The 2001 sampling of coral reef and hard-bottom habitats in Sanctuary no-take zones and reference areas complemented a three-year effort dating back to 1999 to sample all of the shallow-water (< 15 m) hard-bottom habitat types in the Sanctuary, as well as most of the fully protected (“no-take”) zones established in 1997. The goals of the NURC/UNCW zone monitoring effort are three-fold: 1) To assess the community structure and condition of reef benthos at multiple spatial scales, with particular reference to the fully protected zones, but also inter-reef, among habitat type, and among region variations; 2) To track the dynamics of coral reef benthos to assess changes due to protection from fishing within the zones, but also changes due to larger-scale factors, such as regional water quality phenomena; and 3) To complement fishery-independent reef fish surveys with “fine-scale” or detailed habitat information, to facilitate experimental and modeling efforts for evaluating essential fish habitat.

To accomplish these goals, the 2001 sampling built upon existing data collected during 1999 and 2000 to guide the underwater surveys. Our focus during 2001 was also three-fold: 1) To survey mid-channel and offshore patch reefs in the Middle and Upper Keys regions of the Sanctuary, to complement surveys of 12 mid-channel and offshore patch reef sites in the Lower Keys during 2000; 2) To survey high-relief spur-and-groove and low-relief hard-bottom habitats throughout the Florida Reef Tract from 1-7 m depth, with a particular focus on reefs constructed by elkhorn coral (*Acropora palmata*); and 3) To survey the deeper fore reef (20 m) throughout the Keys at 19 locations from Key West to northern Key Largo. Surveys in this habitat type are planned for June 2002.

Methods: A two-stage stratified random sampling design was used to randomly select sites during 2001. A grid system constructed in a geographic information system (GIS) was used to overlay the existing habitat map of the Florida Keys. Cells or blocks 200 m x 200 m in dimension were used to randomly select sites from ten habitat strata (Table 1). Twelve of the Sanctuary’s 24 fully protected zones were sampled during 2001, with all but one of the zones (Cheeca Rocks SPA) located on the outer platform margin. Two sites or blocks were assigned to each zone and a total of 86 sites were surveyed between June 12 and September 4. We were fortunate this year in being able to sample the majority of offshore *Acropora* reefs from Key Largo to Key West, including both high relief and low-relief habitat types. The 2001 sampling effort (86 sites) required 38 field days underwater from mid-June to early September. Although 12 days of field time were lost to inclement weather or other logistical issues, we were able to complete the surveys of all but the deeper fore-reef sites (the deep fore-reef sites will be sampled in June 2002).

The 2001 surveys addressed the same variables measured during 1999-2000, in addition to

several variables added to the existing design (Table 2). Briefly, at each site pre-determined GPS points were used to locate the position of transect deployment. Except for patch reefs (10 m transects), four pairs of 25 m transects were deployed in each block, labeled as 1A, 1B, 2A, 2B, etc. Along four of the primary transects (A), coverage was determined every 25 cm to yield 100 points per transect. Digital video along a 0.4 m swath was also taken along the primary transects. The number of species of stony corals, gorgonians, and sponges was determined on all four primary and secondary transects. Gorgonian density and height distribution using four size classes (< 20 cm, 20-50 cm, 50-100 cm, > 100 cm) were determined along transects 1A and 2A, as were coral density, size, and condition. The condition measurements included an assessment of competition between corals and other taxa, and the extent to which interactions caused tissue damage or mortality. Juvenile corals (< 4 cm maximum diameter) were assessed along transects 1A and 2A by randomly sampling ten 0.68 m x 0.45 m quadrats along each transect. Urchin density and test diameter, as well as the density of incidental marine invertebrates were assessed on all four primary and secondary transects.

We additionally assessed density and predation by the flamingo tongue snail, by noting the number of individuals, gorgonian prey, and gorgonian height on all transects deployed. We continued surveys of fishing gear and other marine debris during 2001, by surveying 1 m on each side of all primary and secondary transects. Noted were the type of gear, dimensions (typically length) to the nearest centimeter, whether the debris was biologically fouled or clean, and the number of sessile invertebrates impacted by the debris that caused tissue abrasion and/or mortality. Finally, in situ measurements of topographic complexity along the four primary transects were undertaken to provide an assessment of substratum angle, maximum vertical relief, and the coverage of different relief categories along 0.4 m x 25 m swaths. These surveys were supplemented by chain transect assessments for comparative purposes, in which a 5 m chain was draped over the contours of the substratum on the four primary transects and compared to the linear distance along the transects.

Also included during the 2001 surveys were surveys of two experimental and two control offshore patch reefs west of Pickles Reef that are being used for *Diadema antillarum* translocation (PIs: Ken Nedimyer and Martin Moe). This is initially a one-year effort to evaluate the efficacy of translocating juvenile urchins from rubble to patch reef habitats in terms of time/effort, mortality, and community structure effects. We have included summary information in this report, although more detailed data for the four sites are presented in a report submitted to K. Nedimyer.

Findings to Date: Benthic cover: Patterns in the coverage of stony corals, total algae, sponges, and the colonial zoanthid *Palythoa mammilosa* (for the 86 sites surveyed) exhibited significant differences among the four habitat types surveyed. Mid-channel patch reefs exhibited some of the highest coral cover we have surveyed in the Sanctuary, often exceeding 30%, but was also variable, ranging from about 5% to almost 43%. Quite unexpectedly, we sampled a mid-channel patch reef south of Sunshine Key, directly in the path of Moser Channel, that had up to 60% coral cover on individual transects. Not surprisingly, massive reef-building corals, namely *Montastraea cavernosa*, *M. faveolata*, *Colpophyllia natans*, and *Siderastrea siderea*, as well *Diploria* spp. on some sites, dominated coral cover on mid-channel patch reefs. Sponges also exhibited the greatest coverage on mid-channel patch reefs, especially at several sites south of

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Vaca Key. Offshore patch reefs exhibited considerable variability in physical structure and geomorphology, from dome-type structures dominated by head corals, to very eroded and small *Acropora* reefs (e.g. western extent of Carysfort SPA) or rubble/hard-bottom matrix communities, to high-relief transitional reefs (e.g. White Banks/Dry Rocks). Coverage by corals was mostly < 10%, and algae were more predominant than on mid-channel patch reefs. Sponge coverage was also generally lower than in central Hawk Channel.

Fore-reef sampling locations included both high-relief spur-and-groove and low-relief hard-bottom habitat types. Nearly all of the 63 locations surveyed were constructed by *Acropora palmata*, and ranged from very high-profile reefs (e.g. most of the SPAs surveyed) to highly eroded or remnant *Acropora* reef flats (e.g. Conch Reef, Davis Reef, and Maryland Shoal). Not unexpectedly, coral cover offshore was greatest on high-relief spur-and-groove reefs, ranging from about 1% to 12.5%. Coral cover tended to be greatest within fully protected zones and was dominated by *Porites astreoides* and *Millepora complanata*. Algae, consisting mostly of algal turf, *Dictyota* spp., *Halimeda* spp., and crustose coralline species, dominated high-relief spur-and-groove reefs throughout the Sanctuary. Sponge cover was mostly < 5% and dominated by encrusting species adapted to higher wave energy. An interesting coverage pattern was evident for *Palythoa mammilosa*. This species exhibited locally high coverage, especially in the Lower and Middle Keys, and was more abundant than reef-building corals at 12 of the 34 (35%) spur-and-groove reefs surveyed.

Offshore low-relief hard-bottom sites were sampled between most well developed spur-and-groove reefs in the Sanctuary. Of the 29 sites, none had greater than 5% coral cover, and algae dominated all of the sites. Sponges and *Palythoa* were locally abundant, but coverage was generally < 6%. Dominant algal functional groups were primarily algal turfs and brown foliose algae, especially *Dictyota* spp., *Sargassum* spp., *Styopodium zonale*, and *Lobophora variegata*.

Species richness: Surveys of the number of species of stony corals, gorgonians, and sponges continued during the 2001 surveys. Similar to results from 2000 and probably contrary to popular perception, patch reefs typically yielded the greatest species density and number of species of reef-building corals. Usually twice the number of sponges were found on patch reefs compared to offshore fore reef areas, despite a 60% smaller sampling area. Notable exceptions were the two sampling locations within Cheeca Rocks SPA, which exhibited the lowest species richness values for stony corals, gorgonians, and sponges of the patch reefs sampled.

Species richness on offshore spur-and-groove reefs exhibited several patterns. In general, coral species richness tended to be similar in the fully protected zones and reference areas. This contrasts with the deeper fore reef (8-12 m) surveyed during 1999, in which the zones had significantly greater coral species richness than the reference areas. Sponges were more speciose than corals at the majority of sites, but exhibited considerably lower numbers of species than mid-channel and offshore patch reefs. Gorgonians exhibited a wide range in species richness, with some spur-and-groove reefs with very few species, to those with abundant and speciose gorgonian faunas. Although coral cover was low on offshore hard-bottom sites, these areas tended to have much greater coral, sponge, and gorgonian species richness than spur-and-groove reefs.

Coral density, size, and condition: Coral density, size, and condition measurements were made using a modified AGRRA approach as in previous years. We also added assessments of competition with measured corals to ascertain the degree of damage caused by interspecific competition. The total area surveyed during 2001 was 971.3 m². Over 4,000 corals were counted and measured from the 86 sites, 1,356 or 34% of which were *Millepora alcicornis* and *M. complanata*, 2,665 or 66% of which were scleractinian corals. Scleractinian corals exhibited marked differences in density and species composition among the four habitat types sampled. Patch reefs, particularly mid-channel sites, exhibited many of the highest densities we recorded this year, reflecting the predominance of massive, reef-building species. Coral densities offshore were highly variable and tended to be dominated by *Millepora* and *Porites astreoides*, especially in high-relief spur-and-groove areas.

The condition measurements during 2001 included assessments of competition, predation, bleaching, and disease. We were encouraged to find very few incidences of bleaching at the areas surveyed. As in previous years, disease incidence in the habitats we surveyed was very low. Of the 2,665 scleractinian coral assessed, only 49 (1.8%) exhibited signs of disease. The percentage of scleractinian corals with symptoms of disease ranged among the habitat types as follows: mid-channel patch reefs (2.5%), offshore patch reefs (2.1%), high-relief spur-and-groove (1.7%), and low relief hard-bottom (1.5%). No incidence of black band disease was recorded from any of the colonies assessed.

We also included more and broader transect surveys of elkhorn coral and staghorn coral during 2001. Surveys assessed the number of colonies, defined as patches of continuous live tissue, with notes on colony size. We were encouraged to find large stands of *Acropora palmata* offshore, especially at Sand Key SPA, Sand Island, Elbow Reef SPA, and South Carysfort Reef. We were also surprised to find some live stands of elkhorn coral in offshore hard-bottom areas. Live patches of staghorn coral, most probably 2-3 years of age, were found on several patch reefs surveyed as well.

Juvenile coral density: Surveys of juvenile coral species composition, density, and maximum diameter continued during 2001. Among the four habitat types sampled, juvenile densities were generally greater on mid-channel and offshore patch reefs, with different dominance patterns than offshore. We noted at several locations that one of the dominant recruiting corals was *Siderastrea siderea*. We were discouraged to find very low densities of juveniles in offshore fore-reef habitats, especially in high-relief spur and groove. Juvenile corals offshore were mostly dominated by smaller brooding species such as *Agaricia agaricites*, *Favia fragum*, and *Porites astreoides*.

Gorgonian density and height distribution: Over 13,000 gorgonians were identified, counted, and measured for colony height in the four habitat types surveyed this year. Colonies were scored into height classes as a further indication of the disturbance history of particular sites. For example, we saw many fore-reef areas, such as Crocker Reef, with high densities of sea plumes, but dominated by small (< 10 cm) colony sizes. At nearly all sites surveyed, gorgonians continue to comprise the dominant sessile macro-invertebrates, often two times or greater in density than stony corals. Many of the mid-channel and offshore patch reefs surveyed yielded some of the highest gorgonian densities (> 30 colonies per m²) we have found in the Sanctuary. One notable pattern in gorgonian density was evident on spur-and-groove reefs. Except for American Shoal,

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which is a moderately eroded *Acropora* reef, gorgonian densities were lower in the Lower Keys compared to the Upper Keys. We noted dominance by sea fans (*Gorgonia ventalina*) and sea plumes, especially *Pseudopterogorgia americana* and *P. bipinnata*, at many Upper Keys reefs.

Urchin density and size: We continued surveys of urchin density and test size at all 86 sites sampled during 2001. Six species were encountered in transect surveys in mid-channel patch reef, offshore patch reef, and shallow fore-reef habitats. Similar to results from 1999 and 2000, all of the sampling locations yielded very low densities of urchins, particularly *Diadema antillarum*. However, we found several locations with large (3.5-5 cm test diameter [TD]) *D. antillarum*, with clear effects of grazing on the substratum, as well as some locations, such as Pickles Reef, with relatively high densities of other urchins. During June surveys in the Lower Keys region, we noted several sites with juvenile (< 0.6 cm TD) *D. antillarum*, even though the peak recruitment apparently occurs in the Florida Keys during August and September. Also noted was a clear shift from a predominance of *Echinometra viridis* on patch reefs to *Euclidaris tribuloides* offshore.

Incidental invertebrates: We assessed density patterns for a variety of sessile and mobile invertebrate species during the 2001 surveys. We continued surveys of anemones and corallimorpharians, in addition to shrimp symbionts. During the first few days of sampling in mid-June, we noted several sites with abundant opisthobranch mollusks, particularly the lettuce sea slug *Tridachia crispata*, so we extended our surveys to include all visible opisthobranch mollusks within the strip transects. We were encouraged to find two nudibranch species, as well as 226 individuals of *T. crispata*, mostly associated with live or remnant *Millepora complanata*. As an additional ecological story, we also surveyed the density of and predation by the flamingo tongue (*Cyphoma gibbosum*). Over 120 individuals were measured in fore-reef habitats. We assessed gorgonian prey, including species and colony height, and will submit a paper on the density and prey utilization of this gorgonian predator.

Fishing gear and other marine debris: We continued last year's surveys of fishing gear and other marine debris at all 86 sites during 2001. Based upon results from 2000, we assumed that relatively little fishing gear would be found throughout much of the shallow (1-6 m) fore reef. We were surprised at the amount of marine debris, represented mostly by recreational hook-and-line gear, which was recovered, even within the fully protected zones. The majority of the debris was recreational hook-and-line gear, represented by monofilament line, wire, leaders, hooks, lead weights, and even a fishing pole, followed by remnant lobster/crab trap debris such as rope, wood slats, buoys, and cement. From all 86 sites representing a total survey area of only 25,200 m², we recovered more than 0.5 km of hook-and-line gear and trap rope. Of the 349 m of hook-and-line gear recovered from the fore reef, 112 m or 32% was recovered from the zones. In fact, many of the fully protected zones surveyed yielded some of the greatest densities of hook-and-line gear in the Sanctuary. While most of the gear within the zones was biologically fouled, clean or freshly lost hook-and-line gear was recovered from Sand Key SPA (7.4 m), Sombrero Key SPA (29.1 m), and Carysfort/S. Carysfort SPA (1.9 m). Similar to 2000, most of the gear found on mid-channel and offshore patch reefs was remnant lobster trap debris, especially buoy lines. However, several patch reefs near Molasses Reef Channel (near Three Sisters) and White Banks/Dry Rocks had significant quantities of hook-and-line gear.

Besides surveying the type, density, and extent of marine debris, we also assessed the number of

organisms impacted by debris, specifically abrasion and tissue mortality to sessile marine invertebrates. On the fore reef alone (63 sites), we noted 319 incidences of damage to fire coral, stony corals, gorgonians, sponges, and the colonial zoanthid *Palythoa mammilosa*. Not surprisingly, most damage was caused by hook-and-line gear on the fore reef, especially to gorgonians, and secondarily to fire coral and sponges. While we recognize that remnant fishing gear is a relatively minor factor affecting Florida Keys reefs, quantitative surveys of this type will at least be one useful measure for assessing compliance to the no-fishing regulations within the zones in the future.

Plans for Use of the Data: We made significant progress in manuscript development since January 2001. Below is a listing of manuscripts in press or published, those submitted for review, and those we intend to submit for publication by June 2002. While many of these reports are descriptive in nature, many of the variables measured by this program have never been assessed at so many sites representing the complement of shallow-water hard-bottom and coral reef habitats in the Florida Keys. Moreover, these products will be timely for the five-year review of the Sanctuary Zoning Action Plan in 2002. Other products planned for the fourth quarter of 2001 and the first quarter of 2002 are the development and dissemination of digital photographs on CD-ROM taken during 2000-2001 in the Keys and Dry Tortugas, and pdf versions of manuscripts published.

Manuscripts in Press or Published

Ault JS, Smith SG, Meester GA, Luo J, Franklin EC, Bohnsack JA, Harper DE, McClellan DB, Miller SL, Swanson DW, Chiappone M (2002) Synoptic habitat and reef fish surveys support marine reserves in the Dry Tortugas, Florida. *Reef Encounter* 31: 22-23.

Chiappone M, Miller SL, Swanson DW (2001) *Condylactis gigantea* – A giant comes under pressure from the aquarium trade in Florida. *Reef Encounter* 30: 29-31

Chiappone M, Swanson DW, Miller SL (2002) Density, spatial distribution, and size structure of sea urchins in coral reef and hard-bottom habitats of the Florida Keys. *Marine Ecology Progress Series* 235: 117-126

Chiappone M, Swanson DW, Miller SL, Smith SG (2002) Large-scale surveys on the Florida Reef Tract indicate poor recovery of the long-spined sea urchin *Diadema antillarum*. *Coral Reefs* 21: 155-159

Chiappone M, Miller SL, Swanson DW, Ault JS, Smith SG (2001) Comparatively high densities of the long-spined sea urchin in the Dry Tortugas, Florida. *Coral Reefs* 20: 137-138

Chiappone M, White A, Swanson DW, Miller SL (In press) Occurrence and biological impacts of fishing gear and other marine debris in the Florida Keys. *Marine Pollution Bulletin*

Miller SL, Chiappone M, Swanson DW, Ault JS, Smith SG, Meester GA, Luo J, Franklin EC, Bohnsack JA, Harper DE, McClellan DB (2001) An extensive deep reef terrace on the Tortugas Bank, Florida Keys National Marine Sanctuary. *Coral Reefs* 20: 299-300

Miller SL, Swanson DW, Chiappone M (In press) Multiple spatial scale assessment of coral reef and hard-bottom community structure in the Florida Keys National Marine Sanctuary. *Proceedings of the 9th International Coral Reef Symposium, Bali*

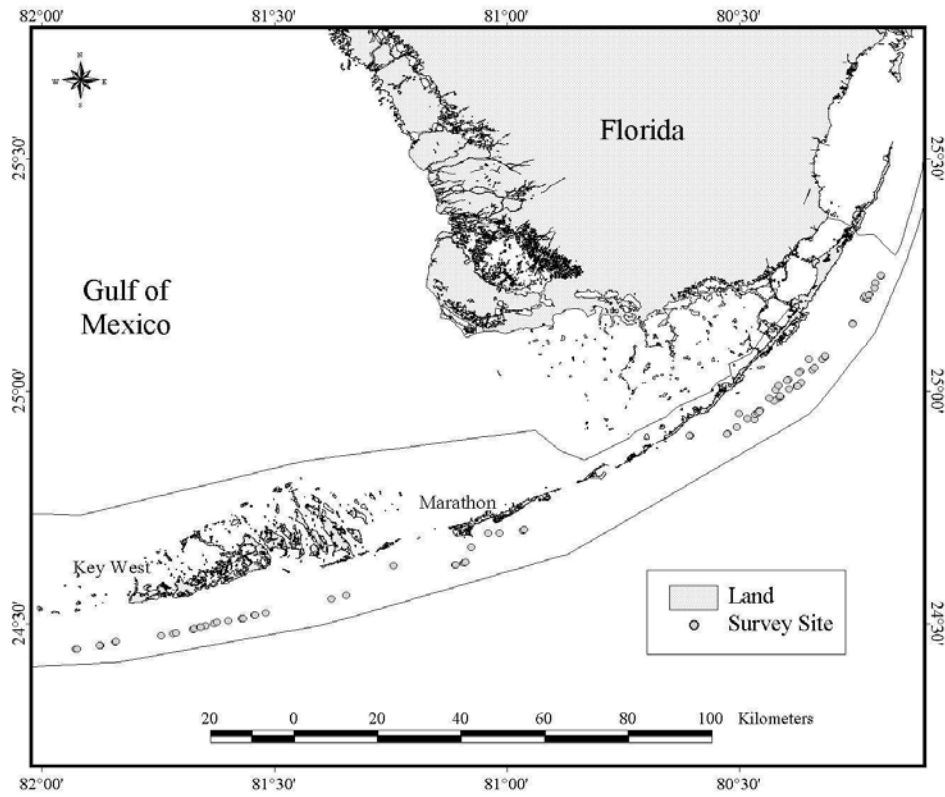


Figure 1. Survey locations in the Florida Keys National Marine Sanctuary during June-September 2001.

Table 1. Sampling effort by habitat type and regional sector in the Florida Keys.

Habitat type	Regional sector	Management type	No. of sites	Effort (%)
Mid-channel patch reef	Middle Keys	Reference areas	6	7.0
	Middle Keys	Fully protected zones	2	2.3
Offshore patch reef	Upper Keys	Reference areas	2	2.3
	Middle Keys	Reference areas	1	1.2
	Upper Keys	Reference areas	10	11.6
	Upper Keys	Fully protected zones	2	2.3
Shallow spur and groove	Lower Keys	Reference areas	9	10.5
	Lower Keys	Fully protected zones	8	9.3
	Middle Keys	Reference areas	1	1.2
	Middle Keys	Fully protected zones	2	2.3
	Upper Keys	Reference areas	6	7.0
	Upper Keys	Fully protected zones	8	9.3
Shallow hard-bottom	Lower Keys	Reference areas	7	8.1
	Middle Keys	Reference areas	9	10.5
	Middle Keys	Fully protected zones	4	4.7
	Upper Keys	Reference areas	9	10.5
Total		11 Fully protected zones	86	100.0

Table 2. Variables measured in Sanctuary fully protected zones and reference areas during 2001. Transects 25 m in length were used in all sites except patch reefs (10 m).

Variable	Method	Factors assessed
Percent cover	Point-intercept along 4 transects	Percent cover, relative abundance
Species richness	0.4 m x 25 m swaths along 8 transects	Species density, total species
Coral density and size	0.4 m x 25 m swaths along 2 transects	Density, size, condition
<i>Acropora</i> coral density	2 m x 25 m swaths along 8 transects	Density
Juvenile coral density	Twenty 0.68 m x 0.45 m quadrats	Species composition and density
Gorgonian density and height	0.4 m x 25 m swaths along 2 transects	Density, height distribution
Urchin density and size	0.4 m x 25 m swaths along 8 transects	Density, test diameter
Marine ornamentals	0.4 m x 25 m swaths along 8 transects	Density
Spiny lobster density	2 m x 25 m swaths along 8 transects	Density
<i>Cyphoma</i> density and prey	0.4 m x 25 m swaths along 8 transects	Density, prey utilization
Fishing gear density	2 m x 25 swaths along 8 transects	Density, length, biological impacts
Topography	0.4 m x 25 m swaths along 4 transects	Maximum relief, substratum slope

Project Title: Sea Stewards: A Volunteer Ecological Monitoring Program

Researcher: Brad Rosov, The Nature Conservancy, Sugarloaf Key, FL.

Goals: In 1998, The Nature Conservancy initiated the Sea Stewards volunteer program to participate in monitoring the Florida Keys National Marine Sanctuary. The objectives of the program are to: 1) target species and ecological processes that are not being monitored by other studies, 2) contribute useful data to the evaluation of the Sanctuary's zoning program, and 3) engage Keys residents and Sanctuary users in evaluating resource condition and the effectiveness of management activities.

Methods: Ten teams of volunteer divers, boat operators, and photographers monitor assigned permanent sites in both Sanctuary fully protected ("no-take") zones and nearby reference areas. Selected targets include 1) all species of reef-dwelling sea urchins (mainly *Diadema antillarum*, *Eucidaris tribuloides*, *Echinometra viridis*, and *E. lucunter*), 2) adult three-spot damselfish (*Stegastes planifrons*), 3) juvenile and adult yellowtail damselfish (*Microspathodon chrysurus*), and 4) all known fish-cleaning species, mainly neon goby (*Gobiosoma oceanops*), Pederson cleaner shrimp (*Perclimenes pedersoni*), spotted cleaner shrimp (*P. yucatanicus*), scarlet-striped cleaner shrimp (*Lysmata grabhami*), juvenile porkfish (*Anisotremus virginicus*), juvenile Spanish hogfish (*Bodianus rufus*), and juvenile bluehead wrasse (*Thalassoma bifasciatum*). Teams collected data on these selected targets every year both during the dry (November-April) and wet (May-October) seasons; due to weather conditions and other constraints, not all of the teams completed data collections for each season.

Sea urchins and target damselfish were identified and counted in 20, one-m² quadrats radiating out from the sites' central feature in belts of five quadrats. In addition, the size of each sea urchin was categorized and recorded. Beginning in May 2000, quantitative data on the number of fish cleaners, active cleaning stations, and clients were collected within two meters on either side of each 25-m belt transect, covering a total area of 400 square meters. In addition, the location of fish cleaners and active fish cleaning stations were mapped for future comparisons.

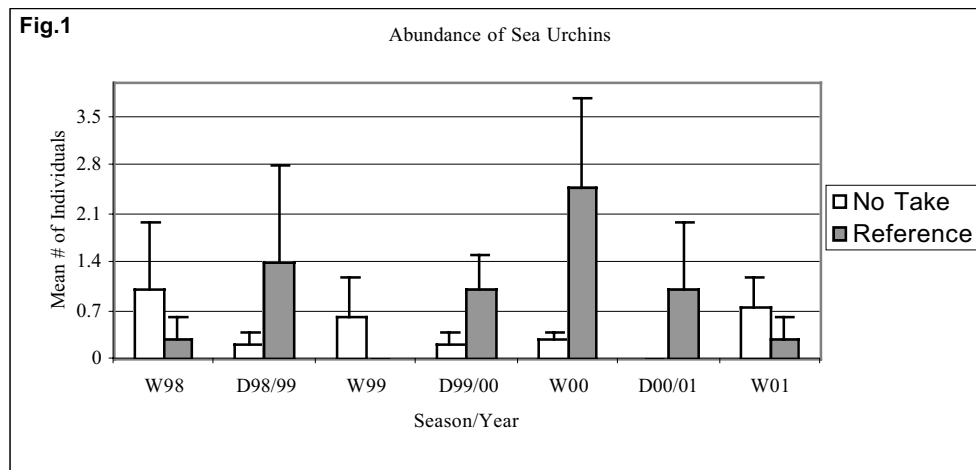
Findings to Date: Until this year (2001), no statistically significant differences were found between the fully protected and reference areas for any of the four targets. The abundance of target species in wet season versus dry season historically has not been statistically different as well. The long-term data documents an overall low density of sea urchins; due to these low numbers all sea urchin species were combined for the statistical analysis. The vast majority of the sea urchins recorded were slate-pencil urchins (*Eucidaris tribuloides*) and very few observations of *Diadema antillarum* were noted (Fig. 1).

