

Sexual reproduction in precious corals (Coralliidae) collected in the Ryukyu Archipelago¹

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Abstract

Little is known about the basic life history of most species of precious corals (Octocorallia: Coralliidae). Three commercially valuable precious coral species (*Paracorallium japonicum*, *Corallium elatius* and *C. konojoi*) were sampled from the Ryukyu Archipelago in order to study their reproductive biology. To determine features of their gamete differentiation, samples were thin-sectioned for examination with a digital light microscope, and the diameters of sperm sacs and oocytes were measured. The sexual reproduction strategy in all three species was determined to be gonochoristic broadcast spawning. Almost all gonads were found to be differentiating in siphonozooids, not in autozooids. The sex ratio of *C. konojoi* was around 1:1, while that of *P. japonicum* and *C. elatius* seemed biased toward females but did not deviate significantly from 1:1. The number of gonads per polyp in these three species was fewer and they were generally smaller in diameter in comparison to other octocorals reported elsewhere, suggesting comparatively low fecundity for these species of Corallidae from southern Japan. Spawning of these three species appears to occur during summer, mostly from May to August.

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Introduction

Species in the genera *Corallium* and *Paracorallium* (Subclass Octocorallia, Order Alcyonacea, Family Coralliidae) are well known for their red or pink skeletons, which have been used since antiquity for ornament, medicine, talismans and currency. As “precious corals”, they have been harvested routinely from the Mediterranean Sea for at least 5,000 yrs and perhaps harvested for as long as 30,000 yrs or more (Kosuge 1987, Liverino 1986). Products made from *Corallium rubrum* were found in an Old Stone Age Monument about 25,000 yrs old in Germany (Kosuge 1987), and precious corals and shells were found in ruins roughly 30,000 yrs old in Lausanne, Switzerland (Liverino 1986).

The first record of collecting precious coral in Japan was in 1812, when a fisherman found a precious coral entangled in his net off Muroto, Kochi Prefecture (Suzuki 1999). The precious coral fishery in Japan continues to the present day in the Kochi, Kagoshima and Okinawa regions.

It is generally recognized that the biomass of precious corals has been reduced by overfishing (Tsounis *et al.* 2006, 2007), but there are few details known about the fishery and the biology of precious coral species in Japan. The first study of any Japanese precious coral was a taxonomic report by Ridley (1882), and next were reports were by Kishinouye (1902, 1903a,b). Kishinouye (1904a,b) made comprehensive studies of Japanese precious corals, including their anatomy, chemistry, morphology, reproduction, symbioses and taxonomy. But, it was

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nearly a century later before studies of Japanese Corallidae resumed including studies of their husbandry (Ueno *et al.* 1993, Iwase 1995, Nonaka *et al.* 2006); of their biology and ecology (Nonaka *et al.* 2009, Iwasaki 2010, Iwasaki *et al.* 2012, Nonaka *et al.* 2012b); and of their taxonomy (Nonaka & Muzik 2010, Uda *et al.* 2011, Nonaka *et al.* 2012a, Nonaka & Muzik 2012b).

Among the most important data needed for successful conservation of any species is information about its reproductive biology. However, the basic life history of most species in the subclass Octocorallia has been poorly known until the last few decades (Simpson 2009, Kahng *et al.* 2011). The remarkable reports almost 150 years ago by Lacaze-Duthiers (1864) on the growth and reproduction of *Corallium rubrum* appear to be the first-ever made about octocoral reproduction. He kept colonies in a bucket on-board fishing boats in the Mediterranean Sea and observed planula larvae escaping from the mouths of living polyps. This species has subsequently been well-studied in the past half century, with multiple studies addressing reproduction and growth (e.g. Vighi 1970, Santangelo *et al.* 2003, Tsounis *et al.* 2006). Regarding other species in the Coralliidae, Hickson (1907) showed a diagram of a transverse section of *C. reginae*, from Indonesia, with gonads in a siphonozooid (a polyp without tentacles). Grigg (1976) reported that a Hawaiian species, *C. secundum* was dioecious, reproducing annually and spawning gametes from around June to July. Most recently, Waller and Baco (2007) reported that two Hawaiian species, *C. secundum* and *C. laauense*, were both gonochoristic spawners, and that their gonads were found within siphonozooids.

Kishinoue (1904a) was the first to report that gametes of *P. japonicum* were differentiated in siphonozooids and that they were more developed in March than in September. However, over a century later, there is still very limited information about reproduction of any precious coral species in Japanese waters. In the three commercially-valuable species (*P.*

japonicum, *C. elatius* and *C. konojoi*) collected from the Ryukyu Archipelago, most gonads were reportedly found in the siphonozooids, not in autozooids (Nonaka *et al*, 2012b). In our study, we focus in more detail on the reproductive biology of the commercial species sampled from the Ryukyu Archipelago.

Materials and Methods

Sampling

Samples were collected from the Ryukyu Archipelago, the southernmost region of Japan (Figure 1). In the northern part of the study area (south of the Kagoshima Islands and the Amami Islands), they were collected by manned submersible at depths from about 100 to 300 m. In the southern part (including Okinawa, Miyako and Ishigaki Islands) they were collected by remotely-operated vehicle (ROV) at depths from about 200 to 330 m. Because collections were made by a private company which was seeking large colonies for commercial purposes, sampling was not random. Basically, larger colonies were selectively harvested, and environmental data was recorded for just the immediate area of the collection. Eighty-three samples were obtained from 4 June 2005 to 1 March 2006. One hundred and five additional samples were obtained after an interval of about 16 months, from 3 June 2007 to 1 September 2009. Environmental data (depth, temperature, type of substratum) was also provided for each sample. Photographs of colonies were first taken *in situ*, and then once collected, photographs were re-taken with a scale on board ship. Heights and widths of colonies were calculated from the photographs. These environmental data and colony sizes have been already published (Nonaka and Muzik 2009). Samples were divided into valuable parts (base parts of colonies) and unvalued parts (broken tips of branches; up to 15 cm long). The former were used for commercial purposes, while the latter were preserved and examined in this study. The study

samples were preserved in 95% ethanol on board right after sampling in order to avoid irregular spawning. All specimens (branch tips) are preserved in the Okinawa Churaumi Aquarium collection.

Identifications

All samples were identified based on accepted morphological characters, such as color, branching pattern, polyp arrangement and size, sclerite shape and size. Color and branching pattern of colonies were examined *in situ* or from shipboard photographs with a scale. Polyp details were observed and measured by light-microscope (Keyence VHX), while sizes and shapes of sclerites were documented by light and scanning electronic microscopy (Keyence VE-8800). Identification follows Kishinouye (1903a, 1904a), Bayer (1956), Bayer and Cairns (2003), Nonaka and Muzik (2010), Nonaka *et al.* (2012a) and Nonaka and Muzik (2012b). Although nine species in the family Coralliidae are found in Japan, this study focused on the three known commercially valuable species: *Paracorallium japonicum* (Kishinouye, 1903), *Corallium elatius* (Ridley, 1882) and *C. konojoi* Kishinouye, 1903.

Bayer and Cairns (2003) established the new genus *Paracorallium* in the family Coralliidae, and they moved seven known species, including *C. japonicum*, to the new genus. This taxonomic decision was initially supported by molecular analysis (Uda *et al.* 2011), but more recently, Ardila *et al.* (2012) have shown that the genus *Paracorallium* is polyphyletic and synonymous with *Corallium*. To avoid confusion, at present we here retain the species *japonicum* in the genus *Paracorallium*, temporarily, as scientific discussion continues about which genus might finally prove acceptable for it.

Observation of sperm sacs and oocytes

Small subsamples (approximately 10-20 mm long) of preserved specimens were first separated from original specimens and decalcified in Bouin's solution (Nakamura and Takahashi 1973). Decalcified subsamples were embedded in paraffin for sectioning. Tissues were then thin-sectioned (7 μm thick), mounted on slides, and stained with hematoxylin and eosin. Prepared specimens were observed and photographs taken with a digital microscope (Keyence VHX). Our current method (fixing in 95% ethanol and decalcifying in Bouin's solution) is not commonly used when making histological sections. However, we chose this method to insure that the specimens were preserved not only for this study, but also for possible future studies including molecular analyses. In past studies, samples were fixed only in Bouin's solution (e.g. Goldberg *et al.* 1974, Farrant 1986). Therefore, we conducted experiments to determine whether there might be any difference between our method (fixing by ethanol and decalcifying with Bouin's) and both fixing and decalcifying with Bouin's, and we concluded there was none.

Based on microscopic observations of these three species, we regarded "sperm sacs" to indicate male gonads, and "oocytes" to indicate females. If minute sperm were visible in the gonads, they were identified as sperm sacs (Figure 2; left). If only conspicuous spots (nuclei) were visible, they were identified as oocytes (Figures 2-4). We classified both types of gonads examined into four developmental stages (Figures 2-4), following Pires *et al.* (2009). Samples with no gonads, or gonads too unripe or too immature to identify to sex, were classified as "Stage I" or as "sex unknown." Once sex was determined, we distinguished developing sperm sacs into three additional stages: spermatoblasts distributed evenly (Stage II), spermatoblasts at the perimeter of the gonad, surrounding a central open space (Stage III), and sperm visibly differentiated and with a tail (Stage IV) (Figures 2-4). Similarly, developing oocytes were distinguished into three additional stages: small oocytes which have no surrounding follicle cells,

no reddish color (Stage II), oocyte which were surrounded by thick follicle cells, dyed pinkish color with eosin, and have conspicuous nucleus (Stage III), and oocytes which were surrounded by thin follicle cells and have an inconspicuous nucleus (Stage IV) (Figures 2-4).

The diameters of gonads (sperm sacs and oocytes) were measured using the accessory software of a digital microscope (Keyence VHX). The largest measurement among the serial sections of the same gonad was selected as a representative of its actual size. Both the major and minor axes of gonads were measured and average gonad diameters then calculated from these measurements. The polyps (almost all of them siphonozooids) harbored various numbers of gonads (Figures 2-4). The number of gonads per siphonozooid was counted by microscopic observation, while being careful to avoid repetitive counts of the same gonad appearing in serial sections.

All siphonozooids with gonads were examined on the slides. There was only a small number of siphonozooids to examine in immature samples. However, in mature samples, a very large number of siphonozooids was examined, e.g., 47 siphonozooids in one sample of *P. japonicum* (male), 32 in a *P. japonicum* (female), 61 in a *C. elatius* (male), 12 in a *C. elatius* (female), 58 in a *C. konojoi* (male) and 58 in a *C. konojoi* (female), the maximum number of fertile gonads counted.

Data analysis

Sex ratios were calculated from the number of colonies having sperm-sacs (male) and eggs (female) and tested for deviation from 1:1 by applying a χ^2 test. A Student's t-test was used to analyze differences found in colony size (height and width) between male and females in all three species. One-way ANOVA and a Student's t-test were used to analyze differences in average gonad size. Similarly, ANOVA was used to test for differences in the frequencies of

polyps possessing gonads, and monthly differences in average gonad sizes. Statistical analysis was performed using MS excel and Statistica software (StatSoft, Inc.).

Results

Reproductive features of samples examined

A total of 188 samples were examined. Ninety colonies were identified as *Paracorallium japonicum*, 55 as *Corallium elatius*, and 27 as *C. konojoi* (Appendix 1-9). The remaining 16 samples were not conclusively identified or belonged to other species. In summary, a total of 172 samples could be positively identified as the three commercial species of interest in this study. We have tentatively concluded that all three species are dioecious, since either sperm sacs or oocytes of almost all of the 172 samples were present in their coenenchyme, (Figures 2-4). However, two hermaphroditic colonies (*P. japonicum* and *C. elatius*) contained a majority of sperm-sacs and a few oocytes in their siphonozooids (Figure 5, Appendix 1, 4). Almost all samples had all gonads located in their siphonozooids. However, in six colonies of *P. japonicum*, seven colonies of *C. elatius* and one colony of *C. konojoi*, a few gonads were also found in their autozooids (Figure 6, Appendix 1-8). No planula larvae were found in any tissues of any colonies.