In 2001, Sea Stewards logged a total of 11 dives; 6 were located in fully protected zones and 5 were located in reference sites. Due to the relatively low number of dives, less data was collected from previous years making statistical analysis difficult. It was determined, however, that there were significantly more adult yellowtail damselfish observed in reference zones compared to fully protected zones (Fig. 2). Adult yellowtail damselfish were significantly more abundant during the wet season versus during the dry season. No significant statistics regarding zones or seasons could be extrapolated for data collected on juvenile yellowtail damselfish and adult

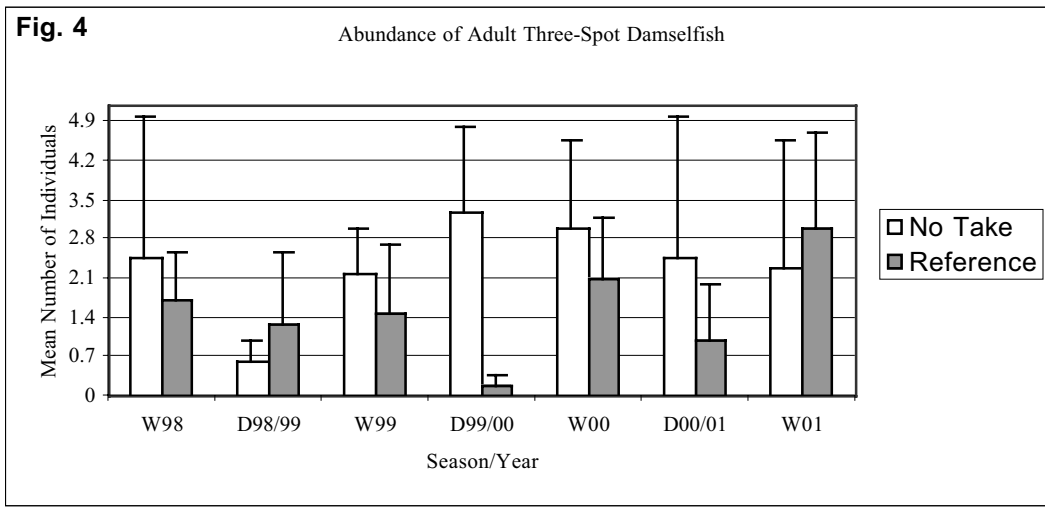
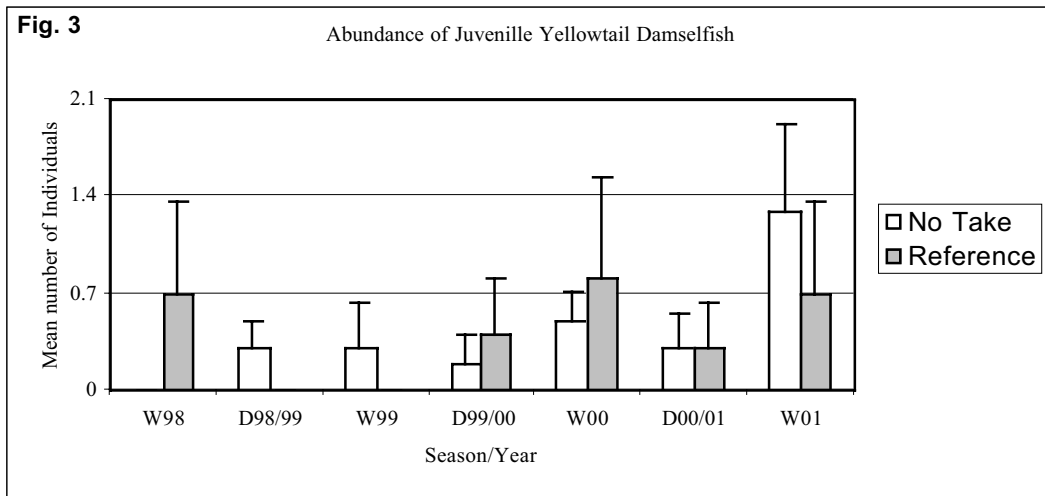
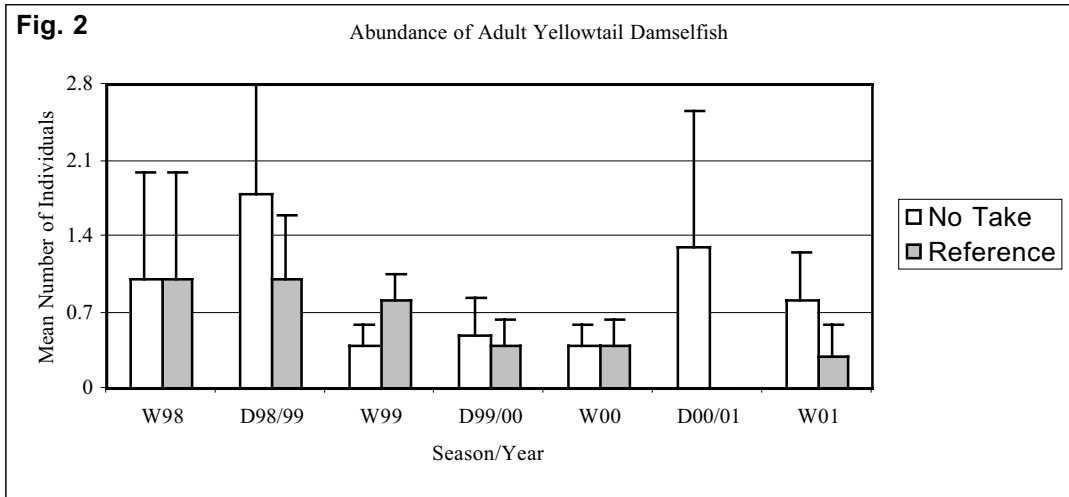
three-spot damselfish (Figs. 3 and 4). There were no observations of long-spined urchins (*Diadema antillarum*) in 2001, which indicates populations have still not recovered from the 1983-1984 massive die-off. The general trend of low urchin densities (all species monitored) continued both within fully protected zones and reference sites.

Sea Stewards collected data on fish cleaners and fish cleaning stations for the first time using the belt transect method during the 2000 wet season. In 2001, 17 active cleaning stations with 706 active cleaners were documented. The most common facultative cleaner documented was the juvenile bluehead wrasse (52% of all cleaner species observed). The most abundant obligate cleaner was the neon goby (39.9% of all cleaner species observed). Given that these two species made up the majority of the cleaners, they were combined for the statistical analysis. The results of the t-test showed no significant difference between the fully protected zones and reference areas for the neon goby/bluehead wrasse or for all other cleaners combined. Seasonal differences did emerge in 2001 with regards to the abundance of cleaner species. A significantly greater abundance of cleaner species was observed during the dry season compared to the wet season. The list of “clients” included species of grunts, groupers, chubs, damselfish, tangs, and eels.

Some trends in the urchin and damselfish densities when comparing fully protected zones and reference areas are beginning to develop; however, it is still too early to speculate about the difference in densities of these targets. Continued monitoring of all four targets is imperative to discern any significant differences or actual trends between the SPA and reference areas.



Figures 1-4: Mean number of individuals per 20 square meters; W= wet season (May-October), D= dry season (November- April). W99 Reference and D00/01 No Take = 0.



Figures 1-4 (continued): Mean number of individuals per 20 square meters; W= wet season (May-October), D= dry season (November- April). Missing bar = 0.

Project Title: Preliminary Analysis of FKNMS Reef Fish Monitoring through 2001

Researchers: James A. Bohnsack, David B. McClellan, and Douglas E. Harper, NOAA/National Marine Fisheries Service, Miami, FL; and Jerry Ault, Steven G. Smith, Geoff Meester, and Jiangang Luo, RSMAS, University of Miami, Miami, FL.

Goal: The goal of this monitoring is to assess changes in reef fish populations in zones under different levels of protective management. On July 1, 1997 the Florida Keys National Marine Sanctuary (FKNMS) established 18 fully protected (“no-take”) Sanctuary Preservation Areas (SPAs) and one Ecological Reserve in the Western Sambo region of the Lower Florida Keys. Field studies since then have been directed at comparing changes in fully protected areas to nearby reference areas with fishing.

Methods: Sampling continued through 2001, the fourth full year of protection. The sampling design was improved in 1999 to include a habitat-based, stratified random sampling design and expanded into other habitats to more efficiently monitor reef fish populations throughout the Florida Keys and to better assess habitat preferences by different species. This expanded effort added two classes of data (random samples of low-relief habitat in protected and fished areas) in addition to the high-relief protected and fished sites previously sampled. In 2001, field sampling was successfully completed for a total of 306 reef blocks and 1,224 dives from Dade County through the Lower Keys (Fig.1). These sites include a total of 278 stratified random blocks and 28 historical reference reef sites. Each block represents 4 stationary fish counts.

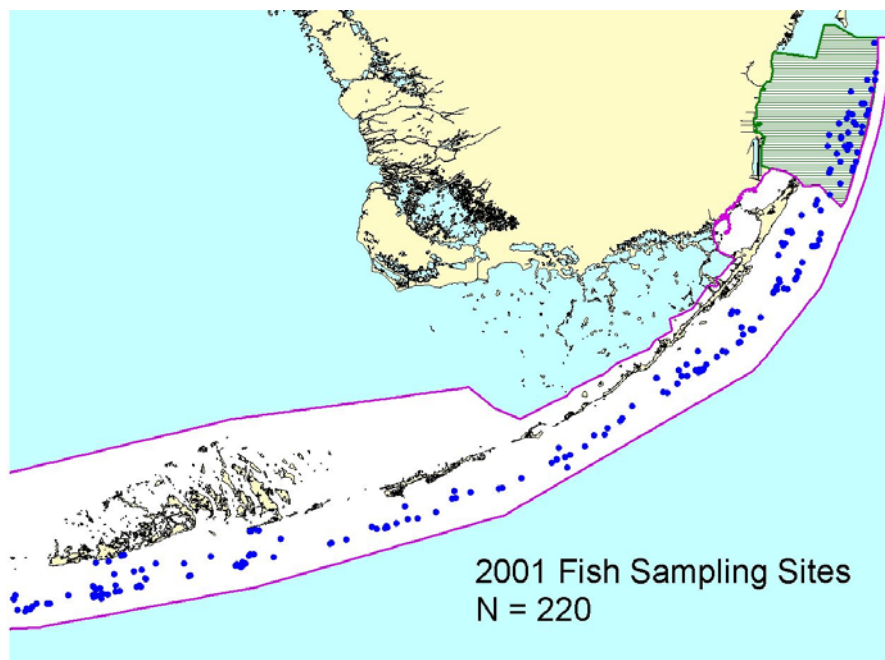


Figure 1. Location of 306 stationary fish sample sites in the Florida Keys National Marine Sanctuary (outlined in purple) and Biscayne National Park (outlined in green) sampled in the Florida Keys in 2001.

Findings to Date: Below we show trend analyses of raw data from fished and unfished areas for selected targeted and non-targeted species. Hurricane symbols show the occurrence of hurricanes impacting the Lower Keys. In the fall of 1998 Hurricane Georges, a large hurricane, and Hurricane Mitch, a small hurricane hit the Florida Keys. In 1999 Hurricane Irene, a small hurricane, passed over the Lower Keys. Yellowtail snapper mean density continued to be significantly higher in fully protected zones than fished sites and further increased above the long-term 1994-1997 performance range relative to fished reference areas (Figure 2).

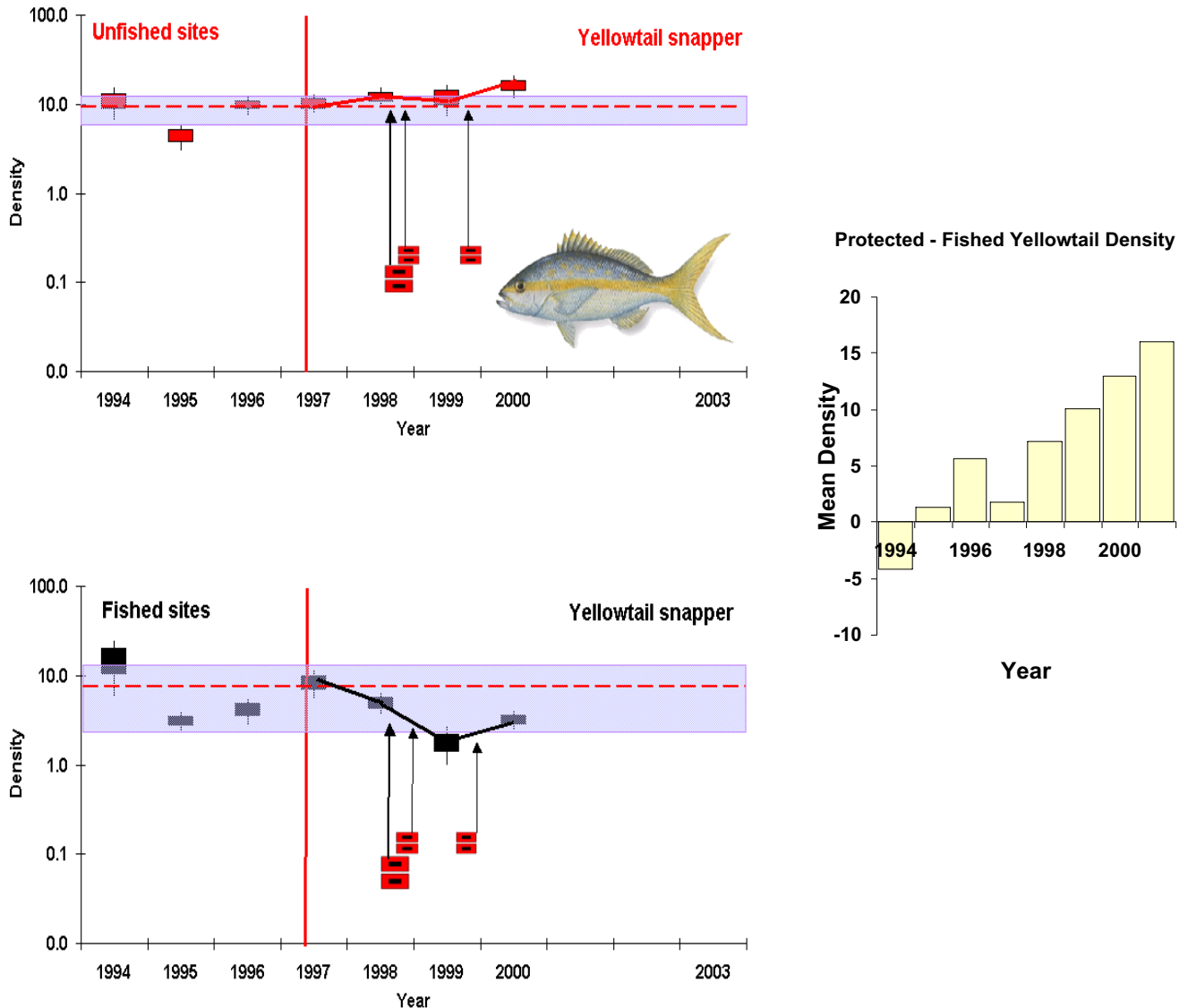


Figure 2. Comparison of yellowtail snapper density trends in the fully protected “no-take” Sanctuary Preservation Areas (SPAs) (top left) and exploited reference areas (bottom left). Vertical red line shows when no-take protection occurred. Horizontal blue bands show null model predictions based on 1994-1997 95% annual performance measures projected to 2003. Boxes show annual standard errors and whiskers show 95% confidence intervals. Flags show hurricane occurrences. Annual density trends obtained by subtracting reference area densities from densities in fully protected zones are shown at right.

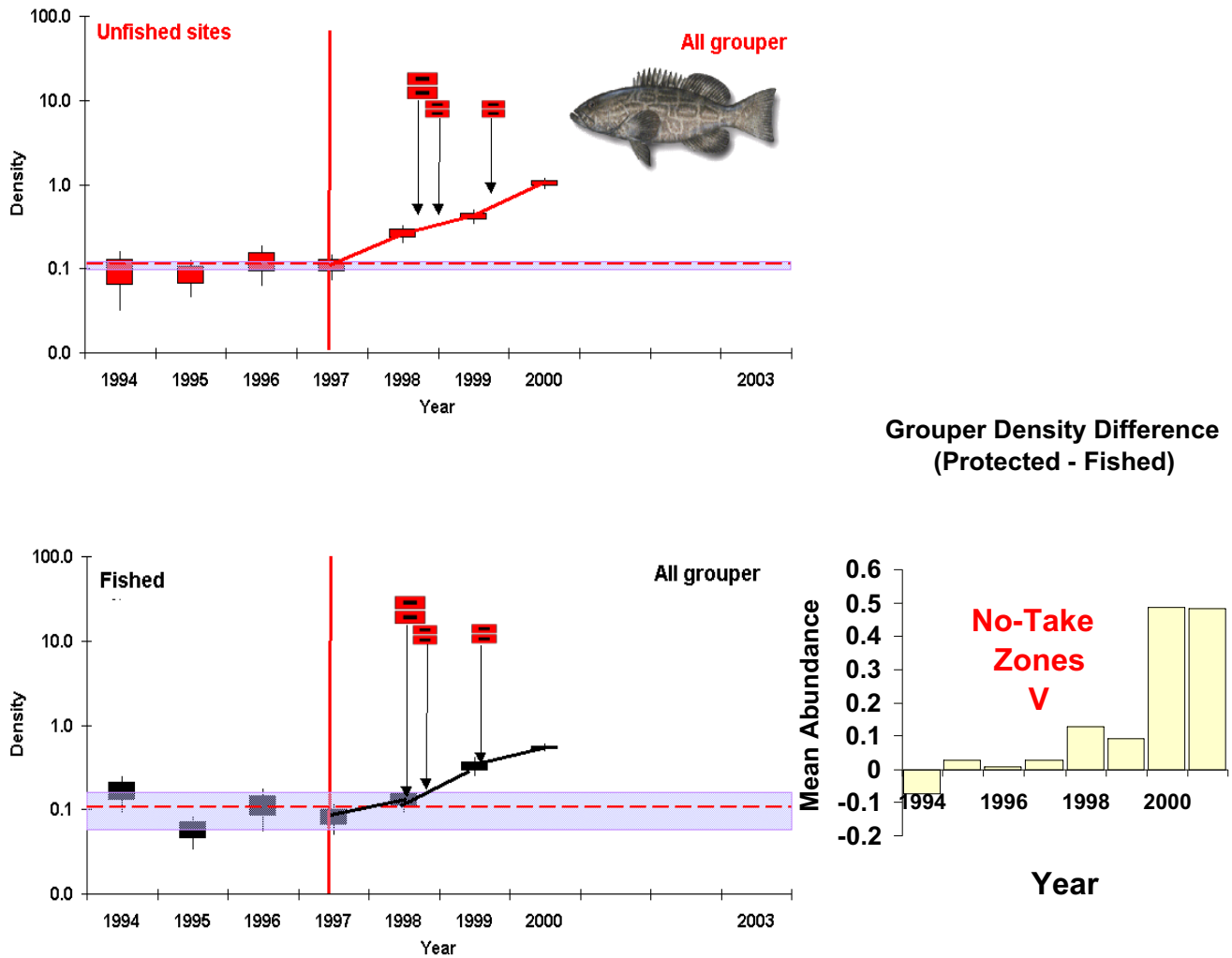


Figure 3. Comparison of combined exploitable grouper density trends in the fully protected “no-take”Sanctuary Preservation Areas (top left) and exploited reference areas (bottom left). Vertical red line shows when no-take protection occurred. Horizontal blue bands show null model predictions based on 1994-1997 95% annual performance measures projected to 2003. Boxes show annual standard errors and whiskers show 95% confidence intervals. Flags show hurricane occurrences. Annual density trends obtained by subtracting reference area densities from densities in fully protected zones are shown at right.

Economically important species of grouper were combined for statistical analysis. The mean combined grouper density has increased in both fished reference areas and fully protected zones since 1997 and currently is approximately an order of magnitude higher than that in the baseline period. Densities in fully protected zones have increased faster than in fished reference areas, especially in 2000 and 2001 (Fig. 3).

Gray Snapper mean density increased in 2001 in fished reference areas and remained stable at

the upper end of the long-term performance range in fully protected zones. Densities have remained higher in fully protected zones than in fished reference areas every year since 1997 (Fig. 4).

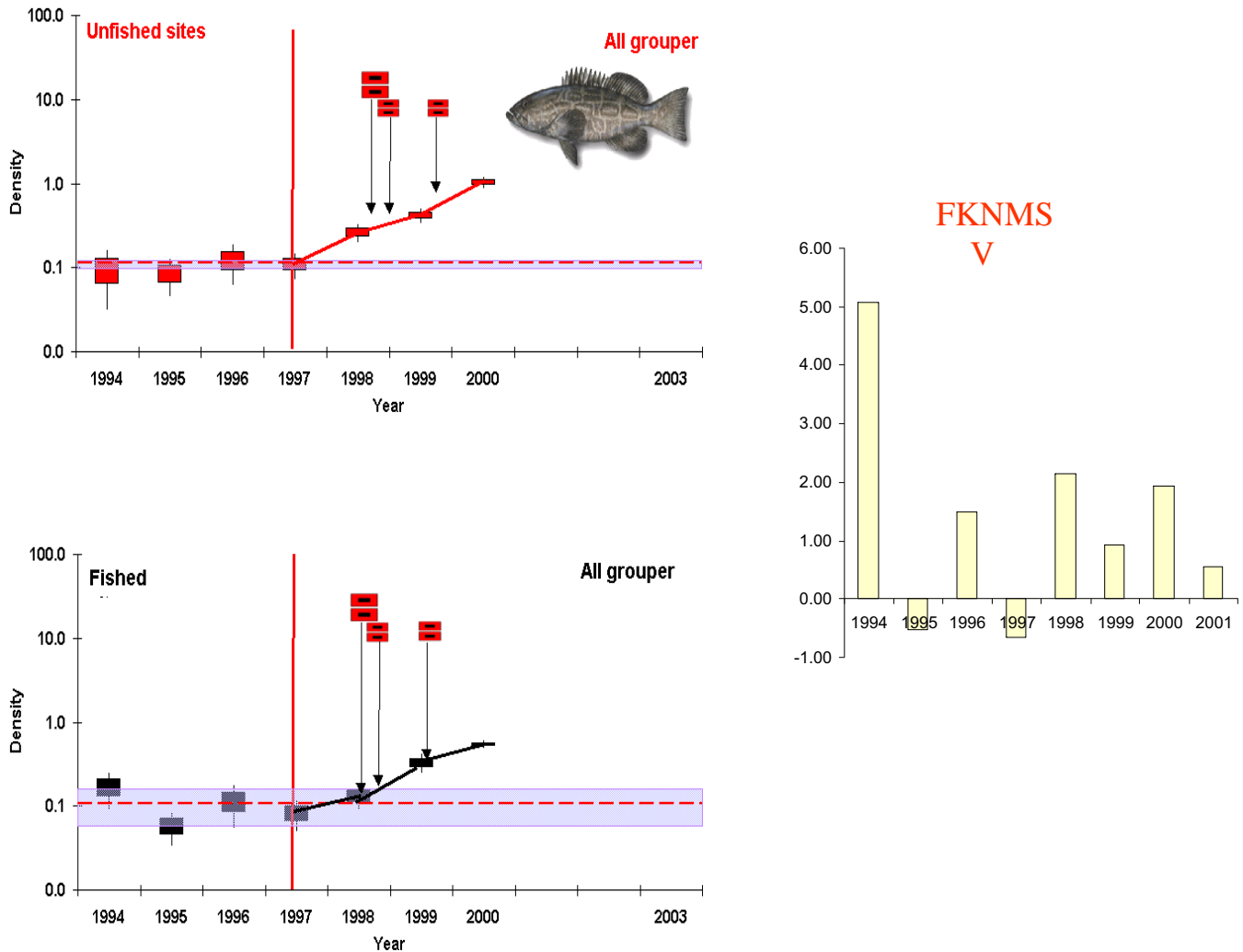


Figure 4. Comparison of gray snapper density trends in the fully protected “no-take” Sanctuary Preservation Areas (top) and exploited reference areas (middle). Vertical red line shows when no-take protection occurred. Horizontal blue bands show null model predictions based on 1994-1997 95% annual performance measures projected to 2003. Boxes show annual standard errors and whiskers show 95% confidence intervals. Flags show hurricane occurrences. Annual density trends obtained by subtracting reference area densities from densities in SPAs are shown at right.

Hogfish mean density increased significantly in fished zones in 2001 and remained relatively constant in fully protected reserves, both at levels above the long-term performance range (Fig. 5). Hogfish mean density remained lower in fully protected zones than in fished reference areas.

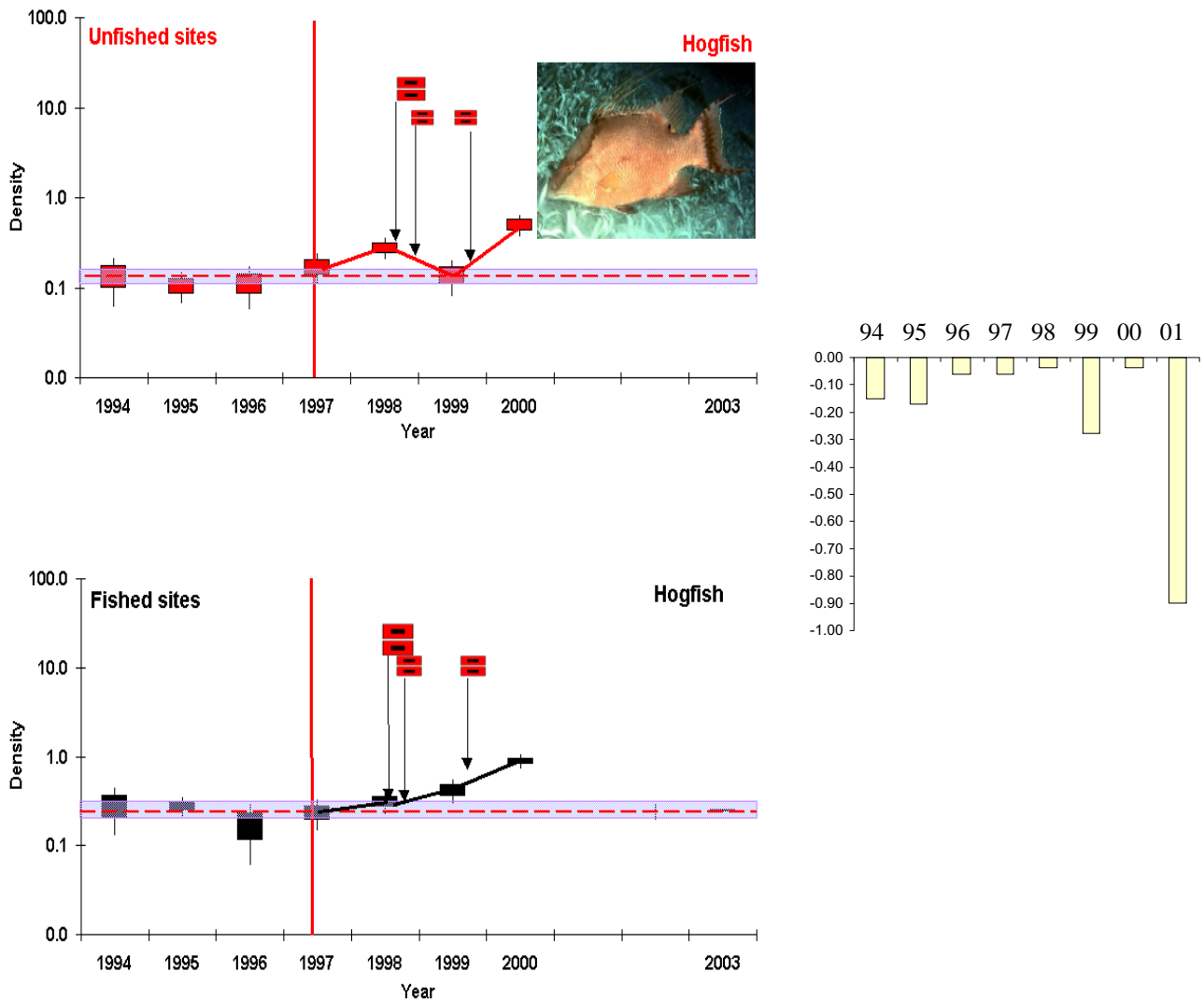


Figure 5. Comparison of Hogfish density trends in the fully protected “no-take” Sanctuary Preservation Areas (top) and exploited reference areas (middle). Vertical red line shows when no-take protection occurred. Horizontal blue bands show null model predictions based on 1994-1997 95% annual performance measures projected to 2003. Boxes show annual standard errors and whiskers show 95% confidence intervals. Flags show hurricane occurrences. Annual density trends obtained by subtracting reference area densities from densities in SPAs are shown at right.

Stoplight parrotfish, a large herbivore not normally targeted by fishing, decreased in mean density (number of individuals per sample) in both fished and unfished areas in 2001 (Fig. 6). Mean density was higher in unfished areas than in fished areas. Both fished and unfished zones showed concordance in relative trends since 1997. Densities in unfished zones were within the long-term, 1994-1997, performance range but remained slightly below in the performance range in fished zones.