The frequency of males and females in *C. konojoi* was equal (Table 1). There were more female colonies of *P. japonicum* and *C. elatius* than male colonies (Table 1); however, the sex ratios (male:female) of the colonies of *P. japonicum* (1:1.4) and *C. elatius* (1:1.7) did not deviate significantly from 1:1 (χ^2 test, $P > 0.05$). The sex ratio of *P. japonicum* was exactly 1:1 during the period after excluding “unknown” colonies from April to June. Similarly, the sex ratio in *C. elatius* was nearly 1:1 during the period excluding sex unknown specimens from October to March and during May and June (1:1.06) (Table 1).

No significant differences in average colony sizes, both height and width, were found among “male”, “female” and “unknown” sexes in all three species (Table 2; ANOVA, $P > 0.05$) except for colony height in *C. elatius*. In *C. elatius*, the average height of colonies of sex “unknown” was significantly larger than those of sexes “male” and “female” (Table 2; ANOVA, $p < 0.01$; Tukey’s HSD test, $P < 0.05$). The smallest specimens having recognizable gonads were: 145 mm high by 180 mm wide in *P. japonicum*; 223 mm by 263 mm in *C. elatius*; and 78 mm by 90 mm in *C. konojoi* (Table 2, Appendix 1-9).

Stages of gonadal differentiation

Gonads (sperm sacs and oocytes) were categorized into four stages (Figs. 2 and 4). Stage I (sex unknown) colonies among *P. japonicum* were not observed from April to June (Figure 7A, Table 1). Stage II colonies were recorded almost all-season long except in June, July and October, and there were relatively fewer (< 40%) Stage II colonies in March to May and in August. In April and June, there were relatively more (50%) classified as stage III. There were relatively more (> 20%) colonies of stage IV appearing in August and September, and fewer in May, June and December (Figure 7A).

Colonies of *C. elatius* classified as stage I (sex unknown) appeared in April, July, August and September (Figure 7B, Table 1). Stage II colonies were recorded more commonly than other stages, but they were not collected in July. No stage III colonies were recorded from July to September, nor in November. In May, June and December, a significant proportion of colonies (50%) was classified as stage III. Stage IV Colonies appeared only in June (Figure 7B).

There were no samples of *C. konojoi* collected in July. Stage I (sex unknown) colonies appeared in February, June, and from October to December (Figure 7C, Table 1). Stage II colonies were recorded from January to May, and in September and December. All colonies

collected in January and September was Stage II. Stage III colonies appeared from March to June, and December. Colonies of stage IV (sperm sacs only) appeared only in August (Figure 7C), and no Stage IV oocytes were found in *C. konojoi*.

Gonad number per polyp

For all three species, the number of gonads per polyp did not to change with season (Figure 8). Also there was no relationship between colony size (average of height and width) and average gonad number per polyp in colonies of the three species ($r^2 = 0.4$).

In males of *P. japonicum*, the largest number of sperm-sacs per polyp recorded was six (Figure 9A), but most polyps had only one or two sperm-sacs per polyp (average 2.09) (Table 3). Comparing male polyp stages, the average gonad number per polyp was greatest in stage III (Table 3; ANOVA, $P < 0.001$; Tukey's HSD test, $p < 0.05$). In *C. elatius* the largest number of sperm-sacs recorded per polyp was also six (Figure 9A). Polyps with a single sperm-sac were most frequent and the average was 1.75 (Table 3). Comparison of stages showed that there were no significant differences among the three stages in the average gonad number per polyp (Table 3; ANOVA, $P > 0.05$). In male colonies of *C. konojoi*, the largest number of sperm-sacs recorded per polyp was eight (Figure 9A), and 34% of polyps had at least one sperm sac (average 2.44) (Table 3). Comparison of the three stages of male polyps showed no significant differences among them in average gonad number per polyp (Table 3; ANOVA, $P > 0.05$).

In female colonies of *P. japonicum*, the largest number of oocytes per polyp recorded was only three (average 1.30) (Figure 9B, Table 3). Polyps with a single oocyte were much more common (74%) than polyps with two or three oocytes (Figure 9B). There were no significant differences among the three stages (Table 3; ANOVA, $P > 0.05$). In female colonies of *C. elatius*, the largest number of oocytes was 7 (Figure 9B). Most polyps (46%) had a single oocyte

(Figure 9B), and their average was 1.91 (Table 3). There were no significant differences among the three stages number of gonads per polyp (Table 3; ANOVA, $P > 0.05$). In female colonies of *C. konojoi*, the largest number of oocytes recorded per polyp was seven; no polyps had 6 (Figure 9B). Most polyps (38%) had only one oocyte (average 2.16) (Figure 9B, Table 3), and there were also no significant differences in gonad number per polyp among the two stages (t-test, $P > 0.05$) for this species.

Comparing the three species, polyps of *C. konojoi* had significantly more male gonads than those of *P. japonicum* and those of *C. elatius* (ANOVA, $p < 0.001$; Tukey's HSD test, $p < 0.001$) (Table 3). There were no significant differences in the number of gonads per polyp between *P. japonicum* and *C. elatius* (Tukey's HSD test, $P > 0.05$). Also, polyps of *C. konojoi* had significantly more oocytes than the other two species (ANOVA, $p < 0.001$; Tukey's HSD test, $p < 0.001$), while polyps of *P. japonicum* had significantly fewer oocytes (Tukey's HSD test, $P < 0.001$) (Table 3).

Diameter of sperm sacs and oocytes

In the three species examined, the mean diameter of gonads was significantly different among the three stages (stage II < III < IV; ANOVA, $p < 0.001$; Tukey's HSD test, $p < 0.05$), except between sperm sacs of stage III and IV (Tukey's HSD test, $p > 0.05$) in *P. japonicum* and *C. elatius* (Figure 10, Table 4). The mean diameter of male gonads at stage IV was significantly different among species (ANOVA, $p < 0.001$), and male gonads of *C. konojoi* were significantly larger than the other two species (Tukey's HSD test, $p < 0.001$) (Figure 10, Table 4). Oocytes (stage IV) of *P. japonicum* were significantly larger in diameter than those of *C. elatius* (t-test, $p < 0.001$) (Figure 10, Table 4). Stage II of both male and female gonads could be distinguished by

their sizes in the three species. However, identification by size alone was difficult for stages III and IV in male colonies (Figure 10, Table 4).

To show the annual cycle of gonad diameter, monthly data was gathered each year, and average and standard deviations were calculated (Figure 11-16). The upper parts of Figures 11-16 show histograms depicting the number of gonads and their diameters in typical colonies.

In male colonies of *P. japonicum*, relatively larger sperm sacs occurred in May (194.6 μm on average), June (261.7 μm), August (235.7 μm) and December (162.3 μm). Smaller male gonads were recorded in September (29.5 μm), October (39.6 μm) and November (53.8 μm) (Figure 11; below). No data was available for this species in January and July. There were significant differences in the average diameter of sperm sacs between the largest-records (e.g. June) and the smallest-records (e.g. September) (ANOVA, $p < 0.001$). However, diameters did not significantly differ between February and April, June and August, and April and November (Tukey's HSD test, $p > 0.05$). Frequency distribution of gonad size in male colonies of *P. japonicum* showed one apparent peak (Figure 11; above). In female colonies of this species, the larger oocytes occurred in May (227.7 μm in average), June (220.7 μm), August (196.9 μm) and September (222.3 μm). Smaller oocytes were recorded in November (102.3 μm), December (111.0 μm) and January (114.7 μm) (Figure 12; below). There was no data for July. During the months when larger oocytes were recorded (May, June, August and September), the gonads were significantly larger than in the months when smaller oocytes were recorded (January, March, November and December) (ANOVA, $p < 0.001$, Tukey's HSD test, between June and March, August and March: $p < 0.05$; the others: $p < 0.001$). Frequency distributions of gonad size in female colonies of *P. japonicum* showed a very wide range of size during the summer season (May, June, August and September) with two peaks and various stages of oocytes in these summer months (Figure 12; above).

In male colonies of *C. elatius*, large sperm sacs were seen in May (250.3 μm on average) and June (242.9 μm). No data was available in July. Relatively smaller male gonads were recorded in September (36.1 μm), October (52.4 μm) and January (51.6 μm) (Figure 13; below). Male gonads recorded in May and June were significantly larger than the other months (ANOVA, $p < 0.001$, Tukey's HSD test, $p < 0.001$). Frequency of sperm-sac size in *C. elatius* showed an almost one peak distribution (Figure 13; above). In female colonies, larger oocytes occurred in May (229.3 μm in average), while smaller oocytes were recorded in August (116.59 μm) and September (112.7 μm). There was no data available in July and November (Figure 14; below). Oocytes recorded in May were significantly larger than those in the other months (ANOVA: $p < 0.001$, Tukey's HSD test, vs. February: $p < 0.01$; the others: $p < 0.001$). The frequency distributions of oocyte size in *C. elatius* showed very wide ranges of size in May, June, October and December with two peaks and various stages of oocytes (Figure 14; above).

In male colonies of *C. konojoi*, large sperm sacs occurred in June (270.0 μm on average) and August (287.2 μm). Smaller male gonads were recorded in January (53.2 μm), April (58.8 μm) and December (50.6 μm). There was no data collected for May, July, October and November (Figure 15; below). During the months when large sperm sacs (June and August) were recorded, the gonads were significantly larger than for the other months (ANOVA, $p < 0.001$, Tukey's HSD test, $p < 0.001$), but there was no significant difference between June and August (Tukey's HSD test, $p > 0.05$). The frequency distribution of gonad size in male colonies of *C. konojoi* was generally one peak (Figure 15; above). In female colonies, larger oocytes occurred in April (154.9 μm on average), May (158.5 μm) and December (168.8 μm). The smallest oocytes were recorded in September (76.3 μm). No data was collected in March, June, July, August, October and November (Figure 16; below). Gonads during months of large oocytes (April, May and December) were significantly larger than during months of smaller oocytes

(February and September) (ANOVA: $p < 0.001$, Tukey's HSD tests, between February and April, February and May, $p < 0.05$; between February and December, $p < 0.01$). Gonads from September were the smallest (Tukey's HSD test, $p < 0.001$). The frequency distribution of gonad sizes formed generally one peak (Figure 16; above).

Discussion

Reproductive Strategy and the Environment

Two basic types of sexual reproduction are known in octocorals: (1) broadcast spawning with subsequent fertilization and development taking place in the water column and (2) fertilization in or on the maternal colony and subsequent brooding of embryos internally or externally on the adult colony (Simpson 2009). Until now, reproduction in only about 150 species of octocoral has been reported: 49% broadcast spawners, 40% internal brooders, and 11% external brooders (Kahng *et al.* 2011).

All three species examined in this study are presumed to be broadcast spawners since no planula larvae were observed, either in their bodies or on their colony surfaces. Kishinouye (1904a) already noted that Japanese precious corals were gonochoristic. However, a few hermaphroditic colonies of *Paracorallium japonicum* and *Corallium elatius* were found in the present study. Being extremely rare, we conclude that they may all be gonochoristic broadcast spawners. The report of a low incidence ($< 1\%$) of hermaphrodites in gonochoric species (Kahng *et al.* 2001) supports our findings.