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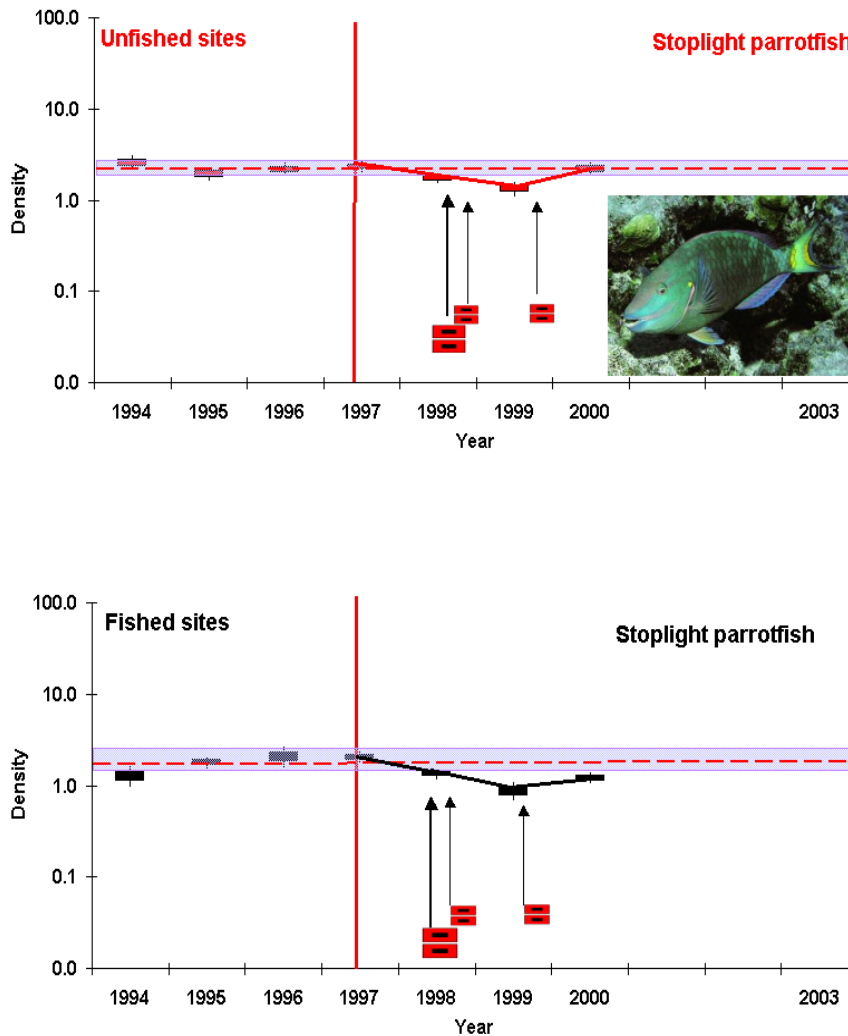


Figure 6. Comparison of stoplight parrotfish density trends in the fully protected “no-take” Sanctuary Preservation Areas (top) and exploited reference areas (bottom). Vertical red line shows when no-take protection occurred. Horizontal blue bands show null model predictions based on 1994-1997 95% annual performance measures projected to 2003. Boxes show annual standard errors and whiskers show 95% confidence intervals. Flags show hurricane occurrences.

Striped parrotfish, a small herbivore not targeted by fishing, showed high concordance in mean density (number of individuals per sample) in both fished and unfished areas over the study period. Density is slightly above the long-term performance range in unfished areas, but similar in fished and unfished areas.

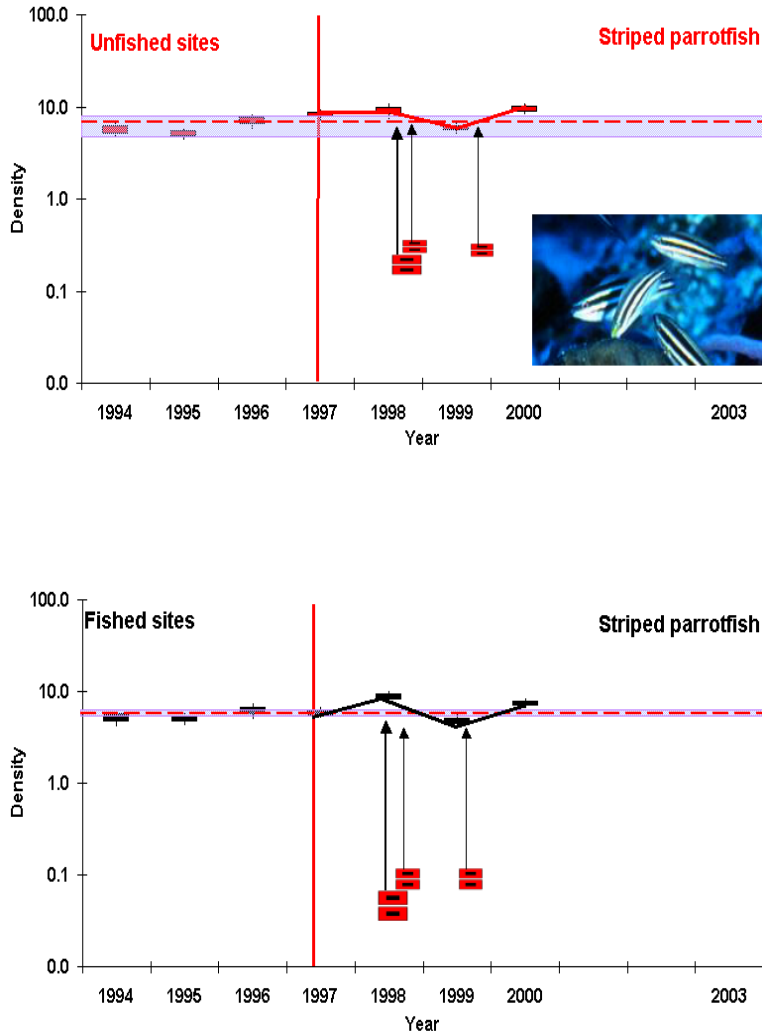


Figure 7. Comparison of striped parrotfish density trends in the fully protected “no-take” Sanctuary Preservation Areas (top) and exploited reference areas (bottom). Vertical red line shows when no-take protection occurred. Horizontal blue bands show null model predictions based on 1994-1997 95% annual performance measures projected to 2003. Boxes show annual standard errors and whiskers show 95% confidence intervals. Flags show hurricane occurrences.

Summary. Since no-take protection was initiated in 1997, significant density increases were observed for several exploited species in fully protected zones compared to fished reference areas. Among exploited species, mean densities were higher in fully protected zones for Gray Snapper, combined grouper, and Yellowtail Snapper. Hogfish densities remained higher in exploited areas than in unfished areas. Concordance was observed in changes in density for Stoplight Parrotfish and Striped Parrotfish, two species not directly exploited. The passage of Hurricane Georges (a strong hurricane) and Mitch (a weak hurricane) in the fall of 1998 resulted in declines of mean density at both fished and unfished sites in 1999 for the two non-exploited parrotfishes and Gray Snapper. No detrimental impacts on fish densities were noted following the passage of Hurricane Irene, a weak hurricane that passed over the Lower Keys in the fall of 1999.

21 March 2003

Project Title: Volunteer Reef Fish Monitoring in the Florida Keys National Marine Sanctuary: 1994 - 2001

Researchers: Reef Environmental Education Foundation (REEF) staff and Advanced Assessment Team

Survey Method: The Roving Diver Technique (RDT) is a non-point visual survey method specifically designed to generate a comprehensive species list along with frequency and abundance estimates. During RDT surveys, divers swim freely throughout a dive site and record every observed fish species. At the conclusion of each survey, divers assign each recorded species one of four \log_{10} abundance categories [single (1); few (2-10), many (11-100), and abundant (>100)]. Following the dive, each surveyor records the species data along with survey time, depth, temperature, and other environmental information on a REEF scan sheet. The scan sheets are returned to REEF, and the data are loaded into the REEF database that is publicly accessible on the Internet at <http://www.reef.org>.

As part of the Florida Keys National Marine Sanctuary (FKNMS) Zone Monitoring Program (ZMP), REEF was contracted to collect reef fish data. This project supports a team of REEF's most experienced surveyors, the Advanced Assessment Team (AAT), to annually survey 37 sites in the FKNMS, including 12 SPAs, 3 Research-only sites, the Western Sambo Ecological Reserve, 10 sites in the Tortugas Ecological Reserve area, and 10 comparison/reference sites. A minimum of six RDT surveys was conducted at each site. These data were collected during a series of cruises in October, and complemented REEF's Fish Survey Project, a continual volunteer monitoring project that involves REEF volunteers conducting RDT surveys during their regular diving activities in the Florida Keys. The field season of 2001 was the fifth year that the AAT has monitored most of these sites and the eighth full year of REEF volunteer data collection in the Sanctuary.

During the 2001 REEF FKNMS ZMP, 473 RDT surveys were conducted by the REEF AAT, documenting 246 fish species. Between 1997 and 2001, 62 AAT members participated in REEF's FKNMS Zone Monitoring Program, contributing 1,626 surveys. Through REEF's ongoing program, a total of 1,329 REEF volunteers have conducted 9,807 surveys from 311 sites in the FKNMS and have documented 415 fish species.

Findings to Date: This report summarizes all REEF data (Expert and Novice) collected at the 27 Zone Monitoring Program sites in the FKNMS between 1994 and 2001 (the Tortugas sites are not included). Table 1 lists the sites included, along with the level of protection (if any) granted in 1997 and annual REEF survey effort.

To estimate richness and evenness at each site, species accumulation curves were generated based on a standardized sample size of 23 Expert REEF RDT surveys using randomized sampling (Table 2). The data were fit to an asymptotic hyperbola using maximum likelihood to estimate the parameters for the Michaelis-Menten equation. The asymptote and the slope of the curve estimated site-level richness and evenness, respectively. This method allows for the estimation of diversity despite differences in survey effort among sites. This analysis was adapted from Semmens et al. (in prep). The inclusion of only REEF Expert data in this particular

analysis was done to minimize the effect of species misidentifications. Sites that exhibited the lowest richness (the Newfound Harbor sites, Cannon Patch, Cheeca Rocks, and Delta Shoals) are all inshore patch reef sites. In all but four of the 16 fully protected/reference site pairs, the fully protected sites had higher richness than the open reference site (see previous REEF Annual Reports for a listing of fully protected/reference site pairs). A more complete GIS analysis of the FKNMS reef fish diversity, including how diversity may be associated with a variety of natural, anthropogenic, and environmental variables is currently being conducted (Semmens et al. in prep).

The basic statistic generated by REEF data is the abundance score, which is a weighted average of the abundance categories reported for each species combined with non-sightings¹. The trends in abundance score between 1994 and 2001 for the top 75 species documented at ZMP sites were estimated. To generate the trend values, ordinal logistic regressions were conducted on each species at each site to evaluate the trend in the likelihood of an observer recording an abundance of either single, few, many, abundant, or absent (Semmens et al. 2000). The likelihood is based on a regression of the ordinal values, and the trend is the slope of the ordinal regression line. This trend analysis is robust to the non-normal distribution of the categorical dataset.

The trend analysis highlighted several sites that are experiencing declines in a majority of the common fishes, and several sites that are experiencing increases in a majority of the common fishes. Sites where at least two-thirds (50) of the species declined more or increased less than at other sites included Grecian Rocks, Looe Key East, Eastern Sambo, and No Name Reef (Fig. 1a). Sites where at least two-thirds of the species increased more or decreased less than at other sites were Molasses Reef, Conch Reef, Hen and Chickens, Sombrero Reef, Sand Key, and Newfound Harbor Open (Fig. 1b).

There was no significant difference in the mean trends of all 75 species between open and protected sites. This is not surprising, as one would not expect reserves to produce changes in abundance across all fishes in a consistent manner. Interspecific interactions yield complex community responses and many species may actually exhibit short-term declines due to trophic cascades and top-down effects. In addition, certain previously exploited species may fail to recover despite reserve designation due to changes in community structure, food web dynamics, and/or habitat and physical parameters.

More species changed in abundance at the protected sites than at the open sites. Using a Wald test of significance, the trend values of each species at each site were evaluated for significance. An alpha value of 0.10 was used as the significance threshold because, for this site level comparison, we were not interested in rejecting specific null hypotheses regarding species trends. Rather, we wished to identify those non-zero slope values that were “reasonably believable”. Twelve of the 27 sites exhibited significant trends (positive or negative) in at least half of the 75 species (Table 3). A majority (9) of those sites were protected as no-take in 1997.

In an effort to pinpoint specific species that appear to be doing exceptionally well or poor, patterns in the slopes of the species’ trends (positive or negative) were evaluated. The following

¹abundance score = $[(n_S \times 1) + (n_F \times 2) + (n_M \times 3) + (n_A \times 4)] / (n_S + n_F + n_M + n_A)$ * percent sighting frequency, where n is the number of times each abundance category was assigned

species increased between 1994 and 2001, defined as positive trends in at least half (14) of the 27 sites – saddled blenny, beaugregory, bridled goby, colon goby, goldspot goby, black grouper, redbelt parrotfish, and bluehead wrasse. The following species appear to be in general decline, defined as negative trends in at least half of the sites – rock beauty, smooth trunkfish, dusky damselfish, sharpnose pufferfish, ocean surgeonfish, and trumpetfish. Possible contributing factors to the general increase in the three sand/rubble-dwelling gobies (bridled, colon, and goldspot; Fig. 2a) are the increase in sand/rubble areas from the hurricanes of 1998 and 1999, the increase in turf algae at many locations, and/or a decrease in predators. The decrease in mean abundance score of trumpetfish beginning in 1998 is shown in Fig. 2b. One possible explanation for this decrease could be the slight decrease in octocoral cover reported by the EPA/FKNMS CRMP study, as trumpetfish often use octocorals for camouflage habitat. The decline in rock beauty was evident at all but a few sites, but rock beauty at protected sites decreased slightly less than at open sites (Fig. 2c). The other common angelfishes (gray, French, and queen) exhibited little change in mean abundance. A likely cause of this change in rock beauty is harvesting for the aquarium industry, as juvenile rock beauty are one of the most collected fish species in the FKNMS.

Of five targeted species (black grouper, hogfish, mahogany snapper, yellowtail snapper, and gray snapper), only gray snapper had significantly different trend values between protected and unprotected sites (one way ANOVA, $p = 0.003$). The difference in trend values between protected and unprotected sites was marginally significant for hogfish (one way ANOVA, $p = 0.065$). The mean annual abundance score values for the three snapper species and hogfish, based on all 27 sites, generally increased or remained unchanged from 1994 through 2002 (Fig. 2d). As stated earlier, black grouper had positive trend values at a majority (20) of the sites and exhibited dramatic increases in mean abundance score and sighting frequency across all sites (Fig. 3). Exceptions were Grecian Rocks and Cannon Patch, where black grouper exhibited statistically significant ($p = 0.041$ and 0.012 , respectively) annual decreases in abundance score between 1994 and 2001. While not a top 75 species, the sighting frequency of red grouper was also evaluated. This species was rarely encountered between 1994 and 1996, but has steadily increased since 1997 (Fig. 4).

Future Plans: The REEF ZMP project in the FKNMS has generated annual data by REEF experts in the protected and reference areas. While the initial five-year project recently has been completed, REEF plans to continue this annual monitoring effort and conducted another round of monitoring in September 2002. REEF will also continue to enable all divers to participate in its volunteer Fish Survey Project in the FKNMS. In the coming year, REEF will continue our partnership with NOAA's Biogeography Office to use the REEF database and the FKNMS Benthic Habitat database to investigate fish-habitat relationships, to map species distributions in the FKNMS, and to evaluate the effect of the zones by analyzing shifts in assemblage composition over time (Jeffrey et al. 2000). In late 2001, a baseline assessment of the proposed Dry Tortugas National Park zones was completed (REEF 2002). In 2002, several new projects were initiated, including 5-year monitoring projects of the *Wellwood* restoration and the *Spiegel Grove*. REEF staff are also currently working with Dr. Tom Gillespe (UCLA Geography Department) on several analyses using REEF FKNMS data.

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- Semmens, B.X., T.W. Gillespe, and C.V. Pattengill-Semmens. in prep. Predictors of fish species richness on coral reefs.
- Semmens, B.X., J.L. Ruesink, and C.V. Pattengill-Semmens. 2000. Multi-site multi-species trends: a new tool for coral reef managers. International Coral Reef Symposium, October 2000.

Table 1. REEF survey effort by location and by year. Effort includes all Species and Abundance RDT surveys conducted during daylight hours (after 7am and before 8pm) greater than 20 minutes in length.

Location	Protection	REEF Survey Effort							
		1994	1995	1996	1997	1998	1999	2000	2001
Ball Buoy Reef	Open	0	0	0	7	5	14	13	14
Grecian Rocks	SPA	27	17	26	30	10	43	74	60
Carysfort Reef	SPA	17	18	0	8	10	21	23	17
Molasses Reef	SPA	31	28	20	47	84	125	85	214
Little Grecian	Open	1	10	3	13	7	10	15	10
South Carysfort Reef	SPA	0	12	14	6	7	15	14	12
Cannon Patch	Open	0	0	0	6	16	1	14	21
Pickles Reef	Open	1	1	1	25	15	12	36	23
Conch Reef	SPA	37	21	7	32	11	19	16	47
Hen and Chickens	SPA	23	8	8	19	15	12	12	22
Tennessee Reef Research	RR	34	0	0	16	9	9	8	12
Cheeca Rocks	SPA	0	0	0	17	11	9	6	13
Sombrero Reef	SPA	87	5	15	20	14	16	13	13
Samantha's Ledge	Open	38	0	6	13	11	12	15	13
Coffins Patch	SPA	35	0	5	6	28	11	10	14
Looe Key East	SPA	19	1	0	10	21	19	39	42
Looe Key Research	RR	18	0	0	6	8	13	9	12
Delta Shoals	Open	0	0	0	12	6	11	9	11
Newfound Harbor SPA	SPA	0	0	0	6	6	10	17	13
Newfound Harbor Open	Open	0	0	0	6	6	10	9	12
No Name Reef	Open	0	0	0	6	6	10	9	12
Western Sambo	ER	40	34	19	7	15	10	14	105
Eastern Sambo	SPA	25	18	0	12	9	8	11	20
Sand Key	SPA	15	45	11	14	17	11	13	29
Middle Sambo	Open	13	18	0	11	9	9	12	20
Pelican Shoals	Open	13	16	10	0	0	0	11	24
Western Dry Rocks	Open	1	0	0	19	19	16	11	37

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Table 2. Fish species richness and evenness estimates based on a sample size of 23 REEF Expert RDT surveys. Lower B values indicate higher evenness.

Location	Protection	M Parameter Estimate (Richness)	B Parameter Estimate (Evenness)
Conch Reef	No-Take	167	1.55
Coffins Patch	No-Take	167	1.56
Molasses Reef	No-Take	166	1.39
Western Dry Rocks	Open	165	1.65
Western Sambo	No-Take	165	1.58
Eastern Sambo	No-Take	165	1.95
Grecian Rocks	No-Take	164	1.93
Looe Key East	No-Take	162	1.44
Middle Sambo	Open	162	1.44
Little Grecian	Open	160	1.62
Sand Key	No-Take	160	1.52
Carysfort Reef	No-Take	159	1.56
No Name Reef	Open	156	1.60
Sombrero Reef	No-Take	155	1.57
Pelican Shoals	Open	153	1.82
Ball Buoy Reef	Open	152	1.40
Samantha's Ledge	Open	152	1.40
Tennessee Reef Research	No-Take	151	2.18
South Carysfort Reef	No-Take	149	1.44
Looe Key Research	No-Take	145	1.85
Hen and Chickens	No-Take	145	1.85
Pickles Reef	Open	145	1.37
Newfound Harbor SPA	No-Take	140	2.38
Delta Shoals	Open	136	1.31
Cheeca Rocks	No-Take	134	2.12
Cannon Patch	Open	131	2.46
Newfound Harbor Open	Open	124	2.12

Table 3. The trend values of each species at each site were evaluated for significance. 12 of the 27 sites exhibited significant trends (positive or negative; alpha value 0.10) in at least half of the 75 species evaluated. A majority (9) of those sites were protected as no-take in 1997.

Site	Protection (as of July 1997)	Proportion of Species
Sombrero Reef	No-Take	77%
Molasses Reef	No-Take	68%
Conch Reef	No-Take	65%
Looe Key Research	No-Take	61%
Samantha's Ledge	Open	56%
Pelican Shoals	Open	55%
Hen and Chickens	No-Take	53%
Coffins Patch	No-Take	53%
Sand Key	No-Take	53%
Grecian Rocks	No-Take	52%
Middle Sambo	Open	52%
Eastern Sambo	No-Take	51%
Tennessee Reef Research	No-Take	47%
Carysfort Reef	No-Take	45%
Looe Key East	No-Take	45%
Western Sambo	No-Take	43%
Little Grecian	Open	37%
South Carysfort Reef	No-Take	36%
Pickles Reef	Open	36%
No Name Reef	Open	35%
Western Dry Rocks	Open	29%
Cannon Patch	Open	27%
Newfound Harbor Open	Open	24%
Cheeca Rocks	Open	21%
Delta Shoals	Open	21%
Newfound Harbor SPA	No-Take	19%
Ball Buoy Reef	Open	12%

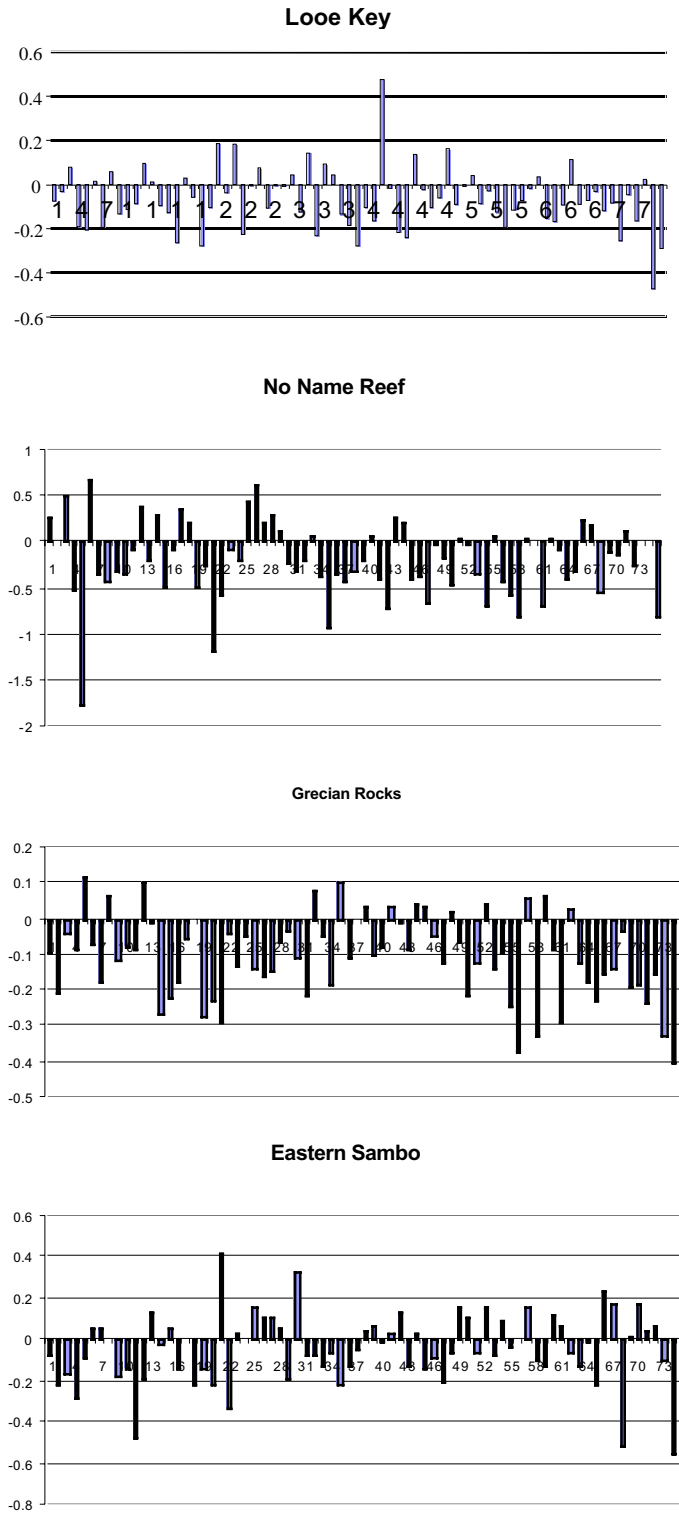


Figure 1. Abundance score trend values for the top 75 fish species, based on REEF data from 1994-2001. Species are listed in order of average sighting frequency: a) sites where at least two-thirds (50) of the species declined more or increased less than at other sites; b) sites where at least two-thirds of the species increased more or decreased less than at other sites.

1a)
continued

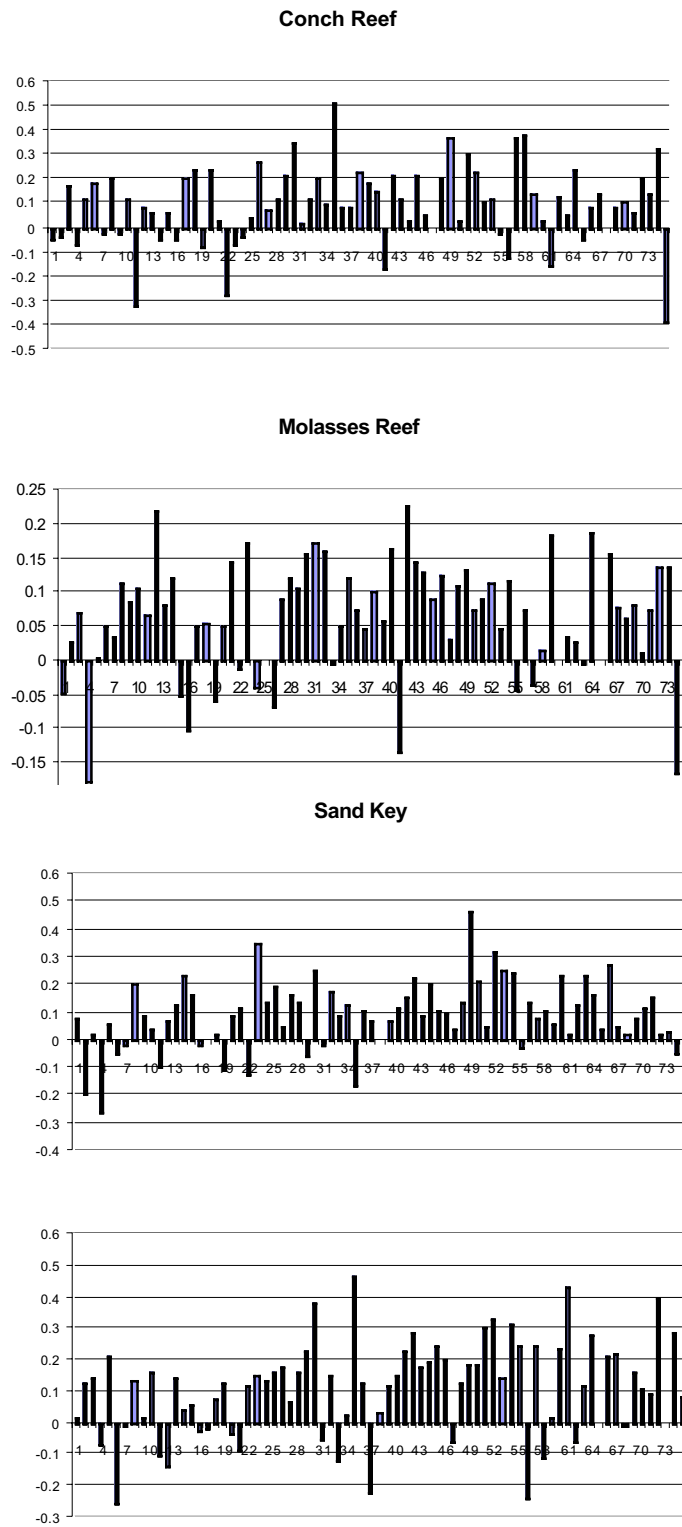
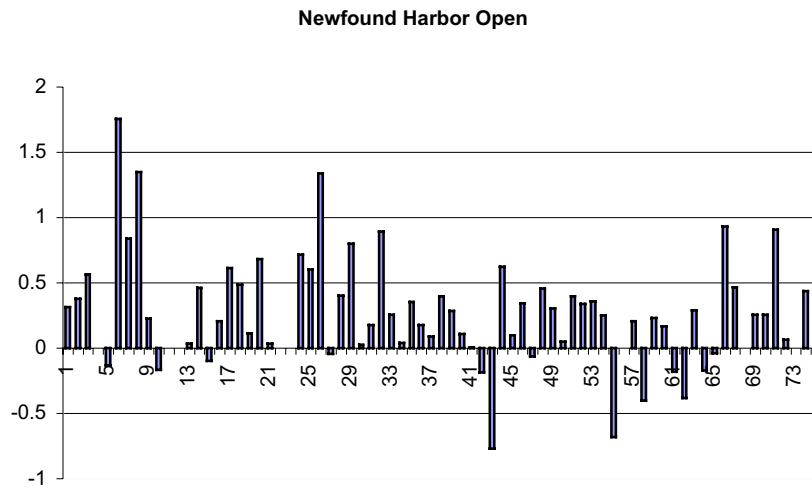


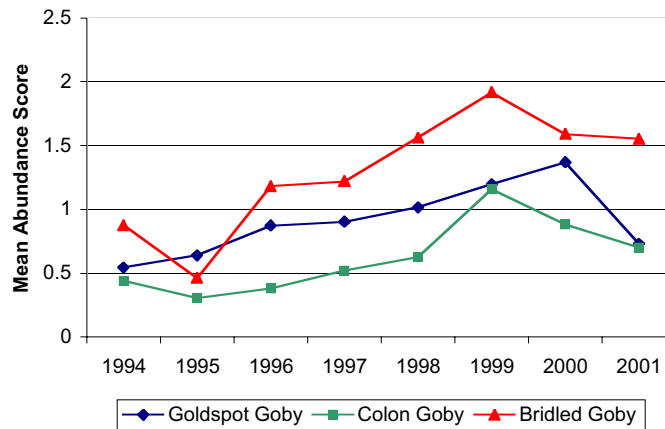
Figure 1 (continued). Abundance score trend values for the top 75 fish species, based on REEF data from 1994-2001. Species are listed in order of average sighting frequency a) Sites where at least two-thirds (50) of the species declined more or increased less than at other sites; b) Sites where at least two-thirds of the species increased more or decreased less than at other sites.

1b)
continued



*Fig. 1 x-axis: 1-French Angelfish; 2-Gray Angelfish; 3-Queen Angelfish; 4-Rock Beauty; 5-Great Barracuda; 6-Saddled Blenny; 7-Smooth Trunkfish; 8-Banded Butterflyfish; 9-Foureye Butterflyfish; 10-Spotfin Butterflyfish; 11-Blue Chromis; 12-Brown Chromis; 13-Beaugregory; 14-Bicolor Damsel; 15-Cocoa Damsel; 16-Dusky Damsel; 17-Longfin Damsel; 18-Sergeant Major; 19-Threespot Damsel; 20-Yellowtail Damsel; 21-Highhat; 22-Scrawled Filefish; 23-Spotted Goatfish; 24-Yellow Goatfish; 25-Bridled Goby; 26-Colon Goby; 27-Goldspot Goby; 28-Masked Goby/Glass Goby; 29-Neon Goby; 30-Black Grouper; 31-Graysby; 32-Bluestriped Grunt; 33-Caesar Grunt; 34-French Grunt; 35-Black Margate; 36-Porkfish; 37-Sailors Choice; 38-Smallmouth Grunt; 39-Spanish Grunt; 40-White Grunt; 41-Butter Hamlet; 42-Hogfish; 43-Spanish Hogfish; 44-Bar Jack; 45-Blue Parrotfish; 46-Midnight Parrotfish; 47-Princess Parrotfish; 48-Queen Parrotfish; 49-Rainbow Parrotfish; 50-Redband Parrotfish; 51-Yellowtail (Redfin) Parrotfish; 52-Redtail Parrotfish; 53-Stoplight Parrotfish; 54-Striped Parrotfish; 55-Sharpnose Puffer; 56-Harlequin Bass; 57-Gray Snapper; 58-Mahogany Snapper; 59-Schoolmaster; 60-Yellowtail Snapper; 61-Longspine Squirrelfish; 62-Squirrelfish; 63-Blue Tang; 64-Doctorfish; 65-Ocean Surgeonfish; 66-Bluehead; 67-Clown Wrasse; 68-Creole Wrasse; 69-Puddingwife; 70-Slippery Dick; 71-Yellowhead Wrasse; 72-Bermuda Chub/Yellow Chub; 73-Yellowhead Jawfish; 74-Glassy Sweeper; 75-Trumpetfish

Sand/Rubble-Dwelling Gobies



2a)

Figure 2. Mean abundance score by year. All sites are combined, unless noted in the legend.

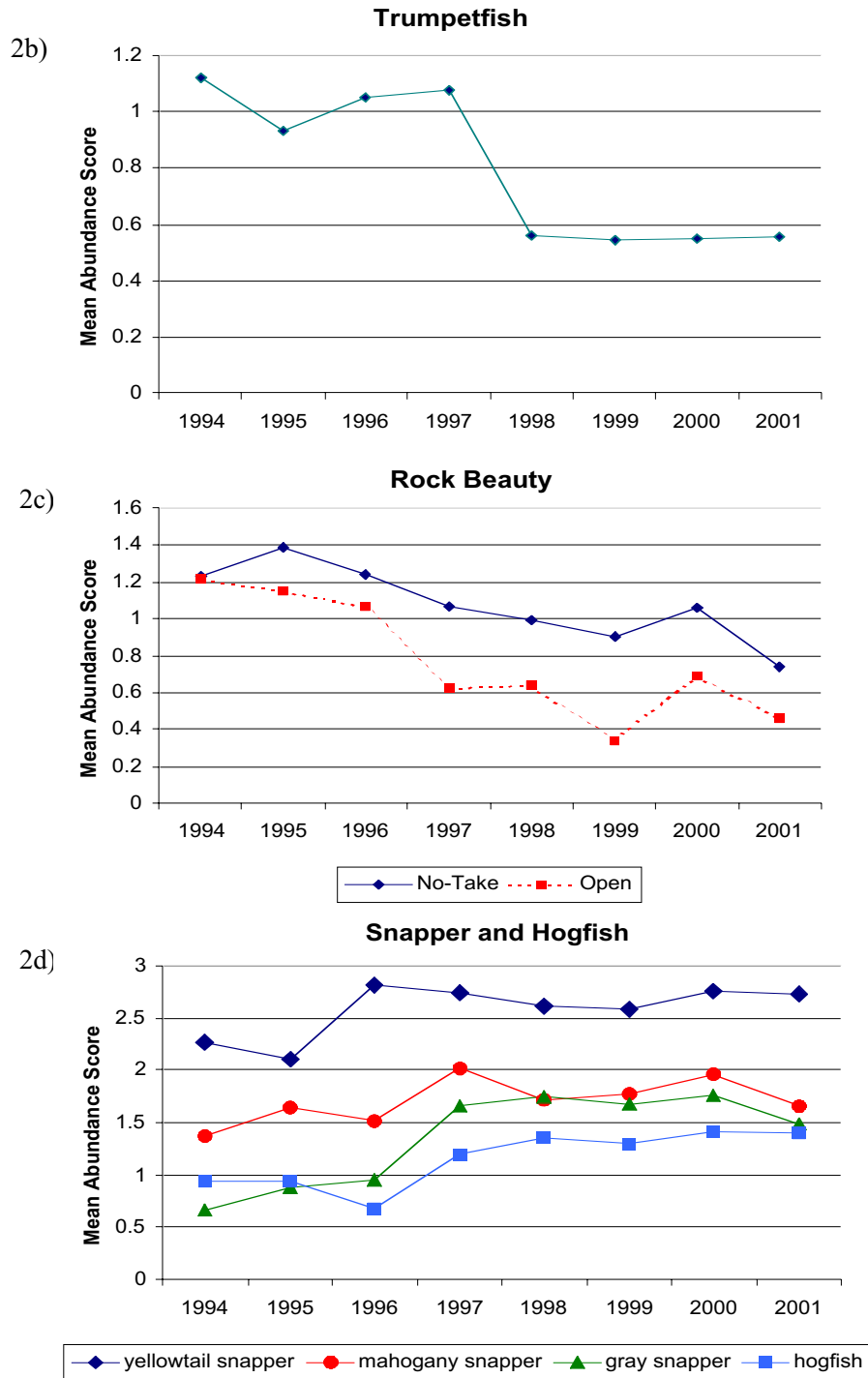
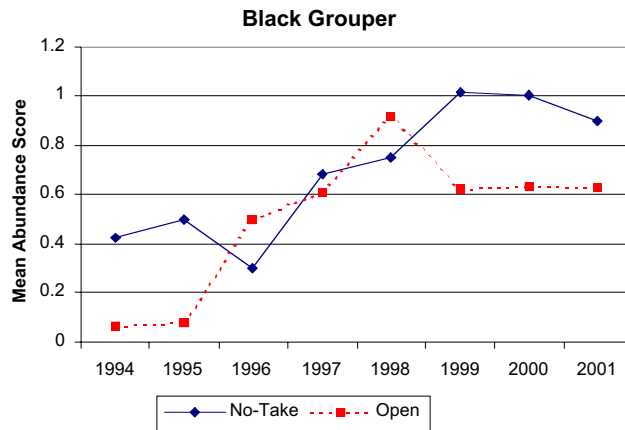


Figure 2. Mean abundance score by year. All sites are combined, unless noted in the legend.

3a)



3b)

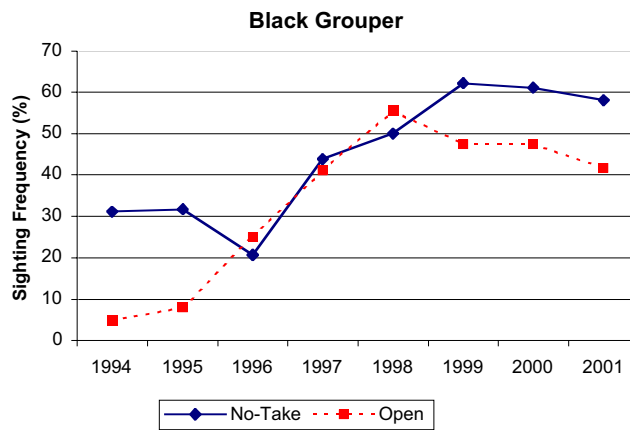


Figure 3. Mean abundance score and sighting frequency by year for black grouper. Sites were grouped by protection (open vs. no-take).

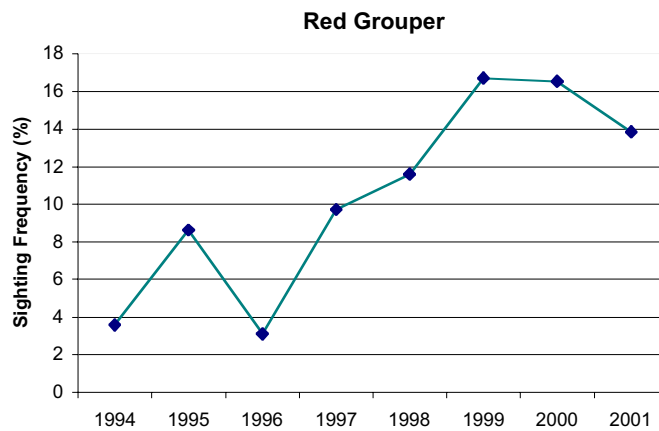


Figure 4. Sighting frequency for red grouper from 1994-2001. Data points represent all 27 sites.

21 March 2003

Project Title: Monitoring Caribbean Spiny Lobsters in the Florida Keys National Marine Sanctuary, 1997-2001

Researchers: Carrollyn Cox, Nathaniel K. Jue, Meaghan C. Darcy, and John H. Hunt, Florida Fish and Wildlife Conservation Commission/Florida Marine Research Institute, Marathon, FL.

Goals: We have monitored spiny lobsters in the marine reserves of the Florida Keys National Marine Sanctuary (FKNMS) since they were closed to fishing in July 1997. Our goal was to determine if the reserves are effective in protecting this highly mobile species from exploitation.

Methods: We sampled thirteen reserves and paired reference areas twice a year, once during July at the end of the closed fishing season, and once in September/October after several months of the lobster fishing season. Reserves were comprised of 10 (Mean= 85 ha) Sanctuary Preservation Areas or Research-only Areas (SPAs), Looe Key SPA (115 ha) which has been a lobster reserve since 1981, one large (515 ha) “super” SPA at Carysfort, and one 3,000 ha Ecological Reserve (ER) at Western Sambo. Sampling was stratified by habitat (fore reef, back reef, offshore patch reef, and nearshore patch reef) in Western Sambo ER, and three subsamples were taken in each habitat. Three subsamples were taken on the fore reef at Carysfort SPA and one sample was taken in primary lobster habitat in each of the SPAs. Samples consisted of a 60-minute timed search during which we enumerated and attempted to catch all lobsters observed. Size, sex, molt stage, reproductive state (of females), den number, and depth were recorded for each lobster encountered. Data from SPAs, Looe Key SPA, Carysfort SPA, and the ER were treated separately and compared with data from their respective exploited reference areas.

Findings to Date: We counted more than 10,000 lobsters during the course of our five-year study (Figure 1). Lobster abundance varied among years with highest abundance in 1999 and lowest abundance in 1998. In most years, the total number of lobsters observed in reserves and references declined during the open season, but the decline was less precipitous for reserves. This decline in total number of lobsters inside reserves between closed and open fishing seasons indicates that most reserves are too small to totally protect lobsters from exploitation. During the closed seasons of 1997-99, lobster abundance was nearly equal in reserves and reference areas indicating that there was redistribution of lobsters along the reef tract during the closed fishing season. Since 2000, considerably more lobsters have been found inside reserves than in reference areas during the closed season. There has been an increase in the percentage of legal-sized (≥ 76 mm carapace length [CL]) in the Western Sambo ER over the last five years, while the abundance of legal lobsters in its reference area continues to reflect the effect of the fishery.

There has been an increase in legal-sized lobster abundance in the small SPAs relative to the reference areas over the last five years. Abundance of legal-sized lobsters is higher on average at Looe Key than at the other SPAs, but it has not increased and is not higher than at its reference area. Abundance of legal-sized lobsters has been very low at Carysfort “super” SPA relative to its reference area and to the small SPAs, and we have not seen an increasing trend in abundance during the last five years. In the large ER, there has been a trend of increasing abundance of legal-sized lobsters on the fore reef.

Overall, mean lobster size was below the legal limit (76 mm CL) in reserves and references in 1997. Since protection, mean lobster size in reserves has been larger than legal size and comparatively larger than in references where it remained below the legal limit. There were no differences in size of legal lobsters between SPAs and references, but SPA lobsters were slightly larger on average. There were no differences in size of legal lobsters between Looe Key SPA and Carysfort SPA and their respective reference areas despite the longevity of the Looe Key SPA and the size of the Carysfort “super” SPA. However, there has been a significant increase in the size of legal-sized lobsters in the large Western Sambo ER. Mean size of male lobsters on ER offshore patch reefs has increased 10 mm in the last five years. Abundance of very large lobsters (≥ 100 mm CL) has increased in the ER relative to its reference area with males becoming larger and more abundant.

Several marine reserves, the best example being Western Sambo ER, exhibited particularly compelling evidence for reserve efficacy by providing at least a temporary refuge for spiny lobsters. Others, such as Carysfort “super” SPA, do not appear to function as lobster reserves at all. Because of its size, habitat diversity, and continuity, the Western Sambo Ecological Reserve may function as a wildlife corridor, protecting spiny lobsters through all life stages from *puerulus*-stage larval settlement in the nearshore through migration of mature lobsters to offshore reefs for reproduction. Effectiveness of marine reserves appears to be a function of reserve size, location, and habitat protected relative to spiny lobster life history.

Total Number of Lobsters Observed

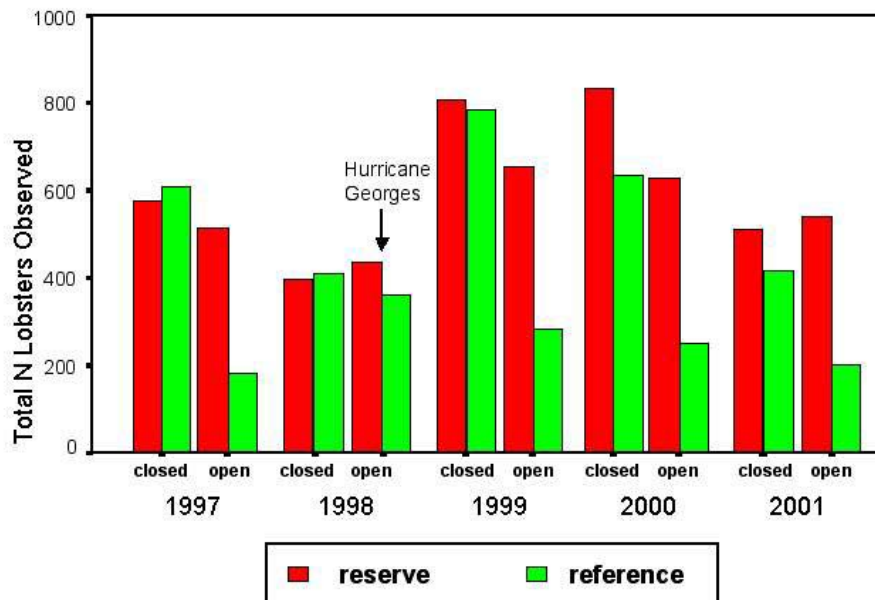


Figure 1. Total number of lobsters observed during closed and open seasons, 1997-2001.

Western Sambo Lobster Abundance

Legal-Sized Lobsters by Habitat

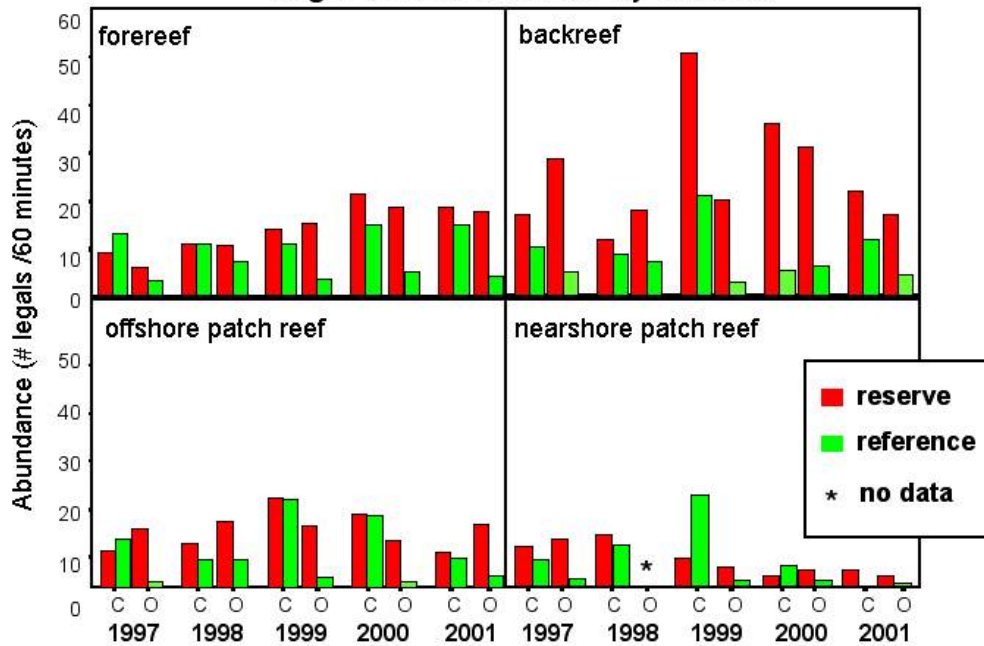


Figure 2. Legal-sized lobster abundance by habitat in Western Sambo ER, 1997-2001. C = closed fishing season, O = open fishing season.

Abundance of Legal-Sized Lobsters

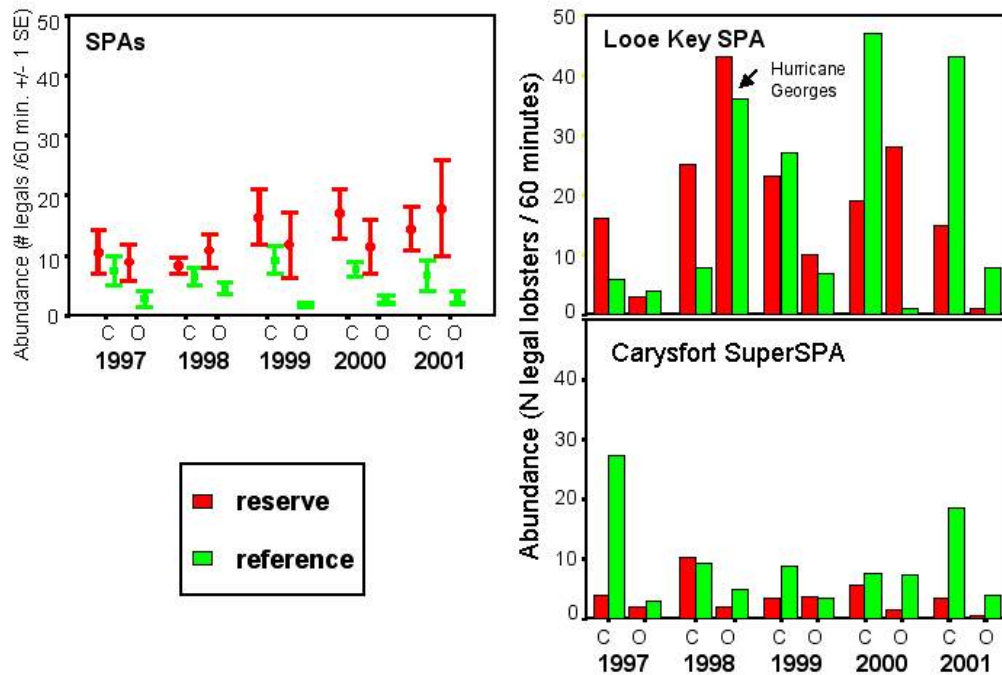


Figure 3. Abundance of legal-sized lobsters in the SPAs and their corresponding reference sites. C = closed fishing season, O = open fishing season.

Size of Legal Lobsters

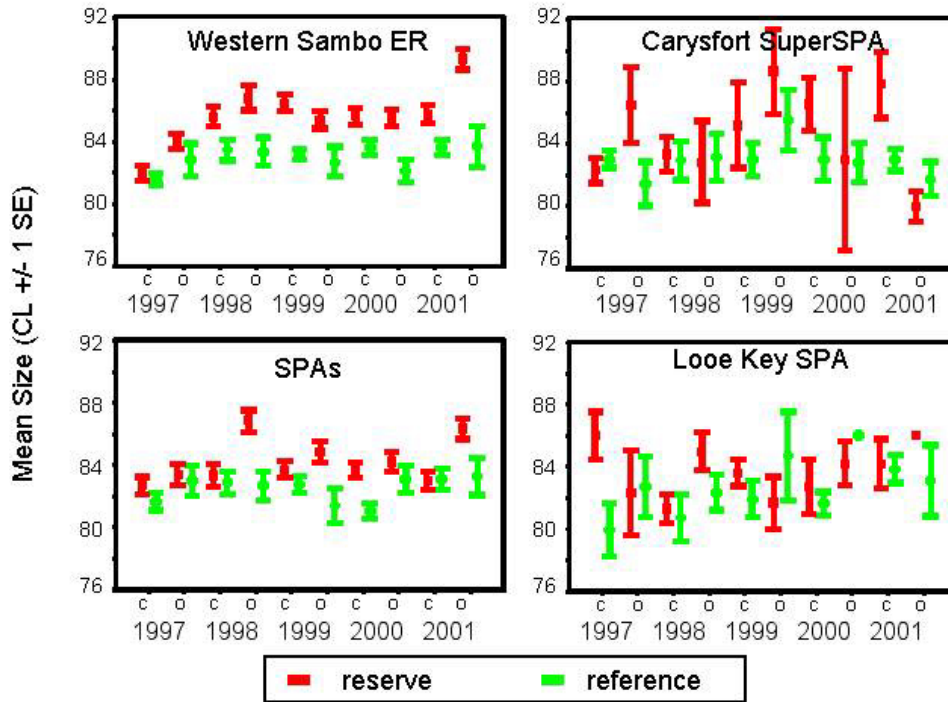


Figure 4. Mean size of lobsters in SPAs and their corresponding reference sites. C = closed fishing season, O = open fishing season.

Western Sambo - Size by Habitat

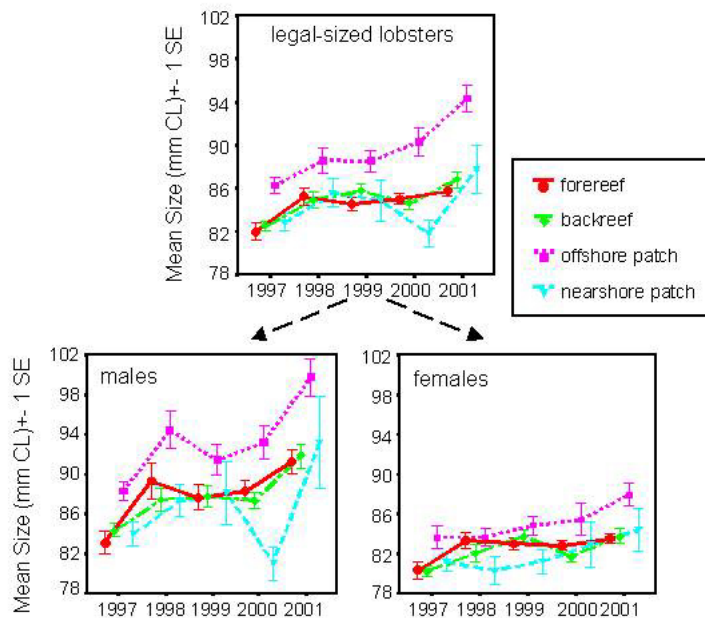


Figure 5. Mean size of male and female lobsters in Western Sambo ER by habitat.

Size-Frequency of Male Spiny Lobsters

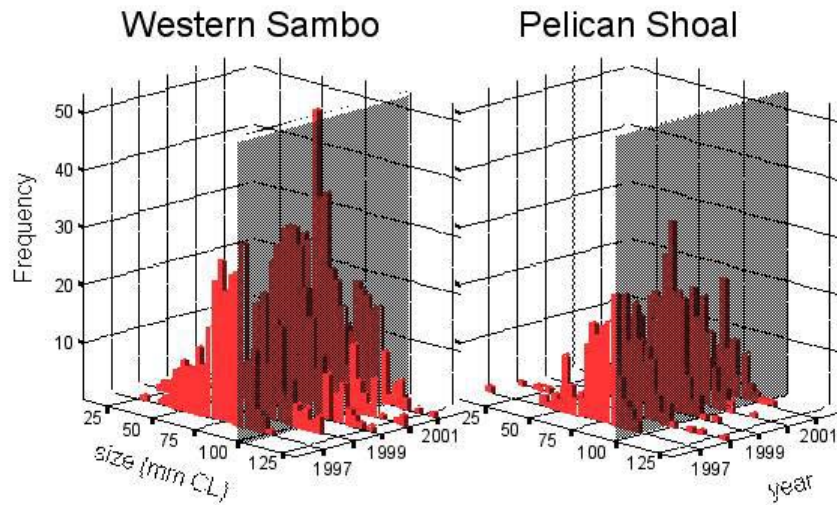


Figure 6. Mean size and frequency of male lobsters in Western Sambo Ecological Reserve and Pelican Shoal (reference site).

21 March 2003

***Project Title:* Sentinel Lobster Fisheries Project for the Florida Keys National Marine Sanctuary, January - December, 2001**

Researcher: Douglas R. Gregory, Jr., University of Florida/Florida Sea Grant, Monroe County Cooperative Extension Service, Key West, FL.