For the Mediterranean *C. rubrum*, all authors have described gonads in the "polyps," or autozooids (Lacaze-Duthier 1864, Vighi 1972, Santangelo *et al.* 2003, Tsounis *et al.* 2006). However, we show here that gonads are usually found developing within the siphonozooids in Pacific species of *Corallium*.

In almost all octocorals, gametogenesis reportedly occurs exclusively in autozooids (Simpson 2009); many dimorphic species (having both autozooids and siphonozooids) still have gonads only in autozooids. Autozooids are essentially “feeding polyps”, so not surprisingly, several authors have postulated that gonads ripening in the autozooids might affect food availability to the colonies (Simpson 2009). Babcock (1990) reported that mature oocytes in *Heliopora coerulea* almost completely obstructed the autozooids and therefore must limit or preclude polyp feeding when mature oocytes were present. In the Antarctic octocoral, *Thouarella variabilis*, a single internally brooded larva may occupy as much as 80% of the volume of the polyp cavity (Brito *et al.* 1997; see Simpson 2009) leaving little or no space for captured food and digestive processes. The temperate-water alcyonacean, *Alcyonium digitatum* exhibits a non-feeding period during the time in which the nearly-mature gonads are present (Hartnoll 1975; see Simpson 2009). These observations suggest that having gonads located in siphonozooids might be quite advantageous for species that depend on feeding from the water column for nutrition, especially in the deep-sea environment. Almost all dimorphic species living in shallow water (e.g. *Sarcophyton* spp., *Lobophytum* spp.) have a symbiotic relationship with zooxanthellae, and thus they can live autotrophically. Consequently, we postulate that gonads may occur mainly in autozooids in shallow-water species, and in siphonozooids in some deep-sea species. The species in the genus *Corallium* and *Anthomastus* with gonads located in their siphonozooids have been shown by molecular analysis to be in the same clade with *Corallium ducale* and *Anthomastus ritteli* (McFadden *et al.* 2006). Kahng *et al.* (2011) analyzed associations with some reproductive strategies (sexuality, sex ratio, mode of reproduction, etc.) and in the phylogenetic clade provided by McFadden *et al.* (2006). For octocorals, it therefore appears that reproductive strategies may be linked to environment in tandem with phylogenetic systems.

Seasonal cycle of gonad differentiation

Past studies of sexual reproduction in Coralliidae species (e.g. Tsounis *et al.* 2006, Waller and Baco 2007) they distinguished gonads only by size (mean diameters), whereas we estimated gonad differentiation and development not only by size but also by classification into four categories by microscopic observation. Both methods are recommended because, for example, the diameter of sperm sacs of stage III and IV were not significantly different in *P. japonicum* and *C. elatius* (Figure 10, Table 4). In our three species, mature colonies (stage IV) appeared during the summer season (Figure 7). Moreover, larger gonads appeared also in the summer season (Figures 11-16). However, we could not find any stage IV oocytes, nor were we able to collect female colonies in June, July and August in *C. konojoi* (Figure 16). It is possible that oocytes of this species were already mature and released during these months. Hence we conclude that summer is the main reproductive season among the three Japanese species in the Ryukyu Archipelago. This conclusion is supported by evidence from the two other species of Corallidae which have been reported: *C. rubrum* reproducing during the summer season in the Mediterranean (Vighi 1970, Tsounis *et al.* 2006), and *C. secundum* reproducing during June and July in Hawaii (Grigg 1976, Waller & Baco 2007).

In the Mediterranean precious coral, *C. rubrum*, the number of both sperm sacs and oocytes per polyp increased in summer (May to July), and was correlated with increase in gonad diameter (Tsounis *et al.* 2006). Brazeau and Lasker (1989) showed that larger oocytes were fewer per polyp in summer time, in a Caribbean gorgonian coral, *Plexaura* sp. but in our study the gonad number per polyp did not change with season (Figure 8). Also we found no significant differences among the three stages (II, III and IV) in gonad number per polyp in the three species (Table 3), except for males of *P. japonicum*. Therefore, for our species, there seems to be no relationship between the gonad number per polyp and gonadal development.

In these three species, differentiated stages and sizes of sperm sacs appeared to be almost equal in the each colony (upper parts of histograms, Figures 11, 13 and 15). Meanwhile, there was more variation in female gonads (Figures 12, 14 and 16), especially in colonies of *P. japonicum* and *C. elatius*. Sizes of their oocytes showed two or more peaks, and there were two or more stages (upper parts of histograms, Figures 12 and 14). There is a similar pattern reported in two cold-water species of octocorals, *Dasystenella acanthina* and *Thouarella* sp., which have two size-classes of oocytes (Orejas *et al.* 2007). That data suggests a two-year reproduction cycle for those two species. Thus, differentiation of oocytes in *P. japonicum* and *C. elatius* may also not be annual but have a two year cycle. The cohort of stage IV oocytes may be released one year, and the next cohort of immature (stage II or III) may be released the following year. This pattern has also been reported for *C. rubrum*, the male gametes developing to maturity within one year whereas oocytes mature over a two-year period (Vighi 1972). Meanwhile, the reproductive cycles of sperm sacs and oocytes were both reported to be annual in *C. secundum* (Grigg 1984), but since oocytes of varying sizes were found in *C. secundum* and *C. laauense*, it is possible that their reproduction might be quasi-continuous (Waller and Baco 2007).

Planula larvae were not observed in any samples examined in this study, which strongly suggests that these three species rely upon external fertilization. To increase fertilization success externally, synchronization would be necessary. However, it appears from our studies that male and female gonads were not in synchrony in 2007. For example, in *P. japonicum*, in September 2007, female gonads were mature but males were absent, and in December 2007, male gonads were mature although female gonads were stage II. In *C. elatius*, female gonads were differentiated but male gonads were immature in October and December 2007. In *C. konojoi*, a female colony had stage III oocytes in December 2007. Our data, though limited, suggests that the rates of fertilization might be comparatively low in the three species we studied.

There are only a few reports about deep-sea environmental factors affecting reproduction. Waller and Baco (2007) pointed out that reproduction of deep-sea organisms relates to available food. Six deep-sea organisms, including two octocoral species, *Gersemia fruticosa* and *Drifa* sp. have been reported as reproducing according to lunar rhythms (Mercier *et al.* 2011b). Reproduction by the deep-sea scleractinian coral, *Flabellum angulare* (Mercier *et al.* 2011a) and an octocoral, *Keratoisis ornata* (Mercier and Hamell 2011) apparently coincided with rising seawater temperature. However, for this study, there is almost no seasonal variation of water temperature in the sampling area (the Ryukyu Archipelago; 100-300 m deep) (Nonaka and Muzik 2009). There is so far no evidence of any other factors which might affect the periodical reproduction of the Japanese precious corals examined in the present study.

Mature gonads size and quantity

The mean diameter of mature oocytes was 362.1 μm in *P. japonicum* (stage IV), 281.1 μm in *C. elatius* (stage IV) and 191.2 μm in *C. konojoi* (stage III) (Table 4). In 119 species of octocorals reporting oocyte size, average size of mature oocytes was $686 \pm 253 \mu\text{m}$, (Kahng *et al.* 2011). Therefore, many octocoral species rear larger oocytes than found in the Japanese precious corals examined in this study. It has also been reported that zooxanthellate species had significantly larger oocytes than azooxanthellate (Kahng *et al.* 2011). For example, the diameter of oocytes in *Heliopora coerulea* was 920 μm (Babcock 1990), 900 μm in *Heteroxenia fuscescens* (Benayahu *et al.* 1989), and 600-825 μm in some coral reef “soft corals”, (Alino and Coll 1989). Cold-water species, however, have gonads more similar in size to those in this study. Antarctic “gorgonians” had small oocytes 183-394 μm (Orejas *et al.* 2002, 2007) and *Anthomastus ritteri* from Monterey Bay (300-450 m deep) had mature oocytes 376 μm in diameter (Cordes *et al.* 2001). It is

probable that fecundity is affected by both physical factors (e.g., water temperature) and biological (e.g., a symbiotic relationship with zooxanthellae).

Gonad production (fertility) can be estimated by counting numbers of gonads per polyp. The average number of both gonads (2.4 sperm sacs and 2.2 oocytes) in *C. konojoi* was significantly greater than in the other two species (Table 3). Tsounis *et al.* (2006) reported on the seasonal cycle of average gonad number per polyp in *C. rubrum*. According to that report, during the reproductive season the average number of sperm sacs was about 2.5, and that of oocytes was about 2. But there have been no other reports about quantity of gonads per polyp in any other species of Coralliidae. In other octocorals, gonad production differs with species. The so-called “soft corals” generally tend to have much higher polyp fecundity than “gorgonian-type” octocorals (Kahng *et al.* 2011). For example, the Antarctic gorgonian, *Dasystenella acanthine* and *Thouarella* sp. reportedly produced only 1.1 or 1.2 oocytes in each polyp on average (Orejas *et al.* 2007) while the Caribbean *Plexaura* sp. had an average of 1.9 oocytes per polyp (Brazeau and Lasker 1989). In contrast, “soft coral” species such as *Sarcophyton glaucum* reportedly grew as large colonies, and their autozooids exceeded 10 cm tall (Benayahu and Loya 1986). They could have enormous gonads containing 25-35 oocytes per 1 cm of autozoid (Benayahu and Loya 1986). Similarly a deep-sea Pennatulacean, *Anthoptilum murrayi*, with comparatively large polyps (approximately 5 mm long; from Figure 2 in Pires *et al.*, 2009) reportedly had up to 90 oocytes per polyp (Pires *et al.* 2009). We suggest that species possessing thicker coenenchyme or larger polyps tend to have more gonads per polyp than species with thinner coenenchyme or smaller polyps. The commercially-valuable species of Japanese Coralliidae have comparatively thin coenenchyme and minute siphonozooids (Nonaka *et al.* 2012b), thus they do not have space for the development of many gonads. The three species examined had relatively smaller and

fewer gonads than other octocorals so far reported, and therefore their reproductive fecundity may be comparatively lower.

Reproductive Maturity

There is no information on the size at reproductive maturity for Japanese precious corals, but the minimum size must be smaller than the smallest fertile specimens studied here (Table 2; Appendixes 1-9; *P. japonicum*; 145 mm high, *C. elatius*; 223 mm, *C. konojoi*; 78 mm). Supposing that these are the minimum reproductive sizes for each species, it is possible to estimate age at maturity. Linear growth rates of *P. japonicum* are reportedly $2.22 \pm 0.82 - 6.66 \pm 5.52$ mm yr⁻¹, *C. elatius*; 2.76 ± 2.09 mm yr⁻¹ and *C. konojoi*; 7.60 ± 0.75 mm yr⁻¹ (Luan *et al.* 2013). Based on this growth rate, age at maturity (the minimum size of colonies having mature gonads) for *P. japonicum* would be approximately 20-60 yrs, *C. elatius* would be 80 yrs, and *C. konojoi* would be roughly 10 yrs.

Santangelo *et al.* (2003) reported that the minimum age at first reproduction of a Mediterranean species, *C. rubrum*, was two years, reaching 100% fertility for colonies over five years old. Another report (Grigg 1976) suggested age at maturity for a Hawaiian species, *C. secundum*, to be 13 yrs. Comparing our data, it is possible *C. konojoi* becomes mature at 10 years, but slower-growing *P. japonicum* and *C. elatius* may be much older at maturity.

Sex ratio

Sex ratios of *P. japonicum* (male:female = 1:1.4) and *C. elatius* (male:female = 1:1.7) seemed biased, but they were not statistically different from 1:1. For these two species, differentiation of oocytes may not be annual but possibly biennial, having a two-year cycle. If true, that may explain why mature female colonies have observable oocytes all year-round. Meanwhile, the

average size of sex-unknown colonies (classified stage I) was not significantly smaller than the others (Table 2), and they appeared to be large enough to be mature. Therefore, it is quite possible that the “sex unknown” colonies reported here were in fact males, sampled just after spawning. Thus, the actual sex ratio (male + unknown : female) could be more correctly recalculated as 1:0.84 in *P. japonicum* and as 1:1.3 in *C. elatius*, closely matching the earlier reported ratios of 1:1.

A 1:1 sex ratio has been reported in some publications (e.g. Benayahu and Loya 1986; Kruger *et al.* 1998; Orejas *et al.* 2002; Ribes *et al.* 2007; Pires *et al.* 2009), but it was only reported in 26 of 56 cases (Kahng *et al.* 2011). Santangelo *et al.* (2003) reported that the sex ratio of the precious coral, *C. rubrum*, in the Mediterranean, was significantly biased towards females; male:female = 1:1.37. They concluded this bias was not natural, but caused by human overexploitation. Accepting this hypothesis, Tsounis *et al.* (2006) reported a sex ratio for this species of 1:1.

For conservation of precious corals, it is important to be able to identify the sex by field observation of external morphology, but there are so far no reports of external differences between sexes. Muzik (personal observation in Okinawan waters, at Kerama and Ishigaki) noted that tentacles of “mother” polyps of shallow-water *Heliopora coerulea* were extremely expanded when guarding their planulae during reproductive season, while adjacent polyps without larvae appeared normal. Outside the reproductive season, no external differences in polyp morphology have been observed, either in this species or any other, anywhere. No obvious external differences between polyps and colony morphology of males and females were found in the precious corals of this study either. Comparisons of average colony size, for example, showed no significant differences between sexes for all three species (Table 2; ANOVA, $P > 0.05$).

Conclusion and proposal for conservation of Japanese precious corals

Table 5 shows the information known to date about sexual reproduction in Coralliidae corals. All species in the Coralliidae reported so far are gonochoristic. The sex ratios of the Japanese three species are almost 1:1. Reproductive strategies are different between the Indo-Pacific and the Mediterranean species of Coralliidae; Pacific species are broadcast spawners, whereas the Mediterranean *C. rubrum* releases planulae. Gonads of the three Japanese species of Coralliidae so far reported are considerably smaller than those of not only other species in the Coralliidae, but also in all other Octocorallia reported. Number of gonads per polyp in the three species studied was also fewer than in others. This data suggests a relatively low fecundity for species of Coralliidae from southern Japan.

Gamete-releasing behavior appears to occur during the summer season, mostly from May to August. In general, to achieve conservation of fishery resources (e.g. fishes, lobsters, sea urchins, etc.), harvesting is prohibited during the reproductive season. However, with such slow-growing, long-lived animals of low fecundity, as these octocorals appear to be, this curtailment may not be applicable. Certainly, harvesting any colonies with mature gametes must be avoided. In order to conserve the species, it is imperative to have more and better information about their reproduction.

Size and age at maturity cannot be definitely determined from our data. However we have made rough estimates of age at maturity from the minimum size of colonies harboring gonads (Table 5). Iwasaki *et al.* (2012) reported that fishermen harvested specimens of *P. japonicum* from the northern Ryukyu Archipelago which he estimated to be 30-40 years old. Accordingly, it is possible that immature colonies of *P. japonicum* were harvested there. Tsounis *et al.* (2010) proposed to decide maximum size limits (maintaining reproductive colonies) and minimum size limits (protecting harvest resource for future) to harvest for their conservation. In

this study, although we do not have the information about “maximum size limits”, we can propose the minimum size limits of our samples as the minimum-safe size limits for harvesting: 145 mm high and 180 mm wide for *P. japonicum*, 223 mm and 263 mm for *C. elatius*, and 78 mm and 90 mm for *C. konojoi*.

The average density of colonies of *P. japonicum* is much lower (~0.5 colonies per 100 m²) than that of *C. rubrum* (Iwasaki *et al.* 2012). Moreover the three species studied here appear to have comparatively low fecundity (fewer and smaller gonads). Therefore, rates of recruitment may also be very low. Establishment of a 10-20 year moratorium area, as suggested by Iwasakai *et al* (2012), indeed has great merit. Meanwhile, many more studies of their biology, such as investigating the quantity of the resource and their reproductive and mortality rates, should be conducted in order to establish an effective conservation program.

Japanese researchers have long advocated for the conservation of precious corals, as early as the beginning of the 20th century (Kitahara 1904, Nagamune 1918 and Kuno 1922; reviewed in Nonaka and Muzik 2012a), but unfortunately, a century later, we have not yet succeeded in achieving their protection. It is imperative that suitable rules for conservation of Coralliidae corals be established and accepted, in order to protect them while maintaining a sustainable fishery.

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Appendix 1. List of samples examined in this study. *Paracorallium japonicum* (male colonies). N=29. Minimum sizes marked in gray.

Catalog Number	Sampling datum				Colony height (mm)	Colony width (mm)	Stage of gonads	gonads number per polyp	Diameter of gonads (µm)				Remarks
	Date	Area	Depth (m)	Temp. (C.)					N	Mean	S.D.	S.E.	
OCA-Cn20050606-005	6-Jun-2005	Okinoerabu Is.	250	16	202	230	III	2.50	12	272.94	54.33	15.68	
OCA-Cn20050606-006	6-Jun-2005	Iou Is.	125	15	393	390	III	1.73	15	275.91	44.27	11.43	
OCA-Cn20050618-016	18-Jun-2005	Iou Is.	130	17	390	275	II III	2.93	49	232.63	23.60	3.37	
OCA-Cn20050618-017	18-Jun-2005	Iou Is.	125	18	260	340	II III	1.59	22	309.63	44.80	9.55	
OCA-Cn20051128-015	28-Nov-2005	Tarama Is.	205	18	373	500	II	1.67	3	35.48	8.39	4.84	
OCA-Cn20051219-023	19-Dec-2005	Izena Is.	204	19	266	260	I II	2.33	15	43.91	4.14	1.07	
OCA-Cn20051220-033	20-Dec-2005	Izena Is.	201	20	228	370	II	1.50	2	59.82	1.32	0.93	
OCA-Cn20051220-035	20-Dec-2005	Izena Is.	206	19	161	300	II	3.00	3	53.43	2.88	1.66	
OCA-Cn20051220-036	20-Dec-2005	Izena Is.	213	19	236	410	II	2.33	6	67.16	9.15	3.74	
OCA-Cn20051220-037	20-Dec-2005	Izena Is.	208	19	190	365	II	1.00	2	38.15	20.09	14.20	
OCA-Cn20051226-023	26-Dec-2005	Tanega Is.	175	17	162	200	II	1.00	3	55.36	10.31	5.95	
OCA-Cn20060210-026	10-Feb-2006	Tanega Is.	195	16	320	265	II	1.67	13	82.42	10.59	2.94	
OCA-Cn20060212-033	12-Feb-2006	Tanega Is.	145	17	366	410	II	2.50	3	59.57	4.34	2.51	
OCA-Cn20060221-042	21-Feb-2006	Mage Is.	125	18	240	245	II III	3.24	40	122.13	18.90	2.99	
OCA-Cn20060222-045	22-Feb-2006	Mage Is.	135	18	239	320	II III	3.20	8	90.91	12.72	4.50	
OCA-Cn20071113-015	13-Nov-2007	Iou Is.	130	17	256	423	II	2.00	29	55.70	10.34	1.92	
OCA-Cn20071202-008	2-Dec-2007	Tarama Is.	203	19	309	640	I II	1.50	3	42.42	8.05	4.65	
OCA-Cn20071211-020	11-Dec-2007	Iou Is.	135	19	216	383	IV	1.58	62	209.96	36.58	4.65	
OCA-Cn20080226-014	26-Feb-2008	Yae-bise	220	20	250	680	II	1.31	21	72.85	9.41	2.05	2 oocytes present
OCA-Cn20080404-021	4-Apr-2008	Yae-bise	237	19	325	529	II	1.86	35	81.85	9.31	1.57	
OCA-Cn20080505-025	5-May-2008	Nakanougan Is.	213	19	307	453	II III	2.82	23	116.40	18.49	3.86	
OCA-Cn20080518-026	18-May-2008	Iou Is.	130	16	250	339	I IV	1.00	2	133.26	54.20	38.33	
OCA-Cn20080522-028	22-May-2008	Yae-bise	225	21	332	396	III	2.00	1	224.07	-	-	
OCA-Cn20080527-029	27-May-2008	Houzanzone	226	20	197	350	II III	2.51	76	171.29	25.96	2.98	2 gonads in 1 autozoid
OCA-Cn20080802-032	2-Aug-2008	Tanega Is.	153	18	391	537	II	2.00	2	43.77	8.91	6.30	
OCA-Cn20080816-034	16-Aug-2008	Kikai Is.	197	19	220	414	IV	1.42	35	246.68	42.40	7.17	
OCA-Cn20081106-040	6-Nov-2008	Kikai Is.	222	19	200	360	II	2.21	23	45.78	7.42	1.55	4 gonads in 2 autozooids
OCA-Cn20090206-045	6-Feb-2009	Kikai Is.	186	21	230	341	II	2.09	21	62.95	9.33	2.04	
OCA-Cn20090504-040	4-May-2009	Iou Is.	140	18	196	455	II III IV	1.96	79	240.91	41.77	4.70	

Appendix 2. List of samples examined in this study. *Paracorallium japonicum* (female colonies), N=41. Minimum sizes marked in gray.