Goals: The purpose of the Sentinel Lobster Fisheries project is to use commercial fishing gear and techniques to evaluate the long-term effectiveness of the Western Sambo Ecological Reserve as a refuge for spiny lobster. The direct involvement of commercial fishermen in this project was an important factor to make the research results as relevant as possible to the commercial fishing community. This past year (2001) was the fourth and final year of the planned four-year project.

The objectives of this study are to: (1) have commercial lobster fishermen directly involved in monitoring potential changes in lobster abundance and size within and adjacent to the Reserve, (2) determine if abundance and size of spiny lobsters within the Reserve were greater than in nearby fished areas during the first four years of protection, and (3) determine if lobsters from within the Reserve were emigrating to the adjacent fished areas and providing yield to fishermen fishing near the Reserve.

Methods: A commercial lobster fisherman was successfully contracted to supply and fish 90 lobster traps during the months of June and November, 1998-2001, to provide observations from both the closed and open fishing seasons. Four sampling trips were conducted during each sampling period (season) with the deployment of 10 traps in each of three different areas (Reserve, Middle Sambo, and Pelican Shoal) and three habitat zones (inshore shallows, channel patch reefs, and outer reef patch reefs; see Figure 1 for approximate locations of each string of 10 traps. During the four years of sampling 1394 (1998), 2238 (1999), 1833 (2000), and 1502 (2001) lobsters were observed in our research traps.

Findings to Date: During 1998-2001, a total of 6,967 lobsters were observed in the Western Sambo Ecological Reserve and adjacent areas. The tag-and-release effort produced 70 tag returns from fishermen, 54 with recapture locations. Four long-distance recoveries to the east of the study site were from shallow water areas south of Big Pine Key, three near Looe Key, and one south of the 7 Mile Bridge. These lobsters came from both the Pelican Shoal and Reserve areas. Similarly, five long-distance recoveries were from west of the study site with two from the Key West Main Ship Channel, two from south of the Marquesas Keys, and one from west of Rebecca Shoal; all of these were originally tagged in the Reserve. Finally, one lobster was returned from the Gulf side, near Calda Light, that was originally tagged at the Pelican Shoal site.

We recaptured 67 tagged lobsters in research traps during the study. From our research recaptures, we did not detect any movement among study areas and most of our research recaptures were from the Ecological Reserve. These limited recaptures with little or no movement indicate that some of the lobsters may not migrate out of the Reserve area.

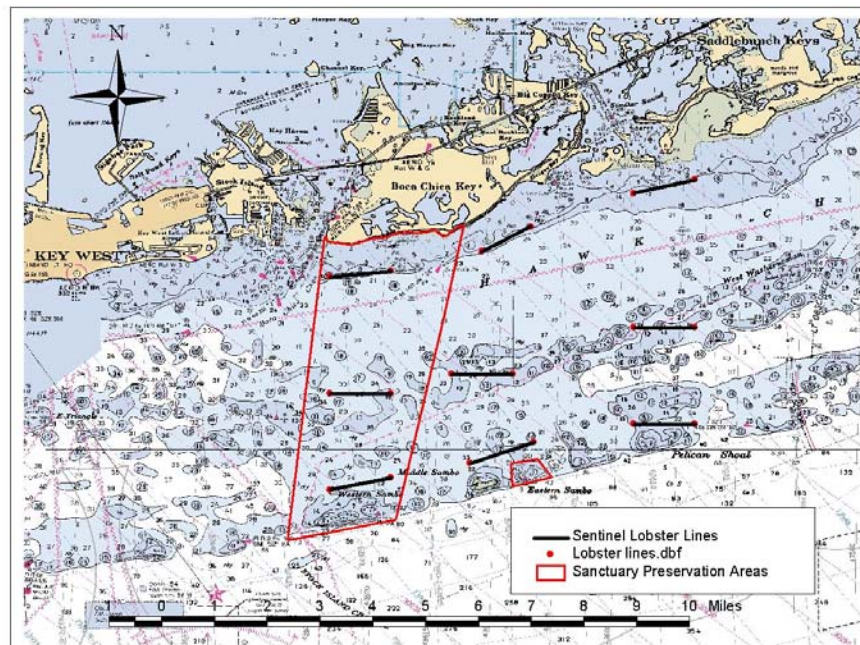
Lobster Size: The average size of the recaptured lobsters within each of the three sample areas were statistically similar to the average size of all lobsters observed in each of the respective areas.

Both catch rates (number of lobsters per trap per soak day) and lobster size during the closed and open fishing seasons indicate that the Reserve is providing some protection to the spiny lobsters within its boundaries.

The mean size of lobsters in the Reserve was significantly larger than in the non-reserve areas in both the open and closed fishing seasons (Fig. 2). The only exception to this trend was that average lobster sizes in the Middle Sambo area during the 2000 and 2001 closed seasons were equal to, or not significantly less than, those of the Reserve. During the open fishing season, lobsters in the Reserve were larger than non-reserve lobsters in each year. In the open season, lobster size in the Reserve declined in 1999 (76 mm carapace length) but recovered in 2000 to 1998 levels (82 mm); a similar, less-pronounced and non-significant trend was observed in the non-reserve areas. In the closed season, a similar significant decline and recovery trend in size was observed; however, in the Reserve the size of lobsters remained depressed through the 2000 closed fishing season, but returned to the 1998 level in the 2000 open fishing season and remained at the 1998 level through 2001.

Inter-annual trends in lobster sizes in the non-reserve areas were similar to the Reserve during the closed seasons, but during the open fishing season the sizes of lobsters were substantially smaller in 2001 than in previous years. Larger observed sizes in the Reserve are directly related to the greater abundance of males in the Reserve relative to the non-reserve areas (Table 1). Size differentials were most evident in male lobsters (Fig. 3), especially during the closed fishing season. Although female sizes among areas during the closed season did not differ statistically in each succeeding year of the study, it appeared that area differences were beginning to occur. These sex-related differences in lobster size by area are probably because male lobsters grow more quickly than female lobsters and thus males responded more quickly to the protection afforded by the Reserve. It is reasonable to expect that female sizes would exhibit greater Reserve versus non-reserve differences over time, unless there is also a sex-related difference in their migratory behavior or home ranges.

Figure 1. Location of Sentinel Fisheries sample sites. Each black line represents a ten-trap string equal to about one mile in length.



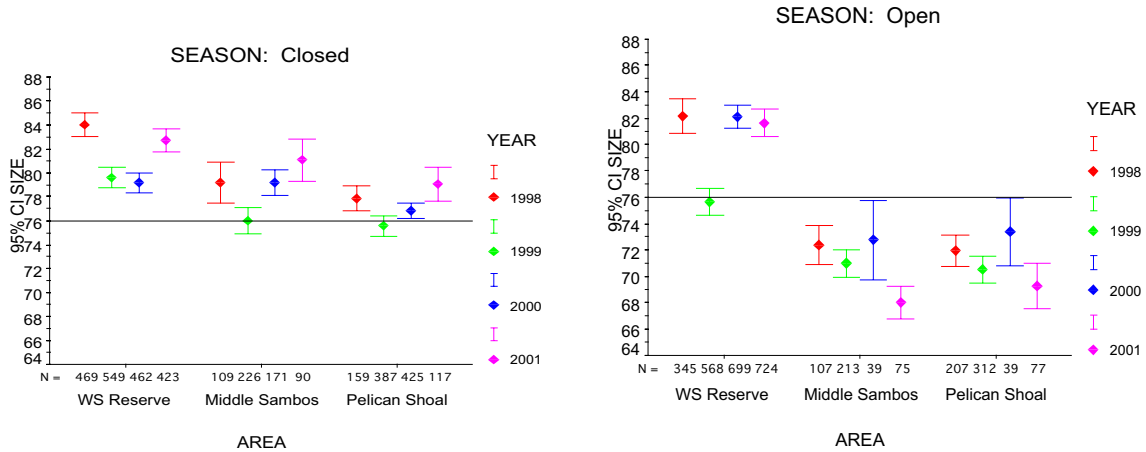


Figure 2. Average lobster size (with 95% confidence intervals) among year, fishing season (closed = April 1-August 5; open = August 6-March 31), and sample areas (Middle Sambo and Pelican Shoal are non-reserve areas).

Table 1. Frequency and percentage sex ratios by sample area within each fishing season, cumulative over the four year sample period (1998-2001).

Season	Area	Count			% Sex Ratio		
		Female	Male	Total	Female	Male	Total
Closed	WS Reserve	207171	678966	886137	23.4%	76.6%	100.0%
	Middle Sambo	78191	168539	246730	31.7%	68.3%	100.0%
	Pelican Shoal	156616	268725	425341	36.8%	63.2%	100.0%
	Totals	441978	1116230	1558208	28.4%	71.6%	100.0%
Open	WS Reserve	438559	643391	1081950	40.5%	59.5%	100.0%
	Middle Sambo	75088	60986	136074	55.2%	44.8%	100.0%
	Pelican Shoal	103179	98569	201748	51.1%	48.9%	100.0%
	Totals	616826	802946	1419772	43.4%	56.6%	100.0%

The best hypothesis at this time for these size differentials among areas is that those lobsters in the Reserve during the February-March pre-reproductive molting period are protected from exploitation during the latter months of the fishing season and most of them probably remain in the Reserve throughout the summer. Most of the long-distance movements in lobsters tend to occur during the fall and early winter months – the non-reproductive season. Thus, it may be no coincidence that the 4- to 5-mm differences in male carapace length observed between the Reserve and non-reserve areas during the closed season is approximately equivalent to the average increase in size of male lobsters during a single molt growth cycle. Females do not grow as fast as males because more of their energy is redirected to reproduction. Lobsters outside the Reserve that molt during the pre-reproductive molting period are probably caught prior to the end the fishing season.

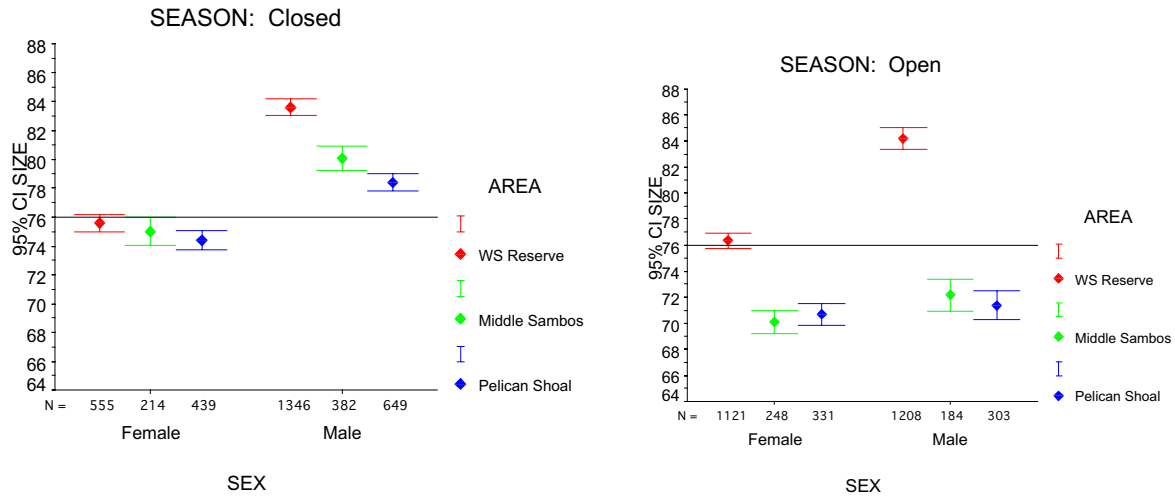


Figure 3. Average lobster size (with 95% confidence intervals) between lobster sex and fishing season.

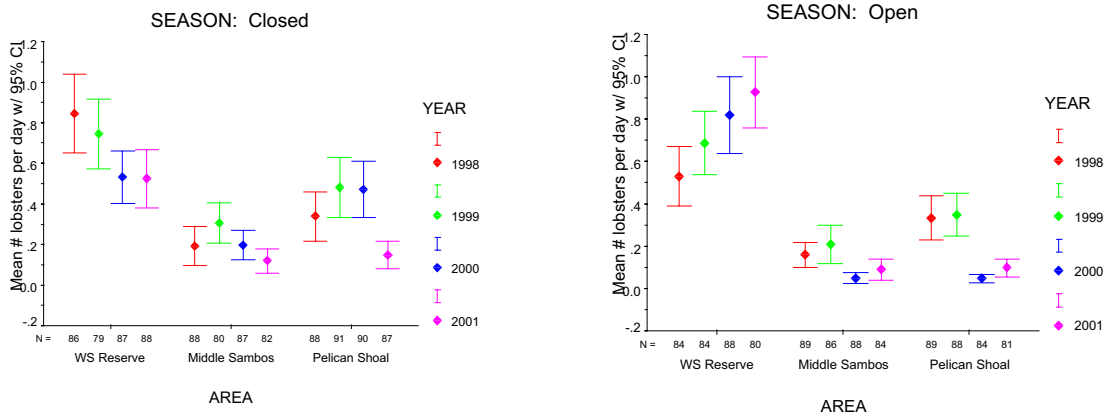


Figure 4. Average lobster catch rates (all sizes), with 95% confidence intervals between lobster sex and fishing season.

Abundance and Catch rate : In all years and seasons, with consistent and comparable sampling effort, we observed more lobsters in the Reserve than in the two non-reserve areas combined (see Table 1). Catch rates, in number of lobsters caught per trap per day of soak time, also were greater in the Reserve than in the non-reserve areas both during the closed and open fishing seasons (Fig. 4 and 5). The Middle Sambo area typically had lower catch rates than the other two areas. Comparison of catch rates between Reserve and non-reserve areas during the open season are confounded by competition with active commercial fishermen in the non-reserve areas. Our research traps were at a decided disadvantage in attracting lobsters because we baited them only with cowhide and commercial fishermen use live lobsters as attractants. Cowhide is a less effective bait than live lobsters, so the catch rates of research traps in non-reserve areas during the open season were not an effective indication of abundance relative to that observed inside the Reserve.

Catch rates in the Reserve during the closed season have trended downward in each year since 1998; however, conversely, during the open season Reserve catch rates have increased in each succeeding year after establishment of the Reserve. No obvious biological characteristics of

lobsters explain these opposing inter-seasonal abundance trends. However, these trends may be the result of inter-annual differences in the timing of weather-induced lobster movements in this area of the fishery. The weather patterns have certainly been different from year to year with 1998 being an El Niño year, 1998 and 1999 had hurricanes that moved across the study area during the fall of each year, and the winters of 2000 and 2001 had fewer than normal cold fronts. Lobsters are typically more migratory with the passage of autumnal and winter cold fronts. The year 2001 also was a time of unusually low lobster abundance and total catch in the fishery. The combined catch rates of the two seasons result in a flat inter-annual trend, suggesting that overall abundance in the Reserve may have remained constant since 1998. Thus, the lack of overall increases in abundance within the Reserve since 1998 supports our observations with respect to lobster size that the Reserve effects of increased size and abundance largely occurred during the first year of protection and the initial benefits of the Reserve have remained constant during the following three years of protection. Perhaps with more years of protection the Reserve effects of larger size and higher abundance may become more cumulative.

To determine if lobsters from within the Reserve were emigrating to the adjacent fished areas and providing yield to fishermen fishing near the Reserve, tag returns and evidence of spillover from differential size and catch-rate observations among the three study areas were analyzed. If spillover were occurring, a gradation of effects among the three areas would be seen with the expectation that, over time, the Reserve would show the largest increases in catch and size and the area farthest from the Reserve (Pelican Shoal area) would exhibit the least changes, with intermediate effects in the Middle Sambo area.

The limited number of tag returns supports only qualitative evaluations, but it seems evident that lobsters within the study area exhibited non-migratory behavior as well as both short- and long-distance movements. Lobsters are capable of moving in and out of the Reserve, and probably do so, to some extent, on a seasonal basis.

Spillover effects were also evaluated through comparisons of the size and catch rate of lobsters within the Reserve to the size and catch rate of lobsters in both the immediately adjacent area (Middle Sambo) and the area farther away (Pelican Shoal). The size distribution of lobsters among areas was variable (Fig. 2). In the first two years of the study average lobster sizes in the non-reserve areas were similar to one another and both were significantly smaller than that of Reserve lobsters. During the closed season of the last two years, 2000 and 2001, average lobster size in the Middle Sambo area equaled that of the Reserve while the average lobster size in the Pelican Shoal area was significantly smaller than the Reserve. No gradation in size from the Reserve was observed during the open season. A comparison of size by sex and area (Fig. 3) shows a gradation in size was more prevalent among male lobsters during the closed season. The increases in lobster sizes in the Middle Sambo area during 2000 and 2001 to levels similar to those observed in the Reserve provides evidence that spillover from the Reserve to the non-reserve area may be occurring.

The catch rate trends did not exhibit the expected gradation if spillover were occurring (Fig.5). It is possible, however, that this lack of a trend in catch rates could be confounded by the residual effects of greater fishing effort adjacent to the Reserve (in the Middle Sambo area)

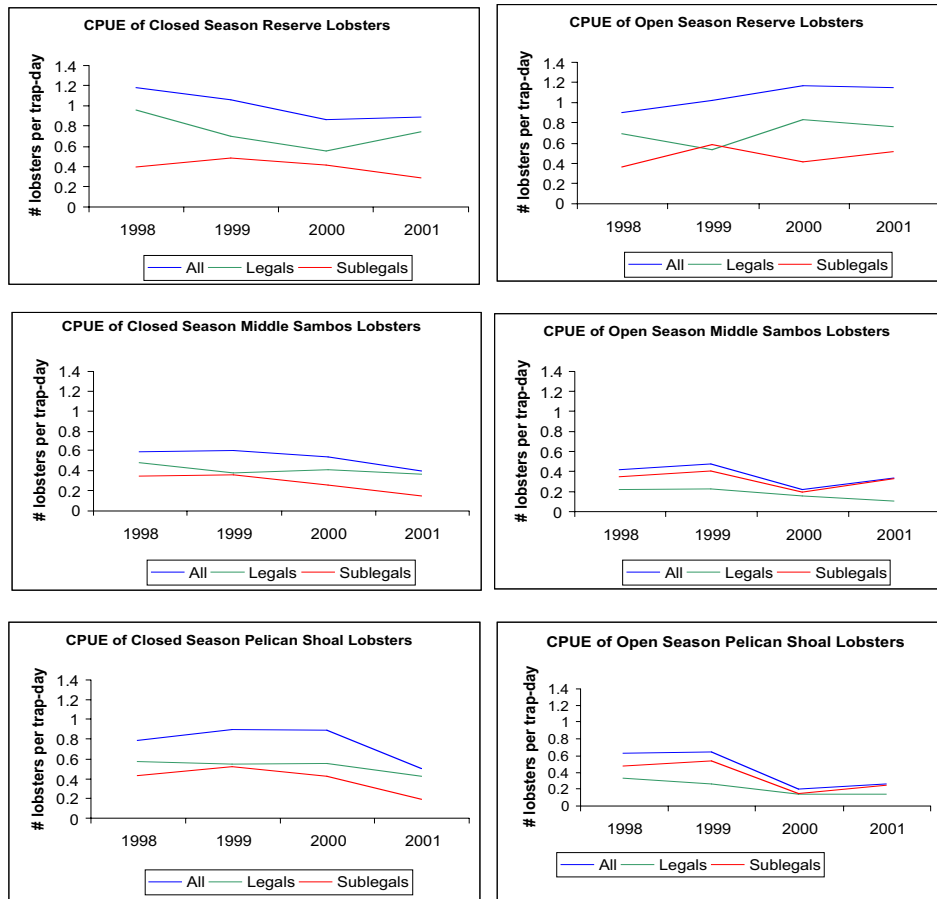


Figure 5. Mean catch per unit effort (CPUE) in number of lobsters caught per trap-day of fishing in non-zero-catch traps. The “All” series represents the catch rate of all lobsters and the legal and sublegal series represent the respective catch rates of lobsters greater than and less than the minimum legal size limit of 76 mm (3 inches) carapace length.

relative to the Pelican Shoal area. The National Marine Fisheries Service, Southeast Fisheries Science Center, conducted a survey of fishing traps in and adjacent to the Reserve in August of 2000 and found that the Middle Sambo area contained substantially more traps than the Pelican Shoal area. It may be that abundance is more sensitive to variations in fishing mortality than is lobster size.

Conclusions: The Reserve has provided some obvious benefits to lobsters both within and adjacent to the Reserve. Average size and abundance of lobsters within the Reserve were consistently greater than the adjacent fished areas. Although our study did not include baseline observations preceding closure of the Reserve, it is not likely that the effects observed are the result of intrinsic differences among the three study areas, but rather are directly related to the reduction in fishing mortality afforded by the Reserve. Some evidence of spillover from the Reserve to the adjacent fishery is also evident. The absence of cumulative Reserve effects over this four-year study may be because the period was too short to detect such effects or that the Reserve, encompassing a very small portion of the overall lobster population in the Florida Keys, provides only limited but consistent benefits due to migration of lobsters between the Reserve and the fishery.

Project Title: Queen Conch Marine Reserve Monitoring

Researcher: Robert Glazer and Gabriel Delgado, Florida Fish and Wildlife Conservation Commission/Florida Marine Research Institute, Marathon, FL.

Goal: Effective evaluation of the Florida Keys National Marine Sanctuary marine zoning plan requires a well-conceived monitoring study to compare resources in protected and unprotected zones. The goal of this project is to determine effects of the fully protected zones on the density, abundance, and area occupied by queen conch in the Florida Keys National Marine Sanctuary (FKNMS). We surveyed queen conch aggregations by conducting belt-transect surveys at offshore reef aggregations within Sanctuary Preservation Areas (SPA's) and the Special-use (Research-only) Areas. Additionally, reef areas without protective status were surveyed (reference areas). The aggregations were surveyed for juvenile and adult density, abundance, and overall aggregation size in order to evaluate patterns of abundance and recruitment. The results from these surveys will also be used to evaluate the effectiveness of the marine reserve concept as a means for protecting and restoring the Florida conch population to historic numbers. This is the fifth annual report on the results of these surveys.

Methods: For the belt-transect surveys, all sampling occurred between July and August 2001 in order to ensure that the surveys were conducted during the period of maximal density associated with spawning. The surveys were conducted at reef locations with SPA designations as well as those reefs without restrictions (i.e., reference areas, Fig. 1). In many cases, the only conch aggregations at the reefs with SPA designations were located outside the SPA boundaries. We defined aggregations as discernible clusters of adult and/or juvenile conch.

An initial survey of each site was made to determine the presence of conch, the approximate size of the aggregation, and to locate an apical edge beyond which conch were infrequent or not observed. If a conch aggregation was estimated to be greater than approximately 100 m in length, a 100-m fiberglass tape (primary tape) was affixed at an apex and was deployed along the margin of the aggregation. Five secondary tapes (i.e., belts) were laid perpendicular to the primary tape at random intervals along the primary tape. Divers then recorded all conch within 1 m of each side of the belt. Densities were determined by dividing the number of conch counted by the area surveyed. Regional (i.e., Upper, Middle, and Lower Keys) and overall (i.e., Keys-wide) densities were calculated using all individuals sampled in the year divided by the total area sampled. Aggregations were mapped to determine overall abundance; we used GPS data to determine the periphery of aggregations. The area encompassed by each aggregation was estimated using ArcView GIS software.

In areas where conch were very sparse, direct counts were made of individuals and belts were not conducted. In those cases, densities were designated as $0.000 \text{ conch} \cdot \text{m}^{-2}$. The counts of individual conch were used to estimate abundance for the aggregation, region, and overall Keys. However, these observations were not included in the subsequent calculations of regional and overall density because densities were not measured. There were two exceptions: 1) the French Reef aggregation contained only adults; therefore, a belt survey was conducted with a resulting measured density of juvenile conch equal to $0.000 \text{ conch} \cdot \text{m}^{-2}$; and 2) the Delta Shoal aggregation

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contained only juveniles; therefore, the belt survey had an adult density equal to $0.000 \text{ conch} \cdot \text{m}^{-2}$.

We examined the overall aggregation area, adult abundance, juvenile abundance, adult density, and juvenile density as a means to evaluate changes in the SPAs and reference areas. The non-parametric Mann-Whitney U test was used to compare SPAs and reference areas in 2001 as well as between years 1997 and 2001 in SPA's and reference areas separately. In addition, we used the non-parametric Kruskal-Wallis test to determine if there were differences in aggregation area, adult abundance, juvenile abundance, adult density, and juvenile density among the regions of the Keys (i.e., Upper, Middle, and Lower Keys).

Findings to Date: A total of 16 aggregations were surveyed (Fig. 1). Densities were measured in 14 conch aggregations and direct counts were conducted in the other two aggregations. In many cases, the conch aggregations were located outside the boundaries of the SPAs.

Juvenile densities ranged from $0.000 \text{ individuals} \cdot \text{m}^{-2}$ at an adult-only aggregation at French Reef to a maximum of $0.415 \text{ individuals} \cdot \text{m}^{-2}$ at Grecian Rocks (Tables 1-3). Excluding French Reef, where no juveniles were found within the belts, the lowest density of juveniles at an aggregation was at Conch Reef where $0.006 \text{ individuals} \cdot \text{m}^{-2}$ were observed. The highest abundance of juvenile conch was observed at the Elbow; approximately 7,003 conch were estimated to be present (Table 1).

The Upper Keys had the highest juvenile conch densities with $0.131 \text{ juveniles} \cdot \text{m}^{-2}$ surveyed (Table 1). The Middle and Lower Keys had similar densities ($0.122 \text{ juveniles} \cdot \text{m}^{-2}$ and $0.056 \text{ juveniles} \cdot \text{m}^{-2}$, respectively) (Tables 2 and 3). Estimated regional abundance for juvenile conch ranged from approximately 11,422 individuals in the Upper Keys to 6,846 in the Middle Keys (Tables 1-3).

Adult conch density was the highest at Conch Reef ($0.138 \text{ adults} \cdot \text{m}^{-2}$) (Table 1) and was lowest at Delta Shoal ($0.000 \text{ adults} \cdot \text{m}^{-2}$) where no adults were surveyed on the belts (Table 2). The highest estimated abundance was at Western Sambo with an estimated 7,644 adults present (Table 3). Of the sites where adult conch were surveyed on the belt transects, Pelican Shoal and Sombrero Reef both had the lowest abundance with an estimated 153 conch present (Tables 2 and 3). The region with the most adults was the Lower Keys by far (approximately 19,085) followed by the Upper Keys (approximately 7,896) and the Middle Keys (approximately 180) (Tables 1-3).

The Western Sambo aggregation was the most extensive in area and was estimated to be $92,645 \text{ m}^2$ (Table 3). The Lower Keys region had the most area encompassed by conch aggregations ($216,462 \text{ m}^2$) (Table 3).

We estimated that there were approximately 27,184 adult conch within the offshore aggregations during 2001 (Table 4). In 1997, we estimated that there were approximately 20,906 adult conch (Table 4). We estimated that there were approximately 26,917 juveniles in the study area in 2001 compared with 10,036 in 1997 (Table 4).

Mann-Whitney U tests indicated that there were no significant differences between SPAs and reference areas during 2001 for overall aggregation area, adult abundance, juvenile abundance, adult density, and juvenile density (Fig. 2 to 4). A comparison between years 1997 and 2001 indicated that there were no significant differences in the SPAs for overall aggregation area, adult abundance, juvenile abundance, and juvenile density; however, adult density was significantly higher in 2001 (Fig. 2 to 4). A comparison between years 1997 and 2001 for reference areas indicated that there were no significant differences for overall aggregation area, adult abundance, adult density, and juvenile density; however, juvenile abundance was significantly higher in 2001 (Fig. 2 to 4). Kruskal-Wallis tests showed there were no significant differences among regions during 2001 (Fig. 5 to 7) or between years 1997 and 2001.

Discussion: The results of the fifth year of queen conch SPA monitoring support those of a year earlier: conch aggregations are distributed in well-defined clusters that, in general, are not entirely encompassed by SPA boundaries. Additionally, they are distributed in marked regional patterns. For example, the Lower Keys region from Looe Key to Western Sambo is a complex containing approximately 19,000 of the 27,000 adults located throughout the Keys. There were few adult conch surveyed in the Middle Keys. The Upper Keys contained many aggregations with about a third of the adult conch found in the Keys. Overall, adult abundance has increased from 1997 to 2001.

A large amount of recruitment seems to have occurred throughout the Keys in 2000 and 2001 (Fig. 3). In the Upper Keys, juvenile abundance nearly tripled from 4,108 conch in 1997 to 11,422 in 2001. In the Middle Keys, juvenile abundance jumped from about 100 in 1997 to approximately 7,000 animals in 2001. Adult abundances in the Middle Keys remain suppressed; however, we expect that this may change next year when this large cohort reaches maturity. In the Lower Keys, juvenile abundance has also increased although not as spectacularly as in the Upper and Middle Keys.

Table 1. Results of queen conch belt-transect surveys conducted in the Upper Keys at the beginning of the study (1997) and 2001. Densities are reported in individuals•m⁻². Areas are for areas encompassed by the aggregations and are reported in m². The mean values reported for overall juvenile and adult densities were derived from the entire data set and not by averaging the mean densities of each aggregation.

Upper Keys										
SPA										
Site	Juv Abund (1997)	Juv Abund (2001)	Juv Density (1997)	Juv Density (2001)	Adult Abund (1997)	Adult Abund (2001)	Adult Density (1997)	Adult Density (2001)	Area (1997)	Area (2001)
Carysfort Reef	0	-	0.000	-	0	-	0.000	-	0	-
The Elbow	3,373	7,003	0.062	0.230	1,214	1,588	0.022	0.052	54,526	30,395
Key Largo Dry Rocks	0	-	0.000	-	0	-	-	-	0	-
Grecian Rocks	472	3,656	0.063	0.415	236	668	0.032	0.076	7,445	8,806
French Reef	56	0	0.003	0.000	992	335	0.054	0.085	18,422	4,198
Molasses Reef	130	634	0.006	0.020	2152	4,039	0.105	0.127	20,480	31,834
Conch Reef	72	49	0.006	0.006	350	1,045	0.029	0.138	11,881	6,783
Mean			0.028	0.134			0.048	0.096		
Total	4,103	11,342			4,944	7,675			112,754	82,016

Table 2. Results of queen conch belt-transect surveys conducted in the Middle Keys in 2001. Densities are reported in individuals•m⁻². Areas are for areas encompassed by the aggregations and are reported in m². The mean values reported for overall juvenile and adult densities were derived from the entire data set and not by averaging the mean densities of each aggregation.

Middle Keys										
SPA										
Site	Juv Abund (1997)	Juv Abund (2001)	Juv Density (1997)	Juv Density (2001)	Adult Abund (1997)	Adult Abund (2001)	Adult Density (1997)	Adult Density (2001)	Area (1997)	Area (2001)
Alligator Reef	48	197	0.010	-	86	27	0.018	-	4,791	-
Sombrero Key	4	939	-	0.149	0	153	-	0.024	-	6,292
Mean			0.01	0.149			0.018	0.024		
Total	52	1,136			86	180			4,791	6,292
Control										
Delta Shoal	33	5,710	0.012	0.101	77	0	0.028	0.000	2,699	56,621
Mean			0.012	0.101			0.028	0.000		
Total	33	5,710			77	0			2,699	56,621
Overall - Mid Keys										
Mean			0.011	0.122			0.021	0.010		
Total	85	6,846			163	180			7,490	62,913

Table 3. Results of queen conch belt-transect surveys conducted in the Lower Keys in 2001. Densities are reported in individuals \cdot m⁻². Areas are for areas encompassed by the aggregations and are reported in m². The mean values reported for overall juvenile and adult densities were derived from the entire data set and not by averaging the mean densities of each aggregation.

Lower Keys										
SPA										
Site	Juv Abund (1997)	Juv Abund (2001)	Juv Density (1997)	Juv Density (2001)	Adult Abund (1997)	Adult Abund (2001)	Adult Density (1997)	Adult Density (2001)	Area (1997)	Area (2001)
Looe Key	1,349	534	0.021	0.038	2,501	747	0.049	0.060	56,451	11,741
Eastern Sambo	773	3,225	0.018	0.080	4,348	3,736	0.101	0.100	42,903	37,561
Western Sambo	411	1,014	0.008	0.011	2,765	7,664	0.055	0.083	50,252	92,645
Eastern Dry Rocks	2	249	-	0.012	21	2,771	-	0.136	-	20,319
Mean			0.016	0.035			0.068	0.095		
Total	2,535	5,022			9,635	14,918			149,606	162,266
Control										
Pelican Shoal	2,455	2,853	0.061	0.219	944	153	0.023	0.012	40,533	13,009
Middle Sambo	767	764	0.014	0.019	3,987	4,014	0.072	0.097	55,370	41,187
Mean			0.012	0.119			0.028	0.051		
Total	3,222	3,617			4,931	4,167			95,903	54,196
Overall - Lower Keys										
Mean			0.023	0.056			0.060	0.089		
Total	5,757	8,639			14,566	19,085			245,509	216,462

Table 4. Summary of queen conch belt-transect surveys conducted in the Florida Keys in 2001.

	Juv Abund (1997)	Juv Abund (2001)	Juv Density (1997)	Juv Density (2001)	Adult Abund (1997)	Adult Abund (2001)	Adult Density (1997)	Adult Density (2001)	Area (1997)	Area (2001)
Mean			0.025	0.092			0.055	0.079		
Total	10,036	26,917			20,906	27,184			383,615	364,285

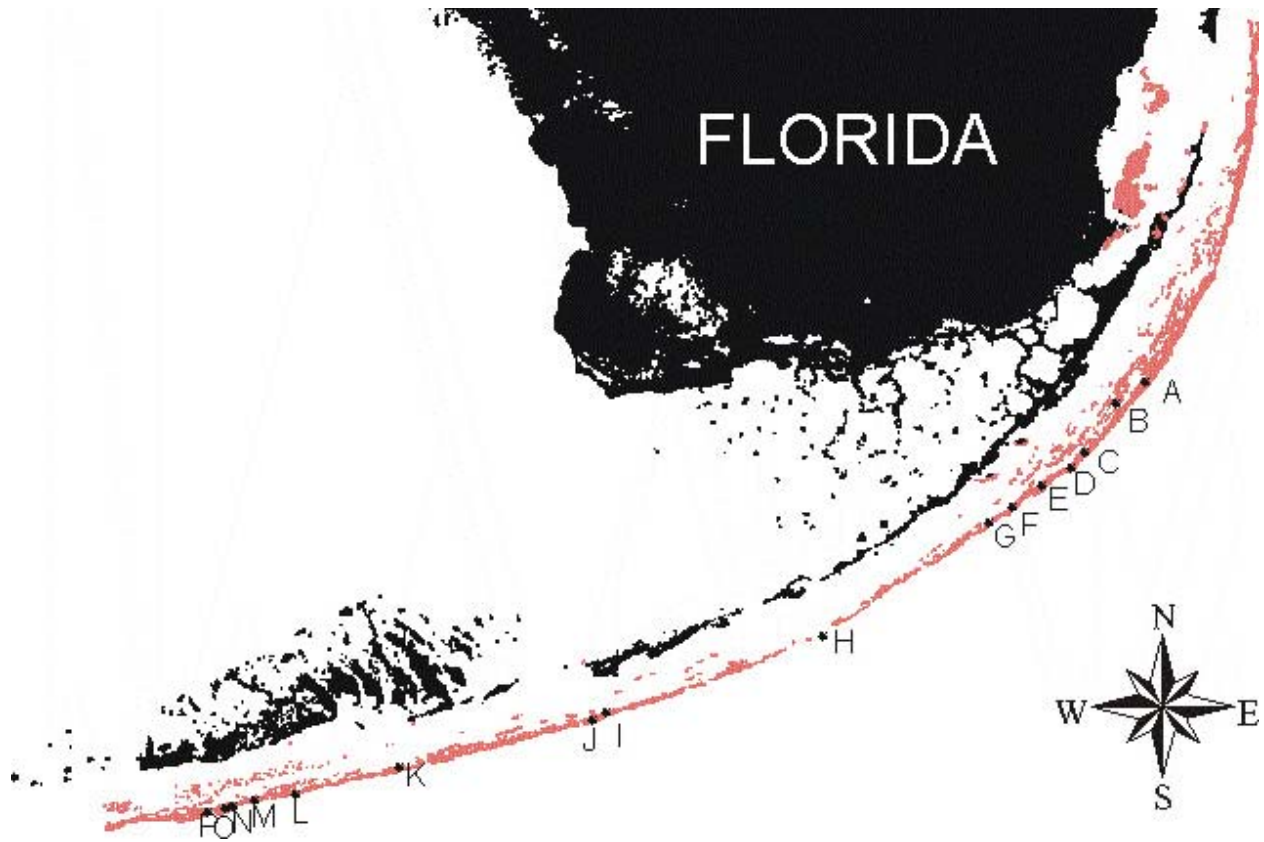


Figure 1. Sampling sites for queen conch SPA monitoring project for 2001. The belt-transect sites included: (A) The Elbow, (B) Grecian Rocks, (C) French Reef, (D) Sand Reef, (E) Molasses Reef, (F) Pickles Reef, (G) Conch Reef, (H) Alligator Reef, (I) Delta Shoals, (J) Sombrero Reef, (K) Looe Key, (L) Pelican Shoal, (M) Eastern Sambo, (N) Middle Sambo, (O) Western Sambo, and (P) Eastern Dry Rocks.

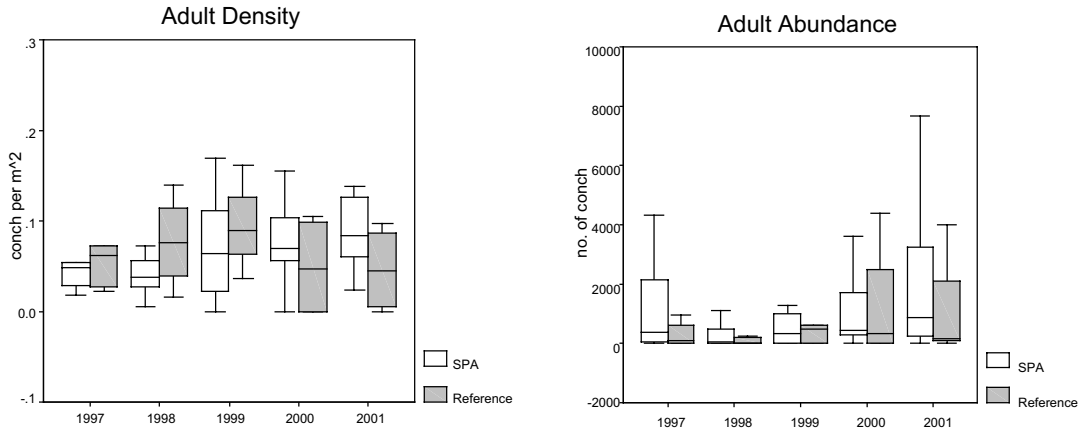


Figure 2. Box plots of the density and abundance of adult queen conch by protective status (i.e., SPA and reference) in the Florida Keys. The box represents the 25th and 75th percentiles. The horizontal line within the box indicates the median. The error bars represent the 10th and 90th percentiles.

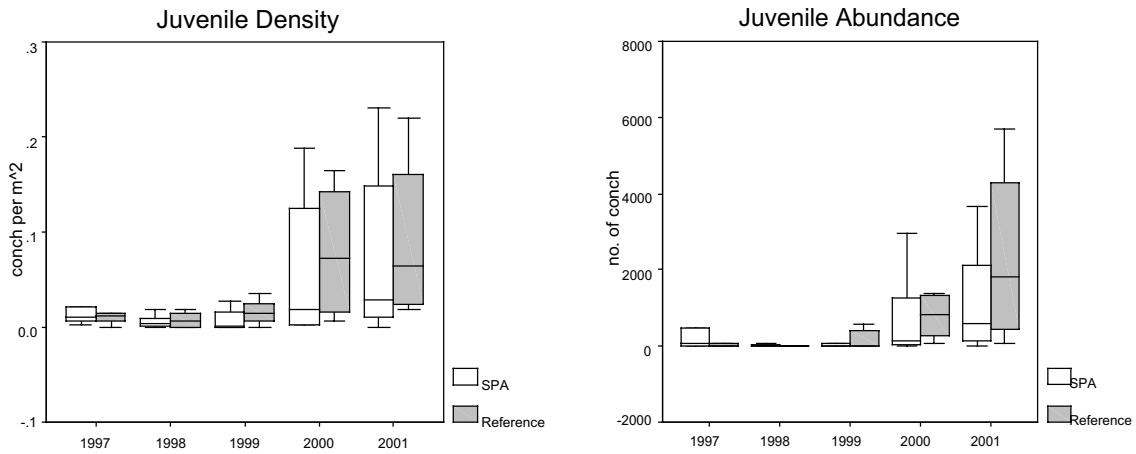


Figure 3. Box plots of the density and abundance of juvenile queen conch by protective status (i.e., SPA and reference) in the Florida Keys. The box represents the 25th and 75th percentiles. The horizontal line within the box indicates the median. The error bars represent the 10th and 90th percentiles.

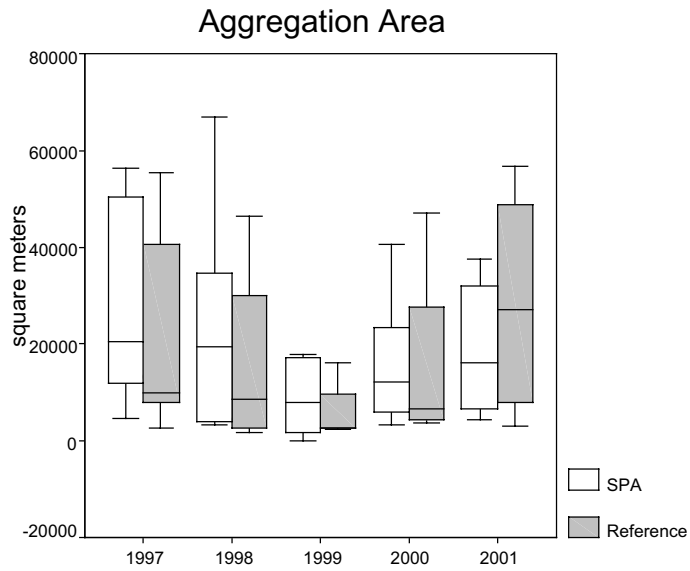


Figure 4. Box plot of queen conch aggregation area by protective status (i.e., SPA and reference) in the Florida Keys. The box represents the 25th and 75th percentiles. The horizontal line within the box indicates the median. The error bars represent the 10th and 90th percentiles.

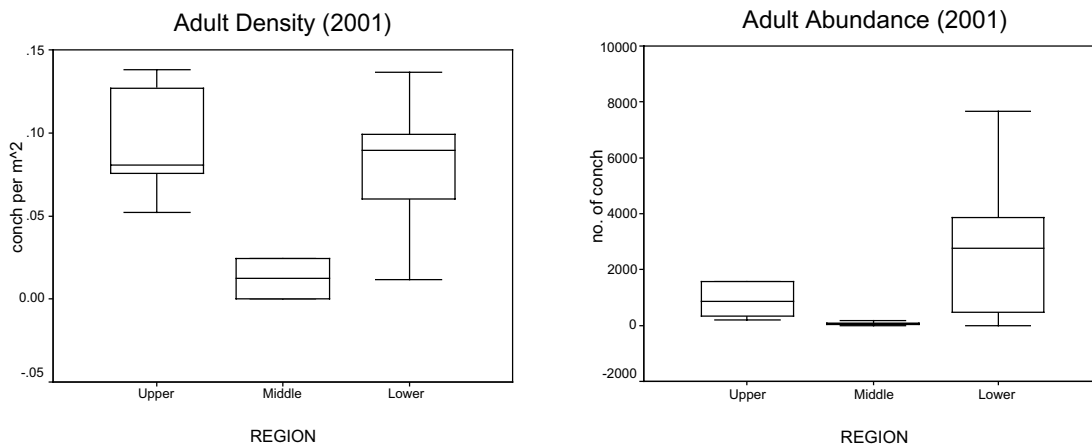


Figure 5. Box plots of the density and abundance of adult queen conch in 2001 by region in the Florida Keys. The box represents the 25th and 75th percentiles. The horizontal line within the box indicates the median. The error bars represent the 10th and 90th percentiles.

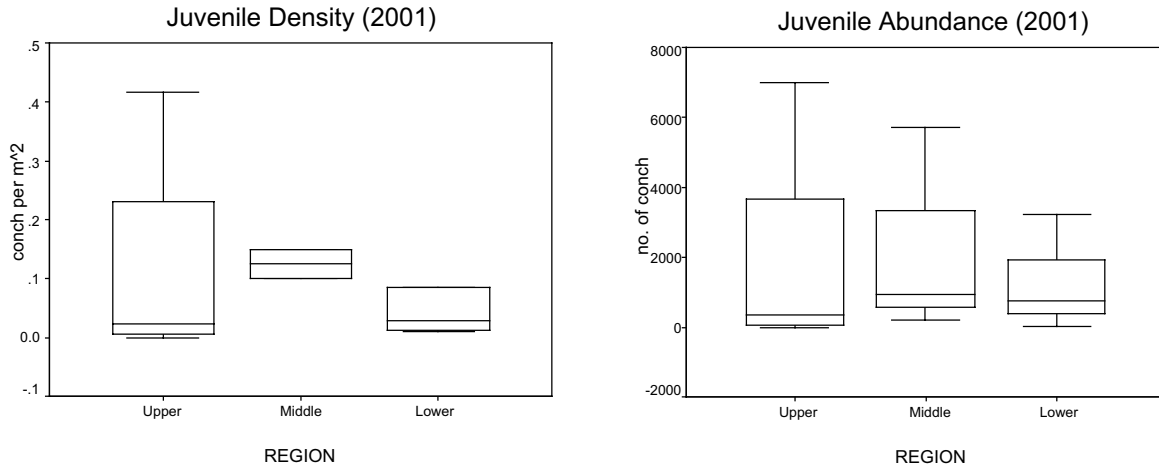


Figure 6. Box plots of the density and abundance of juvenile queen conch in 2001 by region in the Florida Keys. The box represents the 25th and 75th percentiles. The horizontal line within the box indicates the median. The error bars represent the 10th and 90th percentiles.

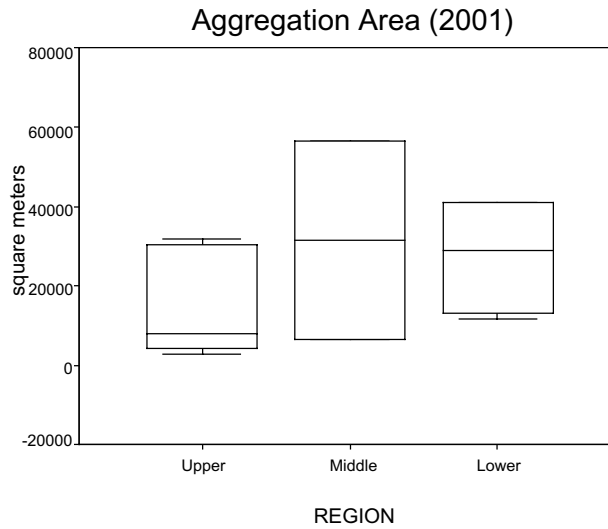


Figure 7. Box plot of queen conch aggregation area in 2001 by region in the Florida Keys. The box represents the 25th and 75th percentiles. The horizontal line within the box indicates the median. The error bars represent the 10th and 90th percentiles.

Project Title: Assessing Coral Health in the Florida Keys National Marine Sanctuary Using a Molecular Biomarker System (MBS)

Researchers: Cheryl M. Woodley, NOAA/NOS Center for Coastal Environmental Health and Biomolecular Research and Medical University of South Carolina, Charleston, SC; Eric R. Lacy, Medical University of South Carolina, Charleston, SC; John E. Fauth, University of Charleston, Charleston, SC; Craig A. Downs, EnVirtue Biotechnologies, Inc, Walnut Creek, CA; Judith Halas, Environmental Moorings International, Key Largo, FL; Pamela Hallock Muller and Elizabeth Fisher, University of South Florida, St. Petersburg, FL; Richard Curry, Biscayne National Park, Homestead, FL; and John Halas, Florida Keys National Marine Sanctuary, Key Largo, FL

Goals: We have designed an integrated Cellular Diagnostic System (CDS) to diagnose whether an organism is stressed and to identify the likely stressor(s) (e.g., heat stress, pesticides, pathogens, etc.). Our goals are to: (1) use the CDS to characterize the health of a coral reef ecosystem in the Florida Keys; (2) verify that the CDS can detect and characterize subtle and chronic effects of environmental stressors on this ecosystem; (3) determine if point-source pollutants or global climate changes (e.g., increased ocean temperatures or UV-B radiation) are stressing coral reef ecosystems; (4) compare the precision, sensitivity, and prognostic capabilities of the CDS to those of traditional measures of ecosystem health, and (5) encourage participation and understanding of the general public, scientific, industrial, and managerial communities in using marine biotechnologies to assess and manage the health of coral reef ecosystems.

Methods: The methods being employed range from established protocols for community-scale assessment (i.e., the AGRRA protocol of Ginsburg et al., 2000), foraminiferal condition (i.e., Hallock Muller et al., 1995), and MBS analysis (Downs et al., 2000; 2001), to methods adapted to monitor coral lesions and sedimentation.

The Cellular Diagnostic System (CDS and formerly known as MBS) is an ELISA-based assay system, specifically used to measure changes in cellular parameters, and allows assessment of cellular-physiological condition, monitoring of cellular stress responses, identification of putative stressors, and forecasting outcomes of environmental problems. The specific cellular and molecular parameters used to assess physiological condition include (but are not limited to): membrane integrity and composition (e.g., lipid peroxidation products), anti-oxidant redox potential (e.g., glutathione redox status), molecular chaperone activity (e.g., heat-shock proteins [Hsp] 60 & 70), enzymatic anti-oxidants (e.g., catalase, superoxide dismutases, glutathione peroxidases), stress-signaling pathways (e.g., MAPK, JANK), xenobiotic detoxification pathways (e.g., cytochrome P450 family, P-glycoprotein 160), metal-regulatory proteins (e.g., metallothionein, ferritin, porphyrin), protein status and turnover (e.g., ubiquitin, protein carbonyl formation), and genomic and translational integrity (e.g., DNA abasic phosphate site formations). These parameters quantify specific cellular physiological functions including (1) whether the structural integrity of the cell is compromised, (2) the type or nature of the stress (e.g., oxidative stress, metal stress, salinity stress), and (3) whether defenses have been mounted against a particular stress (i.e., pesticide, acidity, heavy metal, PAH).

Coral lesions (i.e., partial mortality) of tagged corals (*Montastraea* complex) have been monitored on a quarterly basis (March/April, June, August/September, and October/November) in 2001 and 2002. A lesion is defined as an area on the colony with no live coral tissue. Lesions created by the biomarker sampling are 3 cm² and should regenerate under non-stressful conditions (Meesters et al., 1997). To monitor lesions, each lesion is photographed using a Nikonos V camera with a close-up adapter. Photographs are scanned to digital images, and then the area and perimeter of the lesions are calculated using image analysis software.

The significance of this study, with regard to coral lesions, is that it will link the regeneration or increased mortality in corals with molecular-scale responses of individual coral colonies providing quantitative indicators of stresses. Data on water temperature, nutrient levels, foraminiferal populations, and sedimentation will then enable us to determine if these factors correlate with changes in lesions and with stress levels quantified by the CDS. The hypothesis being tested is that a coral, which the CDS indicates to be more stressed, will be less likely to regenerate than a coral, which CDS indicates to be less stressed.

Findings to Date: Our interim results indicate that this technology can be used to characterize coral health in defined areas of the Florida Keys (Obj. 1), distinguish between global-level stressors (e.g., El Niño/La Niña effects) and local-level stressors (e.g., agricultural runoff) (Obj. 2 and 3), and help predict the condition of corals several months before more obvious symptoms appear (e.g., coral bleaching or coral death) (Obj. 3 and 4). Additionally, comparisons of coral lesion healing with biomarker response have shown significant correlations between the level of biomarker response (representative of the cellular physiological status) and measures traditionally used to assess ecosystem health (Obj. 4). These results support our hypothesis that a coral that the CDS indicates to be more stressed will be less likely to regenerate than a coral that CDS indicates to be less stressed. These preliminary findings have been presented (Obj. 5) to user groups including state and federal resource managers, coral biologists and representatives from the general public who have been encouraged to offer input and collaborations on this and related projects.

Objectives 1-3. The Cellular Diagnostic System (CDS) was developed to focus a comprehensive array of biotechnologies on the diagnosis of a variety of ecosystem health issues; however, it was specifically tailored to coral physiological health and discerning the causes of coral reef system declines (Downs et al., 2000; Woodley et al., 2001). In this study, we specifically applied the CDS to corals in the Florida Keys and demonstrated that the CDS could distinguish whether a local coral population of *Montastraea annularis* was being stressed by a global stressor (e.g., high sea-surface temperatures; Figure 1) or by a stressor that is local in nature (Fig. 2). In conjunction with other technologies and monitoring methods, this biotechnology was able to identify potential stressor(s) responsible for the decline (Fig. 3). The CDS also possessed the ability to predict the progression of a health condition based on key diagnostic markers (Fig. 4). Finally, we believe that the CDS is able to address evolutionary issues in coral reef biology. For example, many researchers hypothesized that the Middle Keys would have experienced the highest rate of coral decline – the CRMP 2001 report indicated that this is not the case. The low rate of coral decline in this area may have, in part, resulted from an evolved increased stress-tolerance capacity of the corals. CDS laboratory studies, which compared corals from the Middle Keys with corals in other areas, were able to distinguish between the processes of cellular-

physiological acclimation and molecular evolutionary adaptation (Downs, Woodley, and Robinson, in preparation).

In Figure 1, *M. annularis* scleractinian Hsp60 and ubiquitin data from the 1999 sampling project can be diagnostically interpreted as follows: the corals, at all four depths, were experiencing a protein-denaturing stress. This was indicated by a positive correlation between the increased ubiquitin levels (a key component of a pathway for degrading 80% of the proteins in the cell) and the abnormally high sea surface temperatures that peaked in the months of July and August (Downs et al., in review A). Hsp60 (for description of function, see Downs et al., 2000) data in 1999 corroborated this diagnostic interpretation. Though the extent of cellular damage differed significantly with depth, the data support the argument that coral cellular damage at all four sites was the result of a global stressor (La Niña sea-surface temperature effects).

In 2000, the patterns of both parameters were radically different than those observed for 1999 and were not correlated with sea-surface temperatures (Woodley et al., in preparation). In March 2000, corals at 3.1 m depth were not experiencing a protein denaturing cellular condition; however, they were experiencing non-adverse changes in mitochondrial function. In June 2000, corals at the 3.1-m site showed signs of cellular stress, which adversely affected mitochondrial function. These diagnostic interpretations for both 1999 and 2000 were corroborated by other diagnostic biomarker data. In summary, the cellular stress experienced by corals at all four sites in 1999 was the result of a global stressor as opposed to a local stressor at the 3.1-m and 9.1-m sites in 2000 (and the stressor was different for these two 2000 sites – Woodley et al., in preparation). In 2000, using only three diagnostic markers (out of 24 biomarkers assayed for each coral sample), we could determine that a coral reef site in Biscayne National Park (BNP) was experiencing a severe cellular stress that was most likely generated by an electrophilically-modifiable xenobiotic (e.g., a fungicide, an organometalloid, endosulfan) (Fig. 2 and 3). The extremely high level of ubiquitin indicates severe rates of protein turnover. This interpretation was corroborated by five other cellular biomarkers. The level of ubiquitin in March 2000 at the Biscayne National Park (BNP) site has been suggested to be near the maximal threshold capacity for this coral species – massive cellular deterioration is beginning to occur and coral death could be predicted (Downs and Woodley, in preparation). In August 2000, significant and punctuated coral coverage loss at the BNP site was observed – no observable coral coverage degradation was observed in March 2000. This partially unidentified stressor adversely affected both scleractinian and dinoflagellate cellular physiology (Fig. 2). Data presented in Fig. 3 can be interpreted as corals: at the BNP site were responding to a xenobiotic stressor and the response pathway included a mono-oxygenase catalytic reaction at the site of olefinic double bonds of the xenobiotic, the conjugation of glutathione to the xenobiotic by glutathione-s-transferase, and cellular exclusion of the GSH-conjugated xenobiotic by a P-glycoprotein 140/160 pump action (a.k.a. MDR: multi-drug resistance gene) (Woodley et al., in preparation; Downs et al., in preparation B; not all data shown for this interpretation).