Catalog Number	Sampling datum				Colony height (mm)	Colony width (mm)	Stage of gonads	gonads number per polyp	Diameter of gonads (µm)				Remarks
	Date	Area	Depth (m)	Temp. (C.)					N	Mean	S.D.	S.E.	
OCA-Cn20050604-001	4-Jun-2005	Okinoerabu Is.	250	16	202	230	IV	1.00	1	233.65	-	-	
OCA-Cn20050608-010	8-Jun-2005	Iou Is.	138	15	256	260	II III	1.00	4	213.39	90.88	45.44	
OCA-Cn20050612-011	12-Jun-2005	Kikai Is.	195	19	145	310	III	1.33	6	254.76	47.08	19.22	
OCA-Cn20050613-014	13-Jun-2005	Kikai Is.	212	19	162	245	II III	1.33	4	161.80	102.78	51.39	
OCA-Cn20050828-008	28-Aug-2005	Ishigaki Is.	228	17	319	490	II	1.14	8	79.27	22.51	7.96	
OCA-Cn20050829-009	29-Aug-2005	Hatoma Is.	219	17	208	350	II	1.17	7	107.30	17.73	6.70	
OCA-Cn20050920-010	20-Sep-2005	Izena Is.	202	20	176	320	II	1.00	4	95.80	8.17	4.08	
OCA-Cn20051128-014	28-Nov-2005	Tarama Is.	214	18	284	505	II	1.00	3	94.58	35.22	20.33	
OCA-Cn20051128-017	28-Nov-2005	Tarama Is.	232	18	363	360	II	1.00	6	113.54	27.50	11.23	
OCA-Cn20051219-019	19-Dec-2005	Izena Is.	204	18	257	350	II	1.25	4	118.84	43.02	21.51	
OCA-Cn20051219-024	19-Dec-2005	Izena Is.	203	20	319	440	II	1.38	11	128.59	31.68	9.55	
OCA-Cn20051219-025	19-Dec-2005	Izena Is.	200	19	322	500	II	1.40	6	128.00	39.31	16.05	
OCA-Cn20051219-029	19-Dec-2005	Izena Is.	205	19	150	395	II	1.50	3	124.78	12.88	7.44	
OCA-Cn20051220-032	20-Dec-2005	Izena Is.	209	20	304	470	II	1.00	1	138.27	-	-	
OCA-Cn20051220-034	20-Dec-2005	Izena Is.	206	20	216	375	II	1.40	7	130.23	23.32	8.81	
OCA-Cn20051220-038	20-Dec-2005	Izena Is.	207	19	150	355	II	1.00	3	133.96	27.48	15.87	
OCA-Cn20051220-039	20-Dec-2005	Izena Is.	214	19	300	630	II	1.08	13	124.55	13.48	3.74	
OCA-Cn20051220-040	20-Dec-2005	Izena Is.	214	20	182	280	II	1.00	1	98.94	-	-	
OCA-Cn20060109-042	9-Jan-2006	Izena Is.	194	21	313	505	II	1.00	2	152.97	6.57	4.65	
OCA-Cn20060109-043	9-Jan-2006	Izena Is.	197	20	277	403	II III	1.50	3	127.42	75.15	43.39	
OCA-Cn20060110-045	10-Jan-2006	Izena Is.	193	20	239	520	II	1.00	8	100.97	25.35	8.96	
OCA-Cn20060222-044	22-Feb-2006	Mage Is.	130	18	171	180	II III	1.22	11	178.98	23.62	7.12	2 gonads in an autozooid
OCA-Cn20070902-003	2-Sep-2007	Nakanougan Is.	219	18	162.5	250	II	1.00	2	99.79	2.66	1.88	
OCA-Cn20070908-007	8-Sep-2007	Kikai Is.	195	22	314	538	II IV	1.45	24	253.54	150.40	30.70	
OCA-Cn20071210-009	10-Dec-2007	Iriomote Is.	251	20	296	440	II	1.13	17	78.20	24.98	6.06	
OCA-Cn20080106-022	6-Jan-2008	Take Is.	130	18	216	323	II	1.50	8	135.05	22.99	8.13	
OCA-Cn20080109-013	9-Jan-2008	Izena Is.	230	18	218	434	II	1.67	3	48.89	6.08	3.51	
OCA-Cn20080226-014	26-Feb-2008	Yaebise	220	20	250	680	III	2.00	2	162.32	25.33	17.91	male colony
OCA-Cn20080307-016	7-Mar-2008	Tarama Is.	210	20	372	390	II III IV	1.46	15	158.81	55.19	14.25	
OCA-Cn20080329-020	29-Mar-2008	Hatoma Is.	219	19	342	503	II	1.42	14	113.38	31.08	8.31	
OCA-Cn20080405-022	5-Apr-2008	Houzanzone	228	20	186	344	II III	1.25	26	131.91	44.41	8.71	
OCA-Cn20080414-024	14-Apr-2008	Nakanougan Is.	232	18	165	250	II III	1.14	8	149.79	54.89	19.41	
OCA-Cn20080521-027	21-May-2008	Iou Is.	145	16	248	320	II III	1.67	13	148.41	74.20	20.58	
OCA-Cn20080522-027	22-May-2008	Yaebise	216	21	318	636	II III IV	1.42	26	266.69	70.91	13.91	
OCA-Cn20080816-033	16-Aug-2008	Kikai Is.	204	19	225	332	II III IV	1.44	13	268.63	133.68	37.07	
OCA-Cn20081106-039	6-Nov-2008	Kikai Is.	221	19	220	307	II	1.00	8	96.80	25.60	9.05	1 gonad in an autozooid
OCA-Cn20081213-041	13-Dec-2008	Kikai Is.	174	21	235	367	II III	1.72	31	109.51	36.69	6.59	
OCA-Cn20090117-043	17-Jan-2009	Kikai Is.	180	21	400	354	II III	1.36	40	115.42	35.91	5.68	
OCA-Cn20090420-036	20-Apr-2009	Iou Is.	135	18	255	280	II III	1.19	20	183.29	77.53	17.34	1 gonad in an autozooid
OCA-Cn20090504-039	4-May-2009	Iou Is.	140	18	292	452	II III IV	1.25	9	229.45	101.95	33.98	
OCA-Cn20090810-048	10-Aug-2009	Iou Is.	125	19	195	437	II III IV	1.23	14	242.41	131.76	35.21	2 gonads in 2 autozooids

Appendix 3. List of samples examined in this study. *Paracorallium japonicum* (sex unknown). N=20. Minimum sizes marked in gray.

Catalog Number	Sampling datum				Colony height (mm)	Colony width (mm)	Stage of gonads	gonads number per polyp	Diameter of gonads (μm)				Remarks
	Date	Area	Depth (m)	Temp. (C.)					N	Mean	S.D.	S.E.	
OCA-Cn20050818-003	18-Aug-2005	Yaebise	239	19	296	355	I	-	0	-	-	-	
OCA-Cn20051128-016	28-Nov-2005	Tarama Is.	217	18	296	430	I	-	0	-	-	-	
OCA-Cn20051219-020	19-Dec-2005	Izena Is.	204	18	221	340	I	2.00	2	57.39	7.62	5.39	
OCA-Cn20051219-022	19-Dec-2005	Izena Is.	201	19	279	400	I	-	0	-	-	-	
OCA-Cn20051219-026	19-Dec-2005	Izena Is.	203	19	274	350	I	-	0	-	-	-	
OCA-Cn20051219-027	19-Dec-2005	Izena Is.	206	19	200	320	I	-	0	-	-	-	
OCA-Cn20051219-028	19-Dec-2005	Izena Is.	206	19	211	390	I	-	0	-	-	-	
OCA-Cn20051219-030	19-Dec-2005	Izena Is.	205	19	176	325	I	1.00	1	31.20	-	-	
OCA-Cn20051220-031	20-Dec-2005	Izena Is.	206	20	234	485	I	1.00	1	82.88	-	-	
OCA-Cn20060111-048	11-Jan-2006	Okinawa Is.	215	19	205	263	I	-	0	-	-	-	
OCA-Cn20060211-031	11-Feb-2006	Tanega Is.	150	17	300	350	I	1.00	1	44.89	-	-	
OCA-Cn20060214-039	14-Feb-2006	Tanega Is.	165	16	250	380	I	-	0	-	-	-	
OCA-Cn20070816-002	16-Aug-2007	Ishigaki Is.	249	19	147	336	I	-	0	-	-	-	
OCA-Cn20070904-005	4-Sep-2007	Take Is.	145	17	260	410	I	-	0	-	-	-	
OCA-Cn20070912-005	12-Sep-2007	Ishigaki Is.	228	18	205	400	I	1.00	1	29.53	-	-	
OCA-Cn20071108-014	8-Nov-2007	Yuse	120	20	315	315	I	-	0	-	-	-	
OCA-Cn20080316-018	16-Mar-2008	Nakanougan Is.	203	19	418	240	I	1.00	1	64.05	-	-	
OCA-Cn20080730-030	30-Jul-2008	Take Is.	147	18	230	316	I	-	0	-	-	-	
OCA-Cn20081020-035	20-Oct-2008	Take Is.	141	19	186	263	I	1.00	2	39.61	6.94	4.91	
OCA-Cn20081101-038	1-Nov-2008	Kikai Is.	205	19	227	300	I	1.00	1	80.43	-	-	

Appendix 4. List of samples examined in this study. *Corallium elatius* (male colonies). N=18. Minimum sizes marked in gray.

Catalog Number	Sampling datum				Colony height (mm)	Colony width (mm)	Stage of gonads	gonads number per polyp	Diameter of gonads (µm)				Remarks
	Date	Area	Depth (m)	Temp. (C.)					N	Mean	S.D.	S.E.	
OCA-Cn20050604-003	4-Jun-2005	Okinoerabu Is.	270	16	453	860	II III	1.47	20	200.40	27.43	6.13	2 gonads in 2 autozooids
OCA-Cn20050606-007	6-Jun-2005	Iou Is.	140	14	295	420	II III	1.23	28	260.17	37.60	7.11	2 gonads in 1 autozooid
OCA-Cn20050620-021	20-Jun-2005	Iou Is.	160	17	472	510	II III	2.78	34	248.09	29.74	5.10	
OCA-Cn20050620-021	20-Jun-2005	Iou Is.	160	17	472	510	II III	2.35	81	251.01	30.47	3.39	
OCA-Cn20051126-011	26-Nov-2005	Ishigaki Is.	290	16	484	530	II III	2.25	10	75.09	8.50	2.69	
OCA-Cn20060210-025	10-Feb-2006	Tanega Is.	190	16	269	455	II III	1.13	9	90.40	22.80	7.60	
OCA-Cn20060214-041	14-Feb-2006	Tanega Is.	220	17	343	770	II III	2.20	16	69.86	6.31	1.58	
OCA-Cn20060225-046	25-Feb-2006	Iou Is.	155	18	295	460	II III	1.81	21	154.32	24.37	5.32	
OCA-Cn20070623-a	23-Jun-2007	Ishigaki Is.	319	16	880	1220	III IV	1.68	51	234.26	46.84	6.56	
OCA-Cn20071029-013	29-Oct-2007	Tokuno Is.	285	17	473	515	I II III	2.30	23	52.43	7.39	1.54	
OCA-Cn20071202-007	2-Dec-2007	Tarama Is.	216	19	478	1000	II III	1.87	28	63.94	12.29	2.32	
OCA-Cn20080108-012	8-Jan-2008	Okinawa Is.	303	17	358	675	II	1.25	5	51.59	6.84	3.06	
OCA-Cn20080325-019	25-Mar-2008	Hateruma Is.	297	17	446	900	II	1.71	9	87.65	11.31	3.77	
OCA-Cn20080518-025	18-May-2008	Iou Is.	130	16	223	263	II III	1.57	33	244.44	37.60	6.55	
OCA-Cn20090424-037	24-Apr-2009	Iou Is.	150	17	569	1123	II III	1.48	29	146.76	46.62	8.66	2 gonads in 2 autozooids. 1 oocyte present.
OCA-Cn20090503-048	3-May-2009	Ikema Is.	326	17	588	723	II III	1.35	22	186.06	70.15	14.96	
OCA-Cn20090509-044	9-May-2009	Yaku Is.	210	15	340	545	II III	1.28	32	259.51	57.44	10.15	1 gonad in an autozooid
OCA-Cn20090509-050	9-May-2009	Ishigaki Is.	270	17	495	575	II III	1.19	34	288.97	68.74	11.79	1 gonad in an autozooid

Appendix 5. List of samples examined in this study. *Corallium elatius* (female colonies). N=31. Minimum sizes marked in gray.

Catalog Number	Sampling datum				Colony height (mm)	Colony width (mm)	Stage of gonads	gonads number per polyp	Diameter of gonads (µm)				Remarks
	Date	Area	Depth (m)	Temp. (C.)					N	Mean	S.D.	S.E.	
OCA-Cn20050606-008	6-Jun-2005	Iou Is.	140	14	310	455	II	1.17	6	126.24	18.12	7.40	
OCA-Cn20050620-022	20-Jun-2005	Iou Is.	145	16	277	370	IV	1.50	6	216.02	33.65	13.74	
OCA-Cn20050821-005	21-Aug-2005	Tarama Is.	282	17	650	1143	II	1.60	6	129.43	15.72	6.42	
OCA-Cn20050828-007	28-Aug-2005	Ishigaki Is.	272	16	473	540	II	1.58	17	125.41	24.22	5.87	
OCA-Cn20060109-044	9-Jan-2006	Izena Is.	195	21	278	900	II III	1.40	6	123.65	30.25	12.35	
OCA-Cn20060115-049	15-Jan-2006	Ishigaki Is.	307	15	468	570	II	1.50	4	71.25	15.22	7.61	
OCA-Cn20060119-050	19-Jan-2006	Tarama Is.	278	17	476	680	II III	2.40	19	131.14	39.92	9.16	
OCA-Cn20060120-051	20-Jan-2006	Tarama Is.	277	14	441	555	II III	1.40	6	117.93	25.01	10.21	1 gonad in an autozoid
OCA-Cn20060210-029	10-Feb-2006	Tanega Is.	200	16	493	500	II III	1.83	16	175.77	28.48	7.12	
OCA-Cn20060213-038	13-Feb-2006	Tanega Is.	220	17	290	490	II	1.40	5	151.69	23.61	10.56	
OCA-Cn20070830-003	30-Aug-2007	Amami Is.	295	18	468	543	II	1.37	24	93.27	21.61	4.41	
OCA-Cn20070921-008	21-Sep-2007	Iou Is.	150	18	348	376	II	1.60	8	96.22	12.05	4.26	
OCA-Cn20070923-009	23-Sep-2007	Take Is.	140	17	320	617	II	1.64	17	108.57	21.38	5.19	
OCA-Cn20071022-010	22-Oct-2007	Tanega Is.	205	17	270	420	II III IV	2.18	40	191.88	131.90	20.85	
OCA-Cn20071022-011	22-Oct-2007	Tanega Is.	205	17	382	482	II III	1.17	7	104.42	33.85	12.79	
OCA-Cn20071205-018	5-Dec-2007	Tanega Is.	175	19	295	490	II III IV	2.02	72	180.14	85.96	10.13	
OCA-Cn20080108-023	8-Jan-2008	Yaku Is.	205	15	660	1570	II III	2.14	34	132.96	44.97	7.71	1 gonad in an autozoid
OCA-Cn20080309-C	9-Mar-2008	Hateruma Is.	298	18	353	269	II	1.33	11	139.10	37.81	11.40	
OCA-Cn20080316-017	16-Mar-2008	Nakanougan Is.	207	19	420	1110	II III	2.06	20	152.65	35.15	7.86	
OCA-Cn20080522-026	22-May-2008	Ishigaki Is.	248	20	590	906	II III IV	2.19	54	267.89	64.10	8.72	
OCA-Cn20080915-031	15-Sep-2008	Take Is.	135	16	300	550	II	2.26	58	116.21	27.89	3.66	
OCA-Cn20081021-037	21-Oct-2008	Take Is.	139	19	271	446	II	1.20	6	86.81	5.97	2.44	
OCA-Cn20090410-046	10-Apr-2009	Ishigaki Is.	293	18	716	1010	II III	1.67	4	150.30	84.09	42.05	
OCA-Cn20090417-034	17-Apr-2009	Take Is.	135	18	253	464	II	1.00	2	76.88	34.25	24.22	
OCA-Cn20090420-035	20-Apr-2009	Iou Is.	135	18	342	463	II III	1.54	48	138.48	55.28	7.98	
OCA-Cn20090424-037	24-Apr-2009	Iou Is.	150	17	569	1123	II	1.00	1	101.73	-	-	male colony
OCA-Cn20090509-045	9-May-2009	Yaku Is.	187	18	420	533	II III	1.58	18	113.66	72.27	17.04	
OCA-Cn20090626-046	26-Jun-2009	Yaku Is.	190	14	400	660	II III IV	1.35	23	139.62	118.50	24.71	
OCA-Cn20090817-049	17-Aug-2009	Yaku Is.	200	15	328	728	II	2.74	99	122.27	32.98	3.31	
OCA-Cn20090831-050	31-Aug-2009	Tokuno Is.	260	18	410	525	II	1.00	1	59.27	-	-	
OCA-Cn20090831-051	31-Aug-2009	Tokuno Is.	260	18	607	1007	II	1.00	7	96.90	11.46	4.33	

Appendix 6. List of samples examined in this study. *Corallium elatius* (sex unknown). N=6. Minimum sizes marked in gray.

Catalog Number	Sampling datum				Colony height (mm)	Colony width (mm)	Stage of gonads	gonads number per polyp	Diameter of gonads (μm)				Remarks
	Date	Area	Depth (m)	Temp. (C.)					N	Mean	S.D.	S.E.	
OCA-Cn20050815-002	15-Aug-2005	Kita Hocho-zone	312	17	530	670	I	1.00	1	121.41	-	-	
OCA-Cn20070705-b	5-Jul-2007	Ishigaki Is.	317	15	623	753	I	-	-	-	-	-	
OCA-Cn20070816-001	16-Aug-2007	Ishigaki Is.	249	19	681	790	I	1.00	2	43.48	6.11	4.32	
OCA-Cn20070822-001	22-Aug-2007	Yaku Is.	170	17	714	650	I	1.00	2	51.89	10.43	7.38	
OCA-Cn20070905-004	5-Sep-2007	Hateruma Is.	296	16	1412	860	I	1.00	1	36.55	-	-	
OCA-Cn20080413-023	13-Apr-2008	Nakano-ugan Is.	212	18	305	475	I	-	-	-	-	-	

Appendix 7. List of samples examined in this study. *Corallium konojoi* (male colonies). N=11. Minimum sizes marked in gray.

Catalog Number	Sampling datum				Colony height (mm)	Colony width (mm)	Stage of gonads	gonads number per polyp	Diameter of gonads (μm)				Remarks
	Date	Area	Depth (m)	Temp. (C.)					N	Mean	S.D.	S.E.	
OCA-Cn20050604-004	4-Jun-2005	Okinoerabu Is.	255	16	422	510	II III IV	2.33	29	270.01	39.03	7.25	
OCA-Cn20060227-047	27-Feb-2006	Iou Is.	130	18	330	510	II	2.90	25	70.45	12.99	2.60	
OCA-Cn20060301-050	1-Mar-2006	Kuchinoerabu Is.	145	17	311	405	II III	2.58	23	88.20	13.80	2.88	
OCA-Cn20070906-006	6-Sep-2007	Kakeroma Is.	250	20	323	317	II III	4.00	7	80.77	61.25	23.15	
OCA-Cn20071209-019	9-Dec-2007	Iou Is.	135	19	300	486	II	5.00	5	50.55	6.98	3.12	
OCA-Cn20080307-015	7-Mar-2008	Tarama Is.	214	20	281	545	II III	2.06	26	77.49	12.54	2.46	
OCA-Cn20080801-031	1-Aug-2008	Tanega Is.	164	15	465	365	II III IV	3.02	129	303.83	59.90	5.27	
OCA-Cn20090119-044	19-Jan-2009	Kikai Is.	200	21	78	90	II	5.00	5	53.21	4.45	1.99	
OCA-Cn20090428-038	28-Apr-2009	Take Is.	135	17	270	512	II	2.00	11	58.85	9.92	2.99	
OCA-Cn20090810-047	10-Aug-2009	Iou Is.	120	19	312	468	II III IV	1.91	85	261.95	56.19	6.09	
OCA-Cn20090901-052	1-Sep-2009	Kakeroma Is.	225	18	372	481	II	1.00	5	57.66	9.14	4.09	

Appendix 8. List of samples examined in this study. *Corallium konojoi* (female colonies). N=11. Minimum sizes marked in gray.

Catalog Number	Sampling datum				Colony height (mm)	Colony width (mm)	Stage of gonads	gonads number per polyp	Diameter of gonads (μm)				Remarks
	Date	Area	Depth (m)	Temp. (°C)					N	Mean	S.D.	S.E.	
OCA-Cn20060111-047	11-Jan-2006	Okinawa Is.	221	19	303	240	II	2.00	2	106.35	26.35	18.63	
OCA-Cn20060221-043	21-Feb-2006	Mage Is.	120	18	225	520	II	1.00	2	162.46	12.24	8.65	
OCA-Cn20060227-048	27-Feb-2006	Iou Is.	135	16	240	470	II	3.50	8	93.55	14.77	5.22	
OCA-Cn20071218-021	18-Dec-2007	Iou Is.	115	20	320	460	II III	2.89	47	168.76	26.39	3.85	
OCA-Cn20080521-028	21-May-2008	Iou Is.	150	16	377	433	III	2.12	59	236.04	54.28	7.07	
OCA-Cn20080911-030	11-Sep-2008	Take Is.	150	16	246	410	II	1.88	14	76.35	16.27	4.35	
OCA-Cn20090410-032	10-Apr-2009	Take Is.	115	17	250	470	II III	2.14	177	140.60	42.94	3.23	
OCA-Cn20090410-033	10-Apr-2009	Take Is.	115	17	350	477	II III	2.28	128	174.79	60.16	5.32	
OCA-Cn20090505-041	5-May-2009	Iou Is.	120	18	175	310	II III	2.42	47	147.05	45.60	6.65	
OCA-Cn20090507-042	7-May-2009	Take Is.	125	19	272	609	II III	1.68	60	134.10	34.27	4.42	
OCA-Cn20090507-043	7-May-2009	Take Is.	115	19	320	340	II III	2.13	207	147.85	50.81	3.53	4 gonads in 1 autozoid

Appendix 9. List of samples examined in this study. *Corallium konojoi* (sex unknown). N=5. Minimum sizes marked in gray.

Catalog Number	Sampling datum				Colony height (mm)	Colony width (mm)	Stage of gonads	gonads number per polyp	Diameter of gonads (μm)				Remarks
	Date	Area	Depth (m)	Temp. (C.)					N	Mean	S.D.	S.E.	
OCA-Cn20050618-018	18-Jun-2005	Iou Is.	120	17	246	235	I	-	-	-	-	-	
OCA-Cn20051226-024	26-Dec-2005	Tanega Is.	160	17	300	280	I	-	-	-	-	-	
OCA-Cn20071106-006	6-Nov-2007	Tanega Is.	235	18	170	287	I	-	-	-	-	-	
OCA-Cn20080205-024	5-Feb-2008	Take Is.	120	17	315	430	I	-	-	-	-	-	
OCA-Cn20081020-036	20-Oct-2008	Take Is.	131	18	333	500	I	-	-	-	-	-	

TABLE 1

Occurrence month of each sex (male, female and sex unknown) in the samples examined.

sampling month		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	N (%)
<i>P. japonicum</i>	male	-	6	-	1	5	4	-	2	-	-	3	8	29 (32%)
	female	6	2	2	3	3	4	-	4	3	-	3	11	41 (46%)
	unknown	1	2	1	-	-	-	1	2	2	1	3	7	20 (22%)
	total	7	10	3	4	8	8	1	8	5	1	9	26	90
<i>C. elatius</i>	male	1	3	1	1	4	5	-	-	-	1	1	1	18 (33%)
	female	5	2	2	4	2	3	-	6	3	3	-	1	31 (56%)
	unknown	-	-	-	1	-	-	1	3	1	-	-	-	6 (11%)
	total	6	5	3	6	6	8	1	9	4	4	1	2	55
<i>C. konojoi</i>	male	1	1	2	1	-	1	-	2	2	-	-	1	11 (41%)
	female	1	2	-	2	4	-	-	-	1	-	-	1	11 (41%)
	unknown	-	1	-	-	-	1	-	-	-	1	1	1	5 (18%)
	total	2	4	2	3	4	2	0	2	3	1	1	3	27

N (%) = number and percentage of each sex

TABLE 2

Colony size (mm) of each sex (male, female and sex-unknown) in the samples examined.