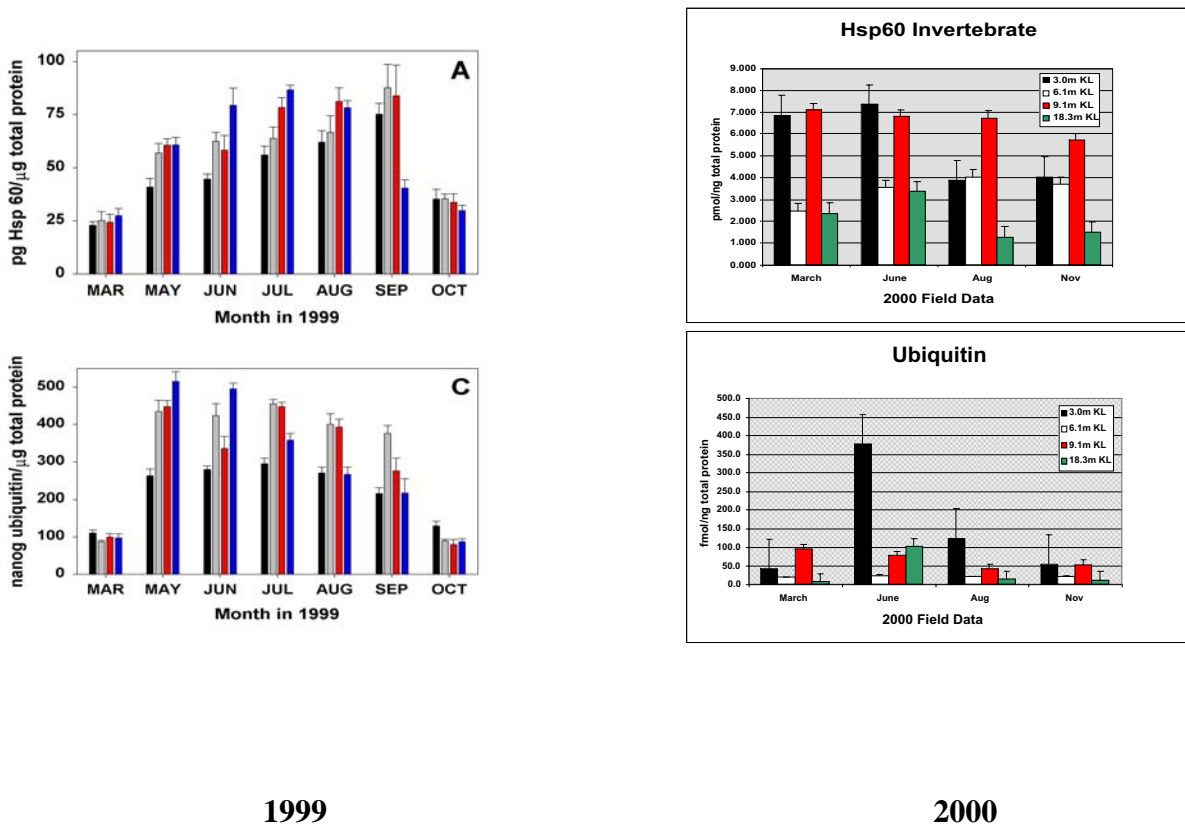


Figure 1. The same coral colonies from four depth sites were sampled on a monthly basis in 1999 and a quarterly basis in 2000. Hsp60 reflect chaperonin levels in the scleractinian; mean concentrations varied significantly with depth, month, and the depth x month interaction in 1999 (repeated measures MANOVAs: all $F > 2.56$, $P < 0.02$;). Ubiquitin levels reflect the rate of protein degradation, which varied significantly with depth, month, and the depth x month interaction (repeated measures MANOVA: all $F > 8.80$, $P < 0.0001$). Bars show untransformed mean (± 1 SE) biomarker concentrations at each depth: for 1999 panel, black = 3.0 m, grey = 6.1 m, red = 9.1 m, blue = 18.3 m. Sites are from a four-mile long transect off the eastern shore of Key Largo.

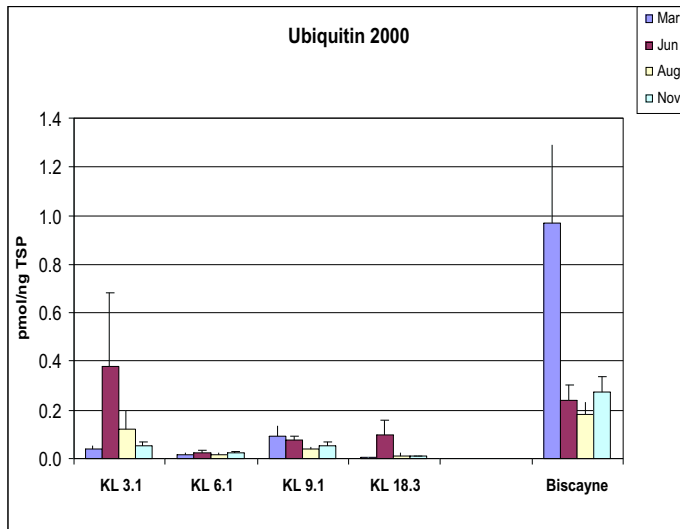
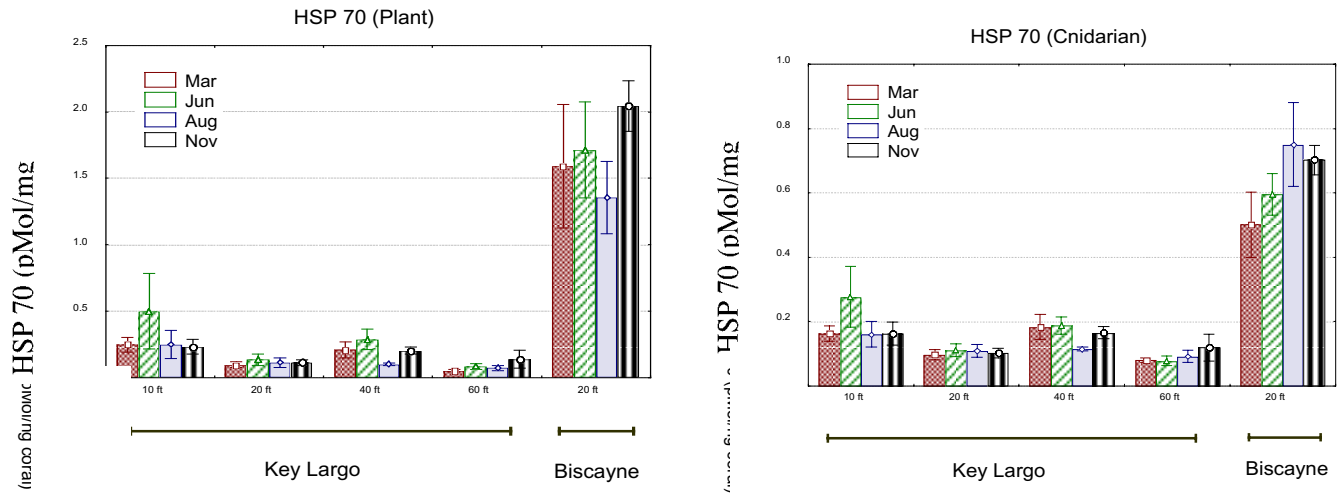


Figure 2. Data from 2000 field collections. Hsp70 is a ubiquitous chaperone, necessary for life. It functions to fold newly synthesized proteins into their active state and refold denatured proteins (resulting from a stressor) into functional enzymes. If a protein is severely damaged and cannot be refolded into a functional enzyme, it must be degraded. Protein degradation occurs mostly through the ubiquitin-proteolytic pathway. Damaged proteins are conjugated with ubiquitin, which designates to the cell that this specific protein is to be degraded. We have developed individual assays that are specific for the Hsp70 homologues found in both the dinoflagellate and scleractinian. Key Largo sites are the same as in 1999. Biscayne site is a patch reef found in southern reaches of Biscayne National Park, 15 nautical miles north of the Key Largo depth transect.

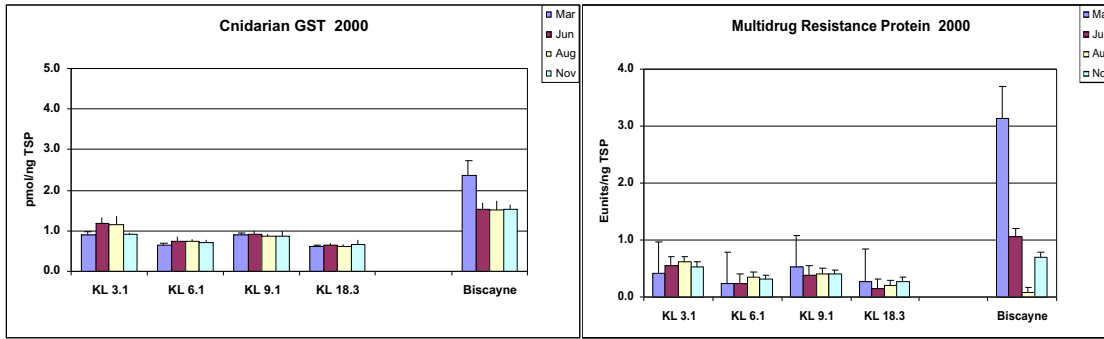


Figure 3. Data from 2000 Field collections. GST Invertebrate = scleractinian homologues of glutathione-S-transferase. GST is an enzyme that will conjugate a xenobiotic with reduced glutathione so that the xenobiotic can easily be managed by the cell. MDR = P-glycoprotein 160, a member of the ABC family of proteins that is up-regulated when an organism has been exposed to specific classes of xenobiotics. Its function is to detoxify the cell of xenobiotics by pumping these xenobiotics out of the organism. Site locations are the same as described in Fig. 2.

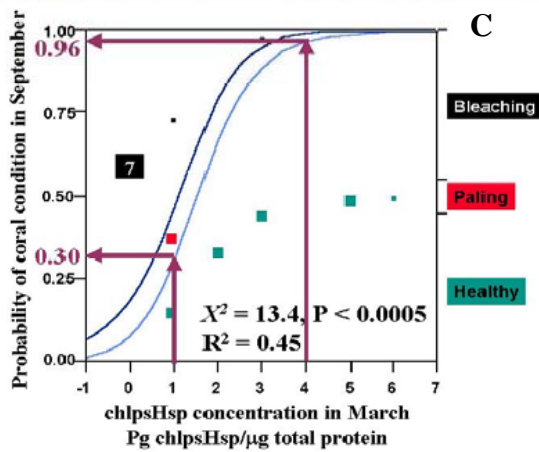
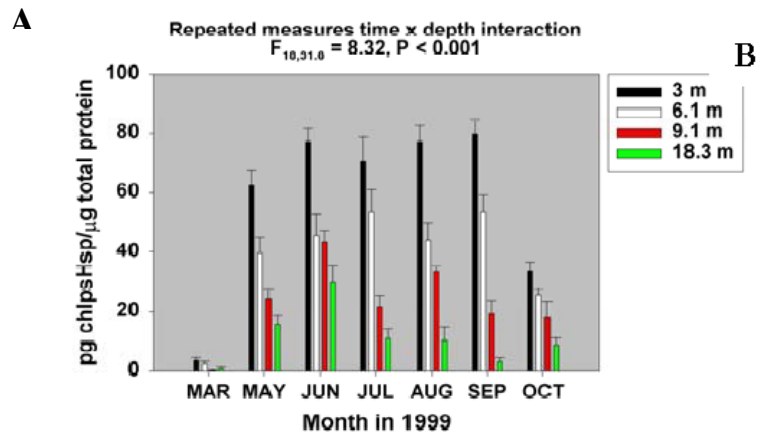
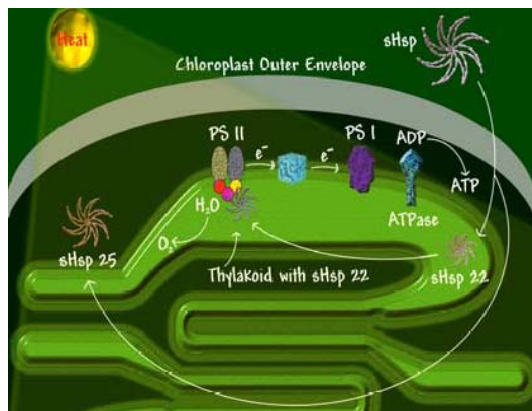


Figure 4. Panel A – Function of the chloroplast small heat-shock protein (ChlpsHsp). This protein is only induced when photosystem II is being damaged. It is a major adaptation of photosynthesis against heat stress, oxidative stress, and photoinhibition (Downs et al., 1999a, b) Panel B – Levels of ChlpsHsp in dinoflagellate of *M. annularis*. Coral samples and sampling scheme the same as in Fig. 2. (Downs et al., in rev.) Panel C- Logistic regression analysis of probability of the level of chlpsHsp in March to predict coral bleaching in September when sea surface temperatures reached 31°C in August (Fauth et al., in prep.)

In 2001, we continued to detect responses that indicated local stressors. Using a principal component analysis, the following patterns emerged: (1) The Key Largo 3.1-m site differs greatly from all the other six sites. The huge upward spike in October suggests a response to metals dominated over a xenobiotic response (data not shown). The Key Largo 6.1-m site had a similar profile, only not as pronounced. One explanation is sedimentation and runoff may have contributed to these responses. Although we cannot unambiguously identify the specific stressor at this time, we do know that it dramatically affects the algal component of the coral, as indicated by markers specific for the chloroplasts and algal mitochondria, and that it is likely associated with runoff and rainfall events. The effect of sedimentation will be tested using sedimentation data collected from sediment traps. In addition, except for the most offshore site, all sites had negative PC2 scores in March, suggesting responses to a xenobiotic predominated over a metal stress. Again, one possible explanation is pesticide runoff. This will be further investigated by examining whether correlations exist between cellular diagnostic parameter levels and water chemistry parameters (chlorophyll a and/or pigments).

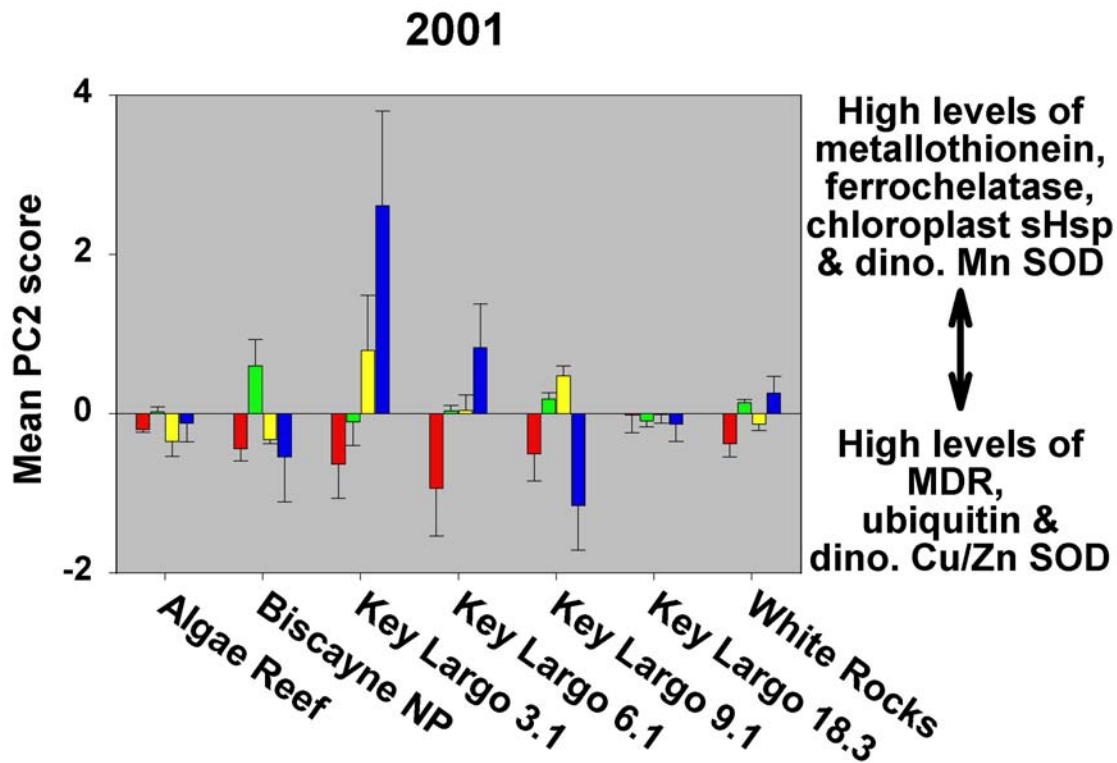


Figure 5. Results of the principal component analysis conducted on data from seven sites during different sampling periods; red = March; green = June; yellow = August; blue = November).

Objective 4: The condition of the corals at selected sites in Biscayne National Park (BNP) and in the upper Florida Keys National Marine Sanctuary (FKNMS) has been assessed at multiple scales in order to compare the precision, sensitivity, and prognostic capabilities of the CDS with measures traditionally used to assess ecosystem health. Community-scale condition of selected patch reefs was assessed using the well established Atlantic and Gulf Rapid Reef Assessment (AGRRA, Ginsburg et al., 2000). This protocol determines the condition of reefs by evaluating major benthic taxa that comprise them: coral and algae. The condition (i.e., mottling or bleaching) of populations of a key symbiont-bearing foraminifer (*Amphistegina gibbosa*), living in the vicinity of the corals, is also being monitored according to Hallock et al. (1995). These data will be used to determine if there is a correlation between bleaching stress in the foraminifer and bleaching or other stress responses in corals. This information will help determine if foraminifer can be used as a surrogate for studies of the mechanisms of coral bleaching. Individual-scale studies include monitoring lesions on the coral (Meesters et al., 1997) and the assessment of the overall condition (i.e., bleaching, disease, overgrowth, etc.) of the sampled corals. These assessments are compared to measures of health status taken at the cellular physiological level in a coral (*Montastraea annularis*), two fishes (*Haemulon plumieri* and *Stegastes partitus*), an alga (*Halimeda opuntia*), and a snail (*Coralliophila abbreviata*) using a Cellular Diagnostic System (CDS) (Downs et al., 2000; 2001). The Cellular Diagnostic System assesses indicators of cell integrity indicative of stressed or non-stressed conditions. Environmental data, including continuous water temperature measurements (using HOBO data loggers) and nutrient levels (taken at the time of biological sampling), sediment-trap data, and data from other ongoing monitoring studies are also being collected. The environmental data will be analyzed in conjunction with community, population, coral condition, and molecular data to develop a more comprehensive overview of coral ecosystem health and provide evidence for the underlying stresses.

To date, we have compared coral lesion healing with levels of cellular parameters at one site in Biscayne National Park (Alina's Reef) and five sites in the upper Florida Keys National Marine Sanctuary that represent both a depth gradient (3.1 m – 18.3 m) and geographic distribution (3 sites each at 6.1 m depth). To accomplish this, we tagged corals (*Montastraea* complex) that were to be sampled. Corals were sampled using a 1.5-cm punch, removing an approximate 3 mm deep divot of tissue from the colony surface. The sampling employed a repeated measures design on a quarterly basis in 2001 (March/April, June, August/September and October/November). The lesions (defined as an area on the colony with no live coral tissue) were monitored by photographing each lesion using a Nikonos V camera with a close-up adapter at each of the quarterly sampling events. Photographs were scanned to digital images, and then the area and perimeter of the lesions were calculated using image analysis software. Tissue samples were analyzed by ELISA for 20 cellular parameters included in the CDS. Our hypothesis was that a coral, which the CDS indicates to be more stressed, would be less likely to regenerate than a coral, which CDS indicates to be less stressed.

In March, lesions from the Key Largo 3.1-m site experienced a large degree of regeneration with some lesions closing completely (Fig. 6A and 7A). Other sites, such as the Key Largo 9.1-m site, experienced very little regeneration with some lesions showing increases in mortality (Fig. 6B and 7A). However, in June, lesions from the Key Largo 9.1-m site appeared to regenerate the best, relative to lesions from the shallower corals at the Key Largo 6-m and 3-m sites (Fig. 7B).

Algae Reef (site 6) and White Banks (site 5) showed the greatest amount of regeneration year round relative to the other two 6-m sites, Alina's Reef and the Key Largo 6.1-m site, which show very little change year round (Fig. 8A and 8B).

Results of a backward stepwise regression, to determine which of the cellular parameters explained significant variation in coral re-growth, indicated that re-growth was correlated with depth and five of the cellular parameters: MDR (multidrug resistance protein), dinoflagellate heat shock protein 60, cnidarian Cu/Zn superoxide dismutase, dinoflagellate Mn superoxide dismutase, and dinoflagellate glutathione peroxidase. Corals with high levels of plant Hsp 60 and plant glutathione peroxidase healed more quickly indicating a healthy status. Lesions in corals with high MDR, cnidarian Cu/Zn superoxide dismutase, and plant Mn superoxide dismutase levels healed more slowly suggesting they were stressed with a xenobiotic, thus allocating less of their energy to regeneration. These analyses indicate that corals located at Algae Reef showed significantly higher re-growth of lesions than those at the 9.1-m and 18.3-m sites off of Key Largo.

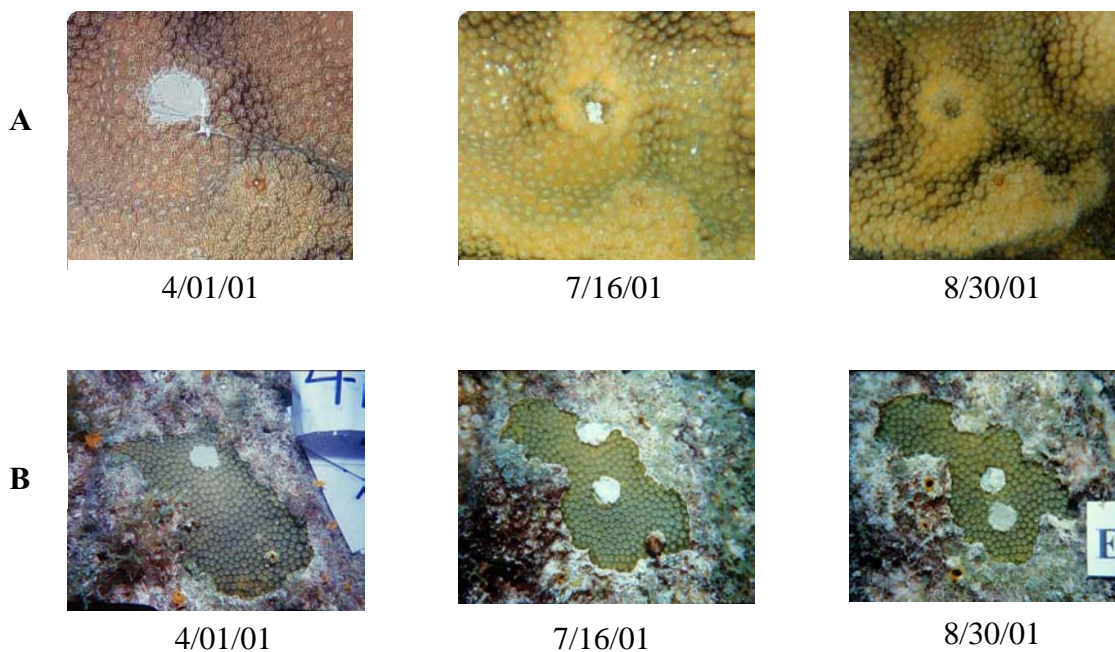


Figure 6. Change in lesion size between March and August 2001: (A) Decrease in lesion size indicating regeneration of lesion at the Key Largo 3 m site. (B) Increase in lesion size indicating increased mortality and algal overgrowth at the Key Largo 10-m site.

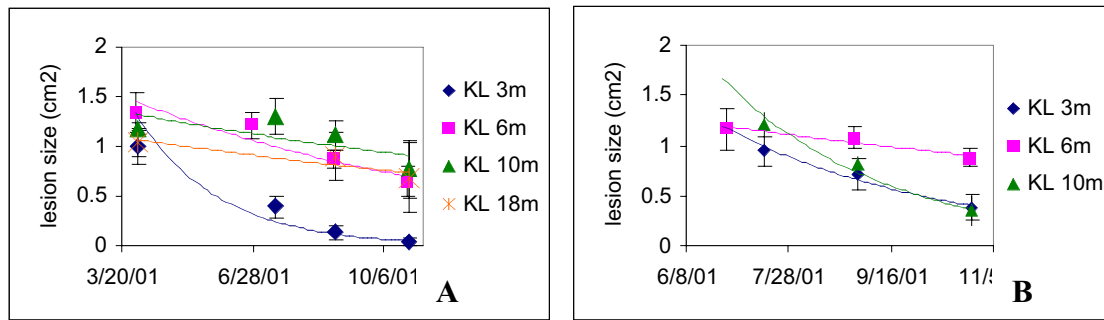


Figure 7. Change in mean lesion size ($\text{cm}^2 \pm \text{SE}$) along a depth gradient of 3 m, 6 m, 10 m, and 18 m in Key Largo. Lines fitted to an exponential model. (A) March sampling lesion (B) June sampling lesion.

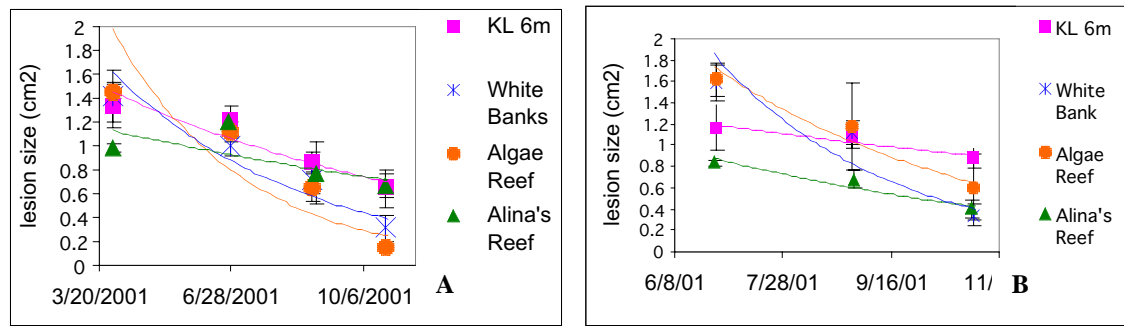


Figure 8. Change in mean lesion size ($\text{cm}^2 \pm \text{SE}$) for the four 6-m sites including Key Largo 6-m (site 2), White Banks (site 5), Algae Reef (site 6), and Alina's Reef (site 7). Lines fitted to an exponential model. (A) March sampling lesion (B) June sampling lesion.

Objective 5: Throughout this project, we have welcomed the participation of individuals from many walks of life. We have had divers participate on various missions including: individuals from a local high school marine biology class, retirees from the local community, resource managers, graduate student volunteers, and industry. Through these interactions, we have been able to communicate and educate others about the novel technology we are testing, the similarity of this technology to modern biomedicine, and the prospects that this technology brings to understanding coral reef degradation and development of science-based strategies to combat them. We have also engaged the scientific and resource management community in evaluating and critiquing our data and experimental design through a recent workshop (March 15, 2002). We had representatives from academia, the State of Florida, Biscayne National Park, USGS, Florida Keys National Marine Sanctuary, National Undersea Research Program, industry, Mote Marine Laboratory, and the National Ocean Service. They were able to review our data and provide critical input to our second-year design. Two significant recommendations from the meeting were to increase the spatial scale of the project and conduct laboratory challenge experiments with suspect stressors.

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Project Title: Ecological Characterization and Experimental Analysis of Disturbance and Recovery Dynamics on Seagrass-*Porites* Coral Banks in the Florida Keys National Marine Sanctuary.