Species	sex	colony height			colony width		
		max	min	average \pm S.D.	max	min	average \pm S.D.
<i>P. japonicum</i>	male	393	161	265.7 \pm 70	680	200	385.5 \pm 114
	female	400	145	249.4 \pm 68	680	180	392.9 \pm 115
	unknown	418	147	246.5 \pm 61	485	240	348.4 \pm 61
<i>C. elatius</i>	male	880	223	440.7 \pm 151	1220	263	669.7 \pm 261
	female	716	253	415.4 \pm 130	1570	269	661.1 \pm 294
	unknown	1412	305	*710.8 \pm 374	860	475	699.7 \pm 135
<i>C. konojoi</i>	male	465	78	314.9 \pm 99	545	90	426.3 \pm 132
	female	377	175	279.8 \pm 60	609	240	430.8 \pm 103
	unknown	333	170	272.8 \pm 66	500	235	346.4 \pm 113

*significantly the largest for each sex (male, female, unknown); $p < 0.05$

TABLE 3Average gonad number per polyp \pm S.E. at each stage for each species

Species	sex	stage II	stage III	stage IV	total
<i>P. japonicum</i>	male	1.95 \pm 0.09	*2.49 \pm 0.09	1.50 \pm 0.09	2.09 \pm 0.06
	female	1.29 \pm 0.04	1.34 \pm 0.05	1.24 \pm 0.10	1.30 \pm 0.03
<i>C. elatius</i>	male	1.58 \pm 0.09	1.84 \pm 0.07	1.68 \pm 0.15	1.75 \pm 0.05
	female	1.88 \pm 0.07	1.94 \pm 0.09	2.11 \pm 0.27	1.91 \pm 0.05
<i>C. konojoi</i>	male	2.33 \pm 0.21	2.23 \pm 0.23	2.57 \pm 0.15	**2.44 \pm 0.11
	female	2.25 \pm 0.10	2.12 \pm 0.06	-	**2.16 \pm 0.05

*significantly the most among the three stages; $p < 0.05$ **significantly the most among the three species; $p < 0.001$

TABLE 4Average diameter \pm S.E. (μm) of gonads at each stage for each species

Species	sex	stage II	stage III	stage IV
<i>P. japonicum</i>	male	*102.2 \pm 4.2	213.7 \pm 4.2	222.2 \pm 4.4
	female	*105.5 \pm 1.9	220.6 \pm 6.3	**#362.1 \pm 10.4
<i>C. elatius</i>	male	*119.6 \pm 5.8	237.4 \pm 3.9	235.0 \pm 6.7
	female	*109.1 \pm 1.4	229.7 \pm 4.9	**281.1 \pm 12.5
<i>C. konojoi</i>	male	*83.8 \pm 5.0	244.8 \pm 9.2	**#291.6 \pm 4.9
	female	*112.8 \pm 1.7	191.2 \pm 2.2	-

*significantly the largest among the three stages; $p < 0.001$ **significantly the largest among the three stages; $p < 0.001$ #significantly the largest among the three species; $p < 0.001$

TABLE 5

Past and present data of reproductive biology in the Coralliidae corals.

Species		<i>C. rubrum</i>	<i>C. reginae</i>	<i>C. secundum</i>	<i>C. laauense</i>	<i>C. elatius</i>	<i>C. konojoi</i>	<i>P. japonicum</i>
Sex		gonochoristic	gonochoristic	gonochoristic	gonochoristic ?	gonochoristic	gonochoristic	gonochoristic
Sex ratio (male : female)		1 : 1	-	-	-	1 : 1	1 : 1	1 : 1
Reproductive type		brooder	-	spawner	spawner	spawner	spawner	spawner
Reproductive season		May-August	-	June-July	Fall ?	May-June	June-August	May-August
Fertile polyp		autozooid	siphonozooid	siphonozooid	siphonozooid	siphonozooid	siphonozooid	siphonozooid
Diameter of mature gonads	sperm sac	460 µm	50 µm ?	410 µm	-	235 µm	292 µm	222 µm
	oocyte	520 µm	-	600 µm	660 µm	281 µm	191 µm (not mature)	362 µm
Mean number of gonads per polyp	sperm sac	2.5	-	-	-	1.8	2.4	2.1
	oocyte	2	-	-	-	1.9	2.2	1.3
Differentiation cycle of gonad	spermsac	1 yr	-	1 yr	-	1 yr	1 yr	1 yr
	oocyte	2 yrs	-	1 yr	-	2 yrs?	1 yr	2 yrs?
Maturity	height	0.2-2 cm	-	12 cm	-	22.3cm?	7.8cm?	14.5cm?
	age	2-5 yrs	-	13 yrs	-	80 yrs?	10 yrs?	20-60 yrs?
Growth rate	length	2-20 mm yr ⁻¹	-	9 mm yr ⁻¹	-	2.76 mm yr ⁻¹	7.60 mm yr ⁻¹	2.2-6.7 mm yr ⁻¹
	diameter	0.24-1.32 mm yr ⁻¹	-	-	-	0.30 mm yr ⁻¹	0.44 mm yr ⁻¹	0.20-0.27 mm yr ⁻¹
Localities		Mediterranean	Indonesia	Hawaii	Hawaii	Japan	Japan	Japan
Reference		Lacaze-Duthiers 1864; Vighi 1970; Santangelo <i>et al.</i> 2003; Marchal <i>et al.</i> 2004; Tsounis <i>et al.</i> 2006	Hickson 1907	Grigg 1976; Waller and Baco 2007	Waller and Baco 2007	Kishinouye 1904; Luan <i>et al.</i> 2013; present study	Kishinouye 1904; Luan <i>et al.</i> 2013; present study	Kishinouye 1904; Luan <i>et al.</i> 2013; present study

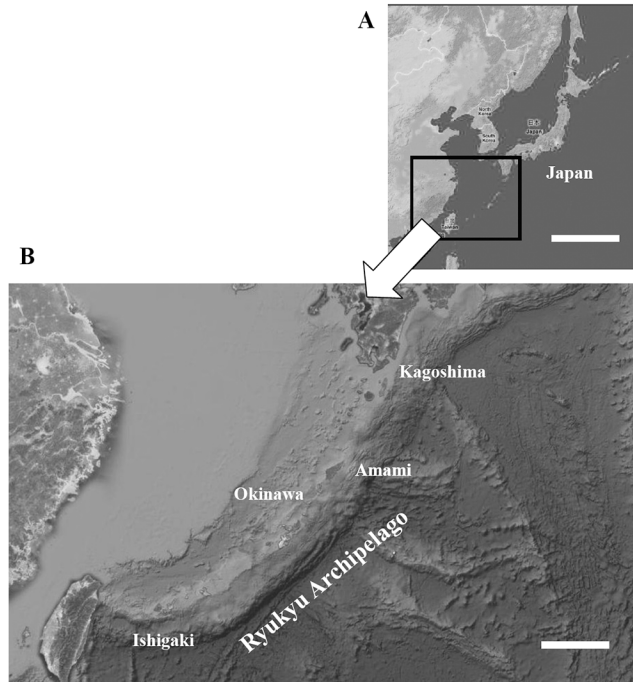


Figure 1. Map of the sampling areas in 2005 and 2009. A: map of Japan. B: magnified map of the black square in A. Scale bars = 1,000 km (A), 200 km (B).

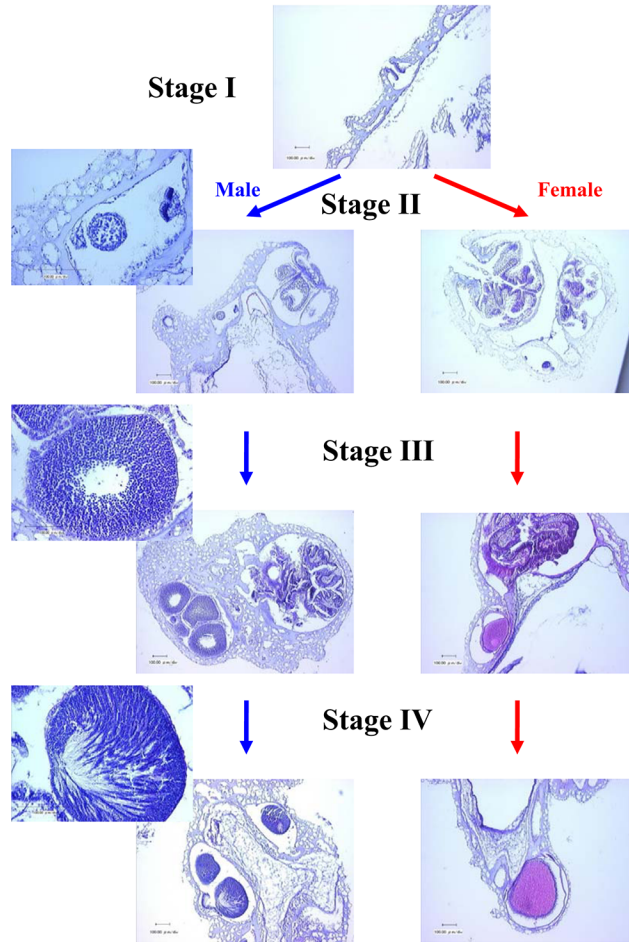


Figure 2. Differentiation stages of gonads of *Paracorallium japonicum*. Magnified images of sperm sac at Stage II (A), Stage III (B) and Stage IV (C). Scale bars are 100 μm (A, B, C), and 200 μm (all others).

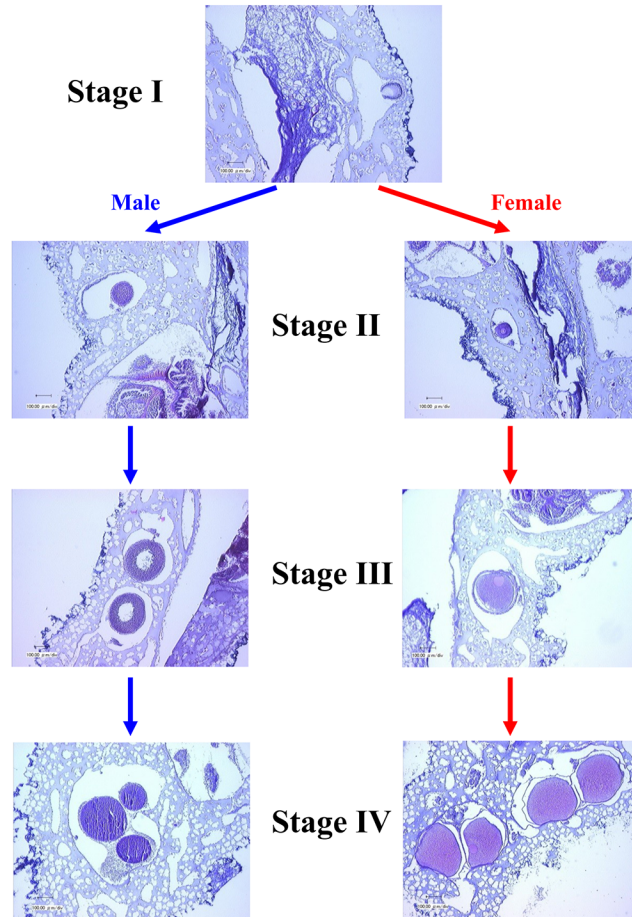


Figure 3. Differentiation stages in gonads of *Corallium elatius*. Scale bars are 200 μm.