Researchers: W. Judson Kenworthy, Paula E. Whitfield, Kamille K. Hammerstrom, and Mark S. Fonseca, Center for Coastal Fisheries and Habitat Research, NCCOS, NOS, NOAA, Beaufort NC.

Project Goals: This research consists of two tasks designed to integrate a habitat characterization study (Task 1) with our ongoing experimental restoration research program (Task 2) in an area of the FKNMS experiencing both intensive power vessel damage as well as extensive changes in regional water quality. This region of the Sanctuary is located at the transition between the Middle and Lower Keys and is known as Red Bay Bank (Fig.1). The Red Bay Bank area is actually a series of extremely unique elevated seagrass-*Porites* coral banks north of Marathon on the Gulf side of the major pass (Moser Channel) connecting the Florida Straits with the Gulf of Mexico. Like many areas throughout the Florida Keys, these seagrass-coral banks stabilize a large volume of unconsolidated sediment while intercepting the flow of water from the Gulf of Mexico to the Florida Straits. Therefore, these banks act as a buffer protecting the reef tract from sedimentation and excess nutrients originating in the southeastern Gulf of Mexico. This area is also a major route for commercial and recreational vessel traffic and is experiencing an unprecedented frequency of vessel groundings that are physically and biologically degrading the habitat (Fig. 2).

The biological and physical characterization of the undisturbed banks will provide a spatially geo-referenced and articulated baseline from which to examine the cumulative impacts of vessel injuries and regional water quality changes occurring on the adjacent shelf. The baseline characterization of injury sites on Red Bay Banks will provide a means of calibrating and verifying current recovery models used to predict *Thalassia testudinum* (turtle grass) recovery rates under a range of injury types. Likewise, these data will improve the Habitat Equivalency Analysis (HEA) by providing a more accurate calculation of the compensation needed to offset damages to Sanctuary resources. The experimental analysis of excavation depths will determine the threshold at which it is necessary to import fill and re-grade severe injuries to accelerate recovery and gain direct benefits from restoration.

Methods: This project documents the physical and biological characteristics of these banks and the impact of vessel damage and deteriorating water quality on this system using a wide range of techniques at different scales of resolution including: satellite imagery, aircraft inspection, vertical aerial photography, in-situ surveys, replicated experimental manipulations, GIS, and spatially articulated ARC View recovery models. Since 1993, the Center for Coastal Fisheries and Habitat Research (CCFHR) has been actively collaborating with the Florida Keys National Marine Sanctuary (FKNMS), NOAA's Damage Assessment (DAC), and NOAA General Counsel (GC) on injury assessment, restoration, and conservation of seagrasses and coral reefs in the FKNMS. The currently funded Red Bay Banks study forms part of an overall research program developing state of the art habitat assessment protocols and injury recovery models to improve the response time of NOAA's assessment teams, and to assure compensation for damages associated with injury claims cases. The foundation for accurate assessments,

defensible compensation calculations, and implementation of successful restoration plans are based on good scientific information (Fonseca et al. 2000). Our restoration research program is providing the Sanctuary with the scientific information necessary to meet the stewardship requirements of the National Marine Sanctuaries Act.

To recover damages from injuries to seagrass resources we have collaborated with Brian Julius (DAC) and the Florida Keys National Marine Sanctuary to develop a Habitat Equivalency Analysis (HEA) procedure that requires knowledge of the baseline of ecological services provided by seagrass habitat, the rates of seagrass recovery, and how environmental factors modify predictions of recovery (Fig. 3). The baseline service levels (Fig. 3, green line) are being quantified through the development of a series of rapid and reproducible assessment protocols. As part of this project, field biologists were trained and the assessment protocols were field tested, refined, and implemented by a joint DAC – FKNMS resource and injury assessment team under the supervision of scientific staff from CCFHR. The protocols are now being used to prosecute vessel injury claims cases under the Mini 312 Injury Assessment program in the Sanctuary. It is estimated that approximately 30 significant injury cases will be processed per year in this program, significantly improving the Sanctuary's capabilities for recovering damages to natural resources.

The second aspect of our HEA-related work requires an understanding of the severity of injuries and the rates of natural (Fig. 3, blue line) and restoration-enhanced (Fig. 3, red line) seagrass recovery. Seagrass response to different classes of injury and recovery rates in different environments are being assessed through controlled field experiments (Kenworthy et al. in press; Kenworthy et al. 2000), long-term monitoring of injury sites in the FKNMS (Whitfield et al. in press), collaborative research in disturbance ecology with the Florida Marine Research Institute (FMRI), and modeling studies (Fonseca et al. 2000; Fonseca et al. submitted; Whitfield et al., 2001).

Findings to Date: In the first two years of this project we have mapped, characterized, and geo-referenced the entire Red Bay Bank system and ten vessel injury sites of different ages and different degrees of physical disturbance. Some of the larger injuries with considerable sediment disturbance have persisted for 10 years (Fig. 4). Where the seagrasses have been removed by displacing the surface sediments the banks are continuing to deteriorate in response to severe storms, suggesting that vessel impacts may have long-lasting effects on the stability of these banks (Fig. 5) (Kenworthy et al. in press; Whitfield et al. in press). The slow rate of seagrass recovery in these physically disturbed bank environments where sediments are excavated suggests that primary restoration (Fig. 3, red line) may be necessary in order to recover losses. The potential benefits of primary restoration (Fig. 3, "BR") are being examined in a series of experiments including: 1) benefits of seagrass transplanting and fertilization of seagrass transplants, 2) recovery under different excavation scenarios, and 3) testing the effects of filling and stabilizing excavations.

In order to understand seagrass recovery dynamics and adopt appropriate restoration techniques, we deployed two experiments mimicking sediment excavations on a mixed species seagrass-*Porites* coral bank near Marathon, FL. The first experiment, initiated in June 2000, was designed to test the effect of excavation depth on seagrass recovery in 50 x 150 cm plots of three depth

treatments: 10, 20, and 40 cm. Initially, the more deeply excavated treatments (40 cm) experienced erosion and became 40% larger. Over time, natural sedimentation processes appear to be gradually filling in the treatments, but the 20- and 40-cm excavations remain significantly deeper than the controls. Recovery in 10 cm treatments was rapid for both *Thalassia* and *Syringodium filiforme* (manatee grass). After seven months, *Syringodium* short-shoot counts in 10- and 20-cm treatments were not significantly different from controls, while *Thalassia* counts were still significantly lower in the two deeper treatments (Fig. 6). Recovery of *Thalassia testudinum* is becoming evident in the 20- and 40-cm excavations, although counts are still much lower than those in control plots. *Syringodium filiforme* results are striking. Short-shoot count has continued to increase in the 20- and 40-cm excavations and now counts are about twice as high as those in the control plots.

The second experiment, initiated in September 2000, was designed to test the effect on seagrass recovery of excavations filled with native limestone pea gravel. Experimental 50 x 150 cm plots were excavated to 30 cm; six plots were filled with gravel and six were not filled. Fill prevented immediate expansion of the excavation; within days of deployment, fill treatments had not changed, but no-fill treatments had expanded by 13%. After 364 days, no-fill treatments were 43% deeper than controls. Again, *T. testudinum* appeared to be starting to recover in both filled and unfilled treatments, although short-shoot counts were still much lower than counts in control plots (Fig. 6). *Syringodium filiforme* displayed the same overcompensation response in the fill experimental plots as in the excavation experimental plots, with short-shoot counts about three times higher than control plot counts. Interestingly, the fill treatment *S. filiforme* counts were less than half those of the unfilled treatment counts. There seemed to be some inhibition of *S. filiforme* growth by the pea gravel, but this was not evident in the *T. testudinum* shoot counts. *Syringodium filiforme* shoot counts were highly variable in both experimental treatments, ranging from 0 to 47 short shoots in a 0.0625 m² quadrat in the filled plots and 0 to 82 short shoots in the unfilled plots. Even though *S. filiforme* short-shoot counts were lower in pea gravel, we are encouraged by the fact that seagrasses were growing into the filled treatments. The possibility that we can stabilize the excavations by re-grading them and still have seagrasses recover is encouraging.

The biological and physical characterization of the undisturbed banks will provide spatially georeferenced and articulated baseline from which to examine the cumulative impacts of vessel injuries and regional water quality changes occurring on the adjacent shelf. The baseline characterization of injury sites on Red Bay Banks will provide a means of calibrating and verifying current recovery models used to predict *T. testudinum* recovery rates under a range of injury types. Likewise, these data will improve the HEA by providing a more accurate calculation of the compensation needed to offset damages to Sanctuary resources. The experimental analysis of excavation depths will determine the threshold where it is necessary to import fill and re-grade severe injuries to accelerate recovery and gain direct benefits from restoration.

21 March 2003

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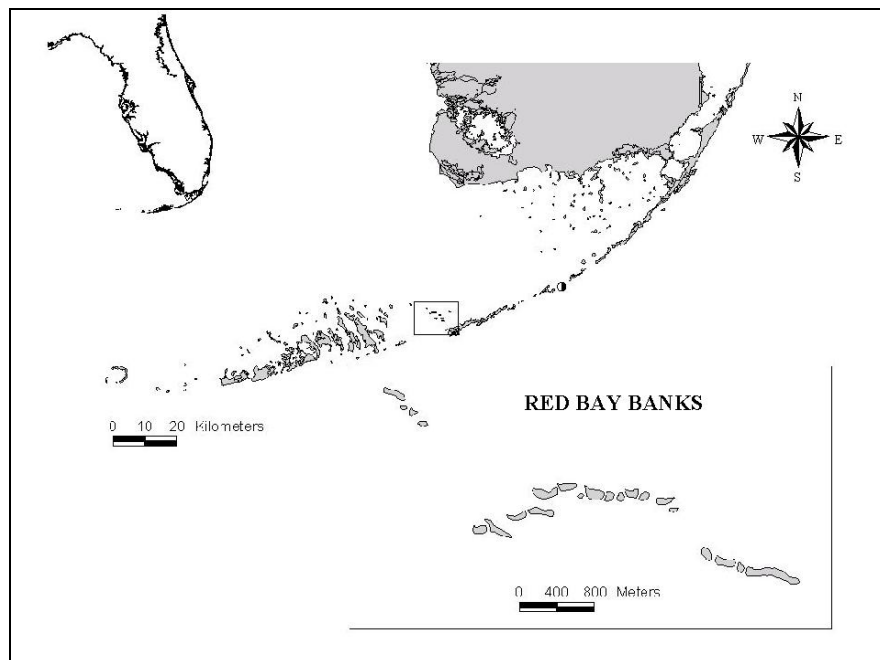


Figure 1. Map showing the general location of Red Bay Banks.

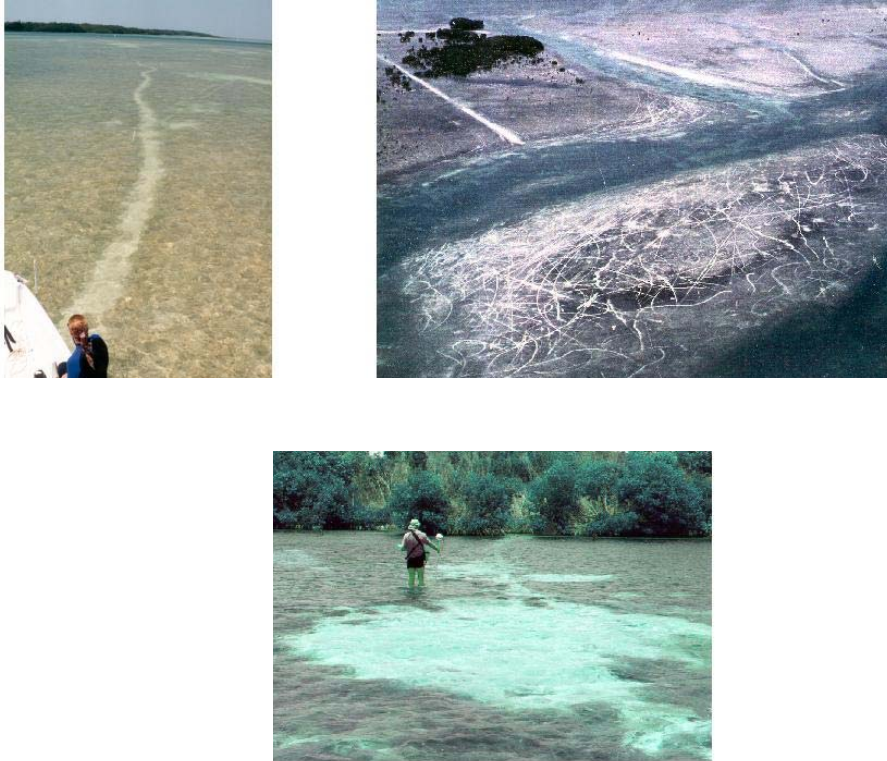


Figure 2. Photographs of typical vessel injuries in seagrass beds: a propeller scar injury (upper left), multiple propeller scars in several adjacent seagrass beds (upper right), and a hull grounding excavation in shallow water (lower middle).

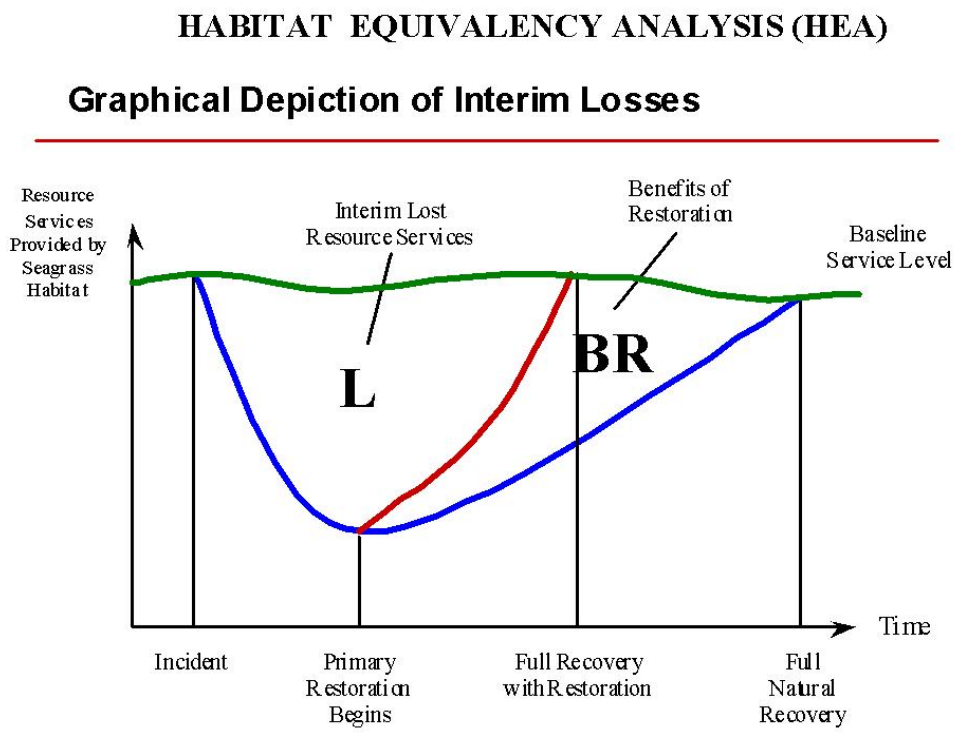


Figure 3. Graphical depiction of the Habitat Equivalency Analysis procedure.

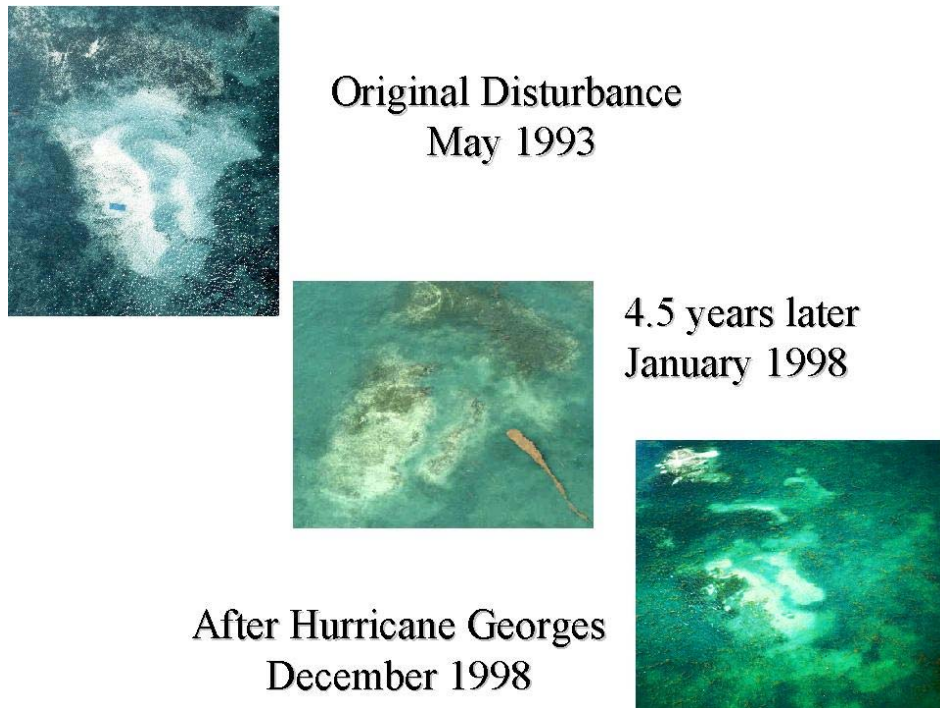


Figure 4. Time series of aerial photographs showing the original tugboat grounding (lighter area = 7200 m²) in May 1993 (upper left), 4.5 years later in January 1998 after some minor recovery (middle), and scouring associated with Hurricane Georges in December 1998 (lower right).

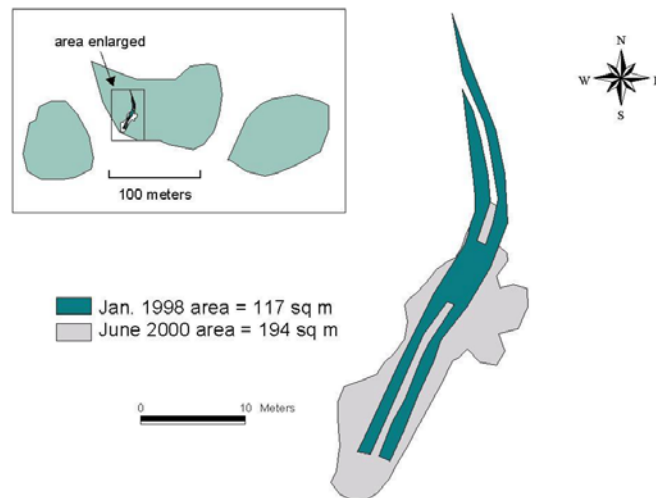
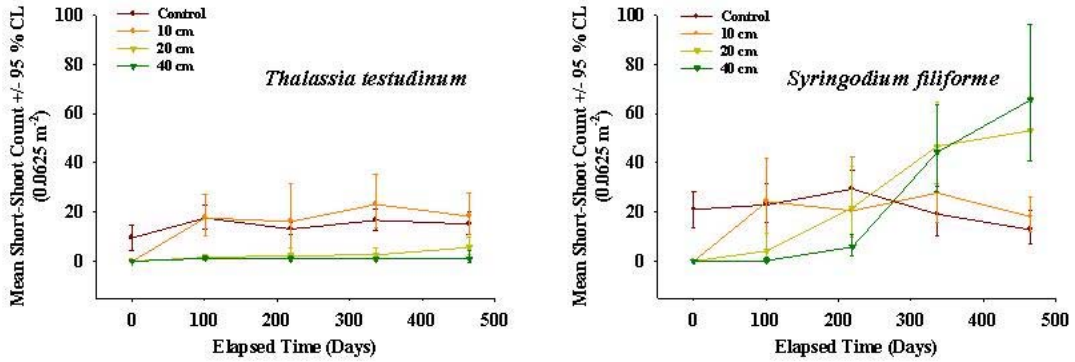


Figure 5. Illustration showing the change in size of a vessel-grounding site during Hurricane Georges. Darker area indicates original grounding (117 m²) and lighter area indicates the increase in size associated with the hurricane (194 m²).

Excavation Experiment



Fill Experiment

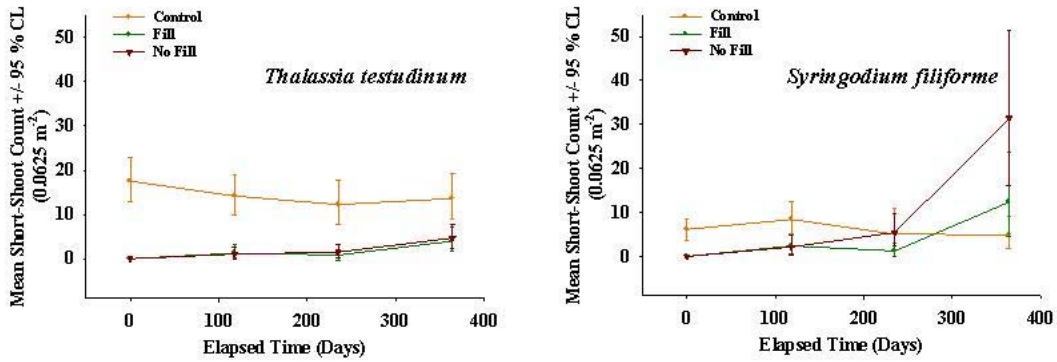


Figure 6. Preliminary results of the excavation (top panels) and fill (bottom panels) experiments showing the short-shoot density of two seagrasses, *Thalassia testudinum* and *Syringodium filiforme*. CI = 95% confidence interval.

Project Title: Fate of Wastewater-Derived Nutrients in Florida Keys Groundwaters

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Background: Shallow injection is the predominant mode of wastewater disposal for most tourist-oriented facilities and some residential communities in the Florida Keys National Marine Sanctuary. Concern has been expressed that wastewater nutrients may be escaping the saline groundwater system into canals and surrounding coastal waters and perhaps to the reef tract 10 km offshore, promoting unwanted algal growth and degradation of water quality. We performed a field study of the fate of wastewater-derived phosphate and nitrate in the subsurface of a Florida Keys residential community (Key Colony Beach, Florida) that uses this disposal method, analyzing samples from 21 monitoring wells and two canal sites.

Findings to Date: The results indicate that wastewater injection at 18 m into saline groundwaters creates a large buoyant plume that flows quickly (within days) upward to a confining layer 6 m below the surface, and then in a preferred flow path toward a canal 200 m to the east (within 2 months, based on a companion tracer study). Phosphate behaves non-conservatively, in part because of fast adsorption followed by slower precipitation of amorphous phosphate minerals (Fig. 1A), as confirmed by laboratory uptake experiments.

Low-salinity groundwaters along the preferred flow path have nitrate concentrations barely reduced from that of the injected wastewaters (ranging from 400-600 mmol/kg). Portions of the low-salinity plume off the main axis of flow have longer residence times (> 90 days) and have had their nitrate concentrations strongly reduced by a combination of mixing and denitrification (Fig. 1B). These waters have N₂/Ar mole ratios as high as 64 (1.6 times atmospheric equilibration values), and d¹⁵N_{air} values of the nitrate of 16-26‰. The calculated isotope fractionation factor for denitrification in the groundwater was about -12 ± 4‰, within the range of previous estimates. Estimated rates of denitrification range from 1.4 - 4.4 mmol/kg N d⁻¹.

Despite rapid scavenging of phosphate and extensive denitrification, substantial quantities of wastewater nutrients are reaching the surface waters of the Florida Keys following wastewater injection. The buoyant nature of the wastewater plume, together with the highly permeable nature of the substrate and its penetration by dredged canals, facilitates the transport of wastewater-contaminated groundwater to surface waters.

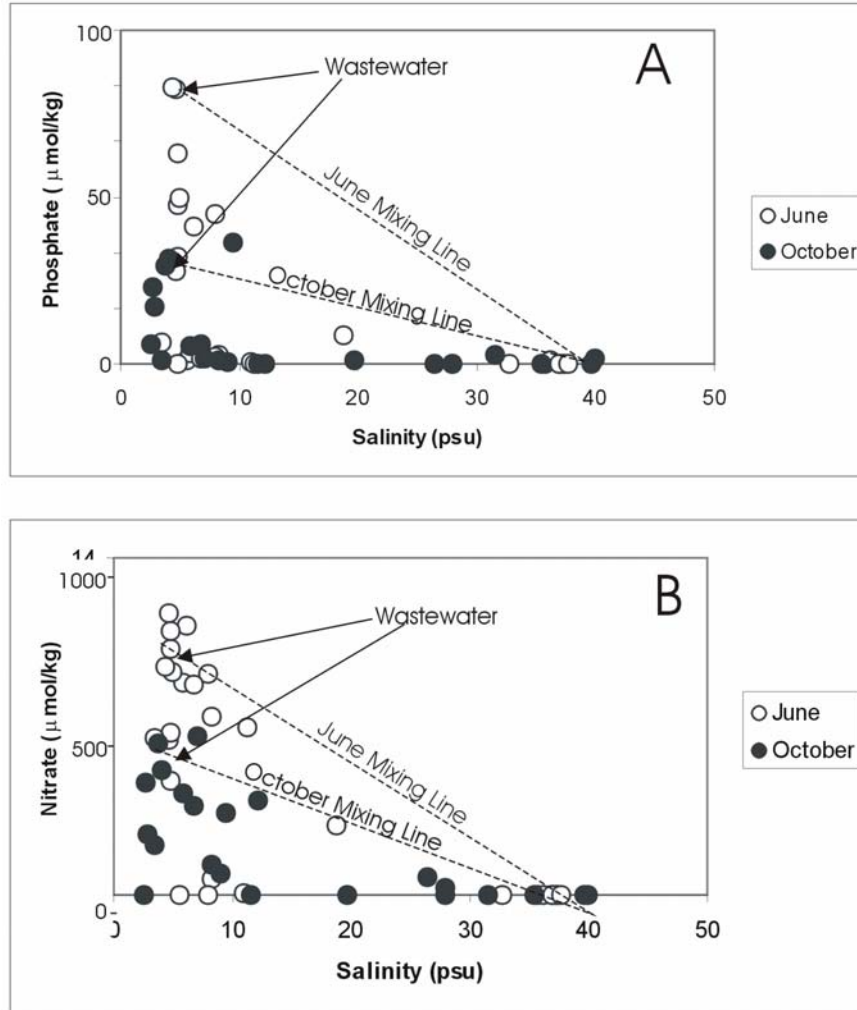


Figure 1. June and October, 1999 inorganic phosphate (A) and nitrate (B) concentrations in Key Colony Beach, Florida groundwaters, plotted against salinity (expressed in practical salinity units, equivalent to mg/kg or ‰). The mixing lines connect the concentration of phosphate in the wastewater with the concentration in the ambient groundwater.

21 March 2003

Project Title: Phosphate Dynamics Surrounding a High Discharge Wastewater Disposal Well in the Florida Keys

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Abstract: Two artificial tracer experiments (June, 1998 and October, 1999) were conducted in a groundwater system surrounding a high-volume (500-1200 m³/d) wastewater disposal well in the Florida Keys. SF₆ served as the conservative groundwater tracer. Groundwater transport rates were bimodal, both horizontally and vertically. Slow, dispersive-type flow rates were estimated to be below 0.3 m/d while the most rapid conduit type flow was characterized by flow rates as high as 20 m/d and even higher, up to 123.3 m/d, immediately adjacent to the point of injection. Typical transport rates were approximately five times higher in the 1999 experiment than those observed in 1998 commensurate with a wastewater discharge increase from 0.63×10^6 to 2.32×10^6 L/d. Salinity data indicate that the wastewater plume extends beneath the entire well field, as far as 175 m from the injection well.

Radiolabelled phosphate experiments showed that ³²PO₄ was rapidly adsorbed onto Key Largo limestone. Recirculation experiments using core material from the site and phosphate-rich water showed a rapid initial uptake of phosphate followed by a slower adsorption until an equilibrium concentration of approximately 26 μM is reached. Addition of phosphate-free water to the same core material showed a release of PO₄ into solution until the same equilibrium concentration was reached. The limestone matrix underlying the study site appears to act as a phosphate buffer once exposed to phosphate-rich water. The mechanisms controlling this buffering capacity are poorly understood at this time.

21 March 2003