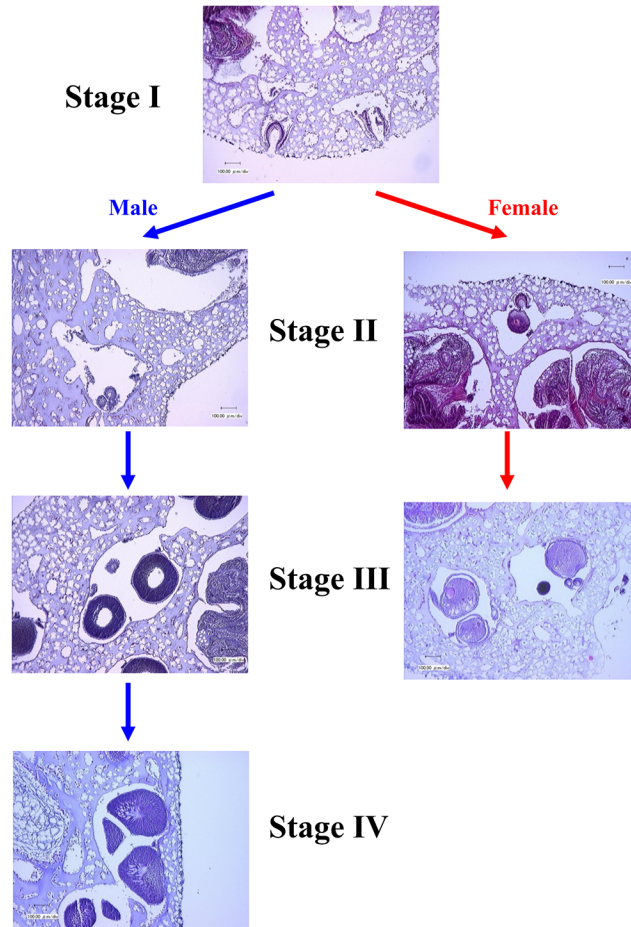


Figure 4. Differentiation stages in gonads of *Corallium konojoi*. Scale bars are 200 μm.

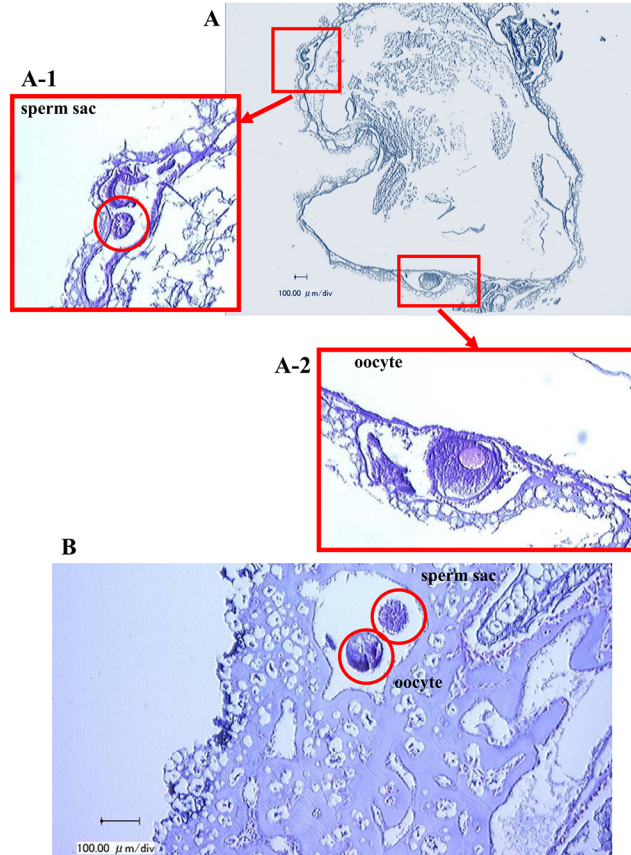


Figure 5. Hermaphroditic colonies. A) Thin section of branch of *Paracorallium japonicum*, enlarged to show a sperm sac (A-1) and an oocyte (A-2). B: Thin section of one siphonozooid of *Corallium elatius*, with both sperm sac and oocyte.

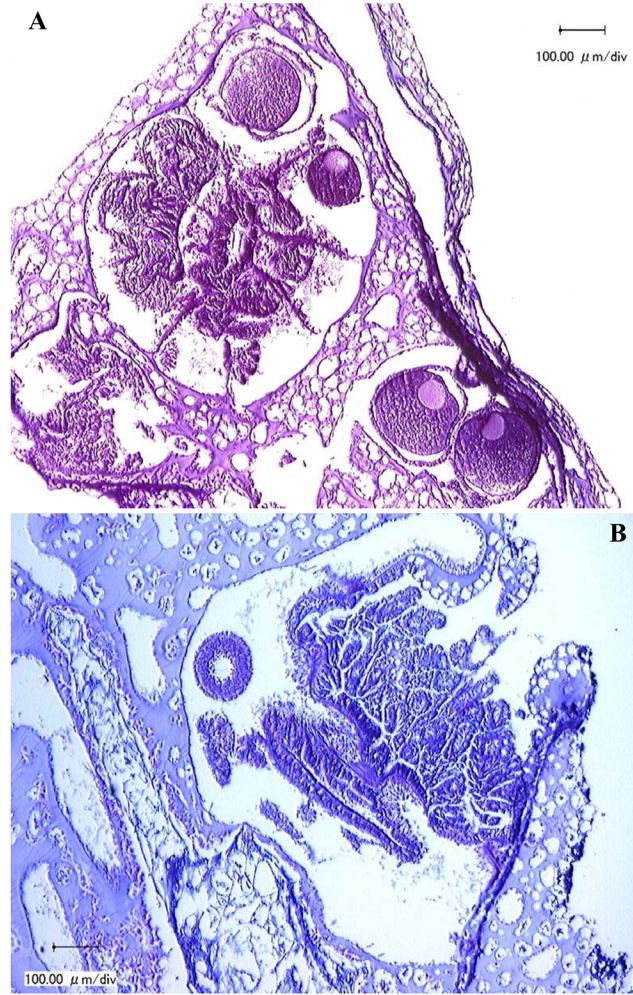


Figure 6. Gonads in autozooids. A: *Paracorallium japonicum*. B: *Corallium elatius*.

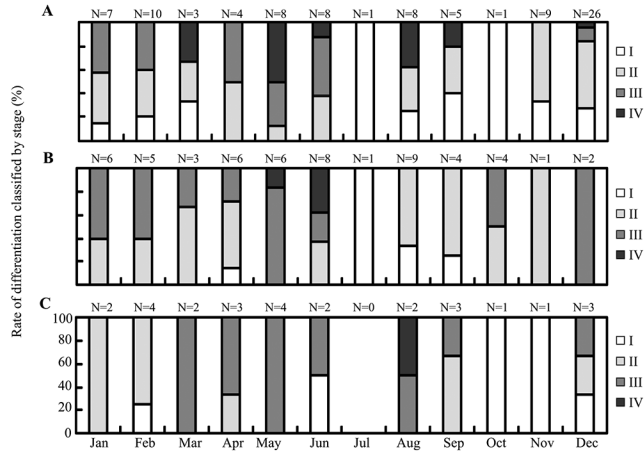


Figure 7. Annual cycle of differentiation of gonads (both sexes) shown by stage for three species. Image A: *P. japonicum*, B: *C. elatius* and C: *C. konojoi*.

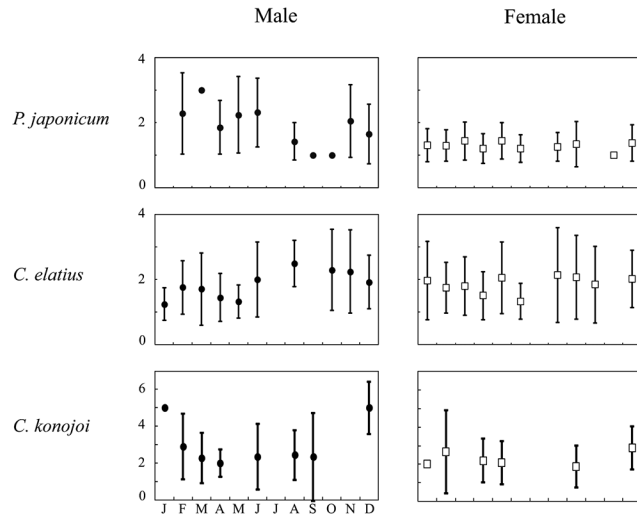


Figure 8. Annual cycle of number of gonads per polyp in the three species examined (average \pm standard deviation).

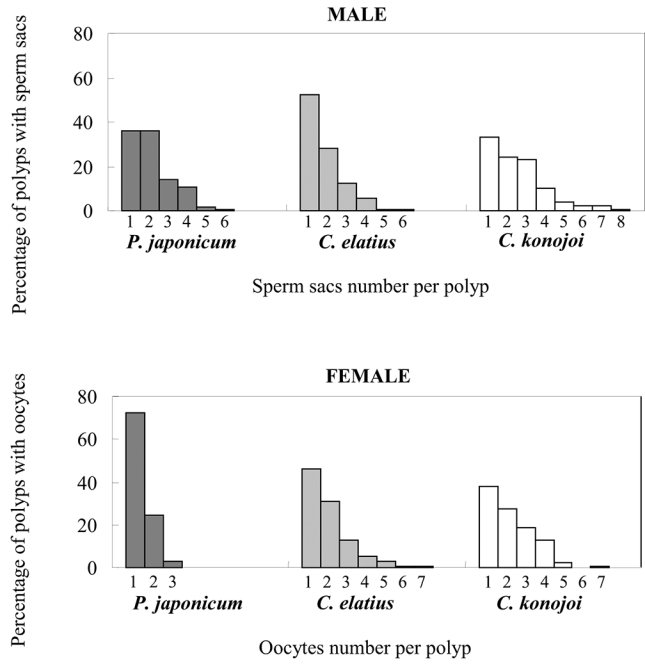


Figure 9. Frequency of number of gonads per polyp for each species. Image A shows male colonies and B shows females.

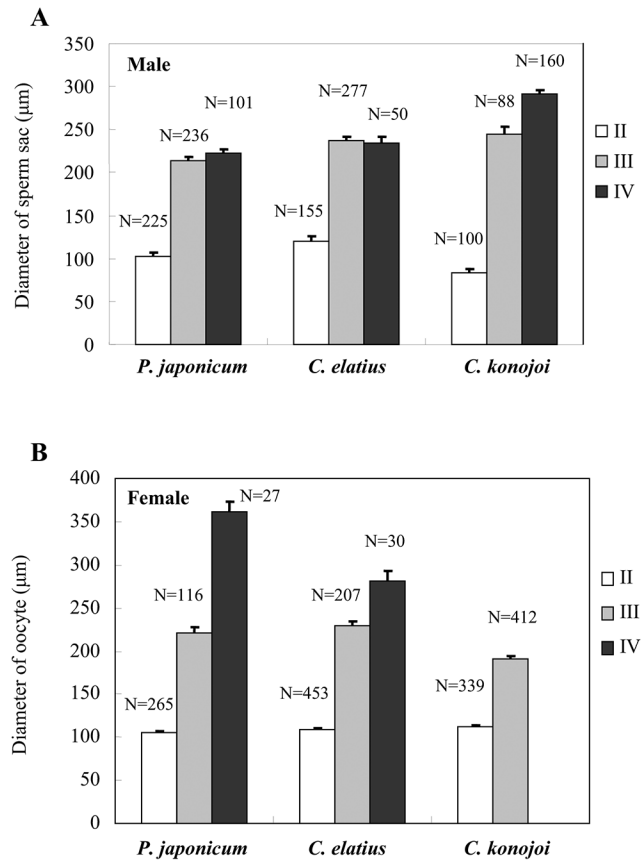


Figure 10. Average diameter of gonads at each stage for each species. Image A shows sperm sacs and B shows oocytes (average \pm standard error).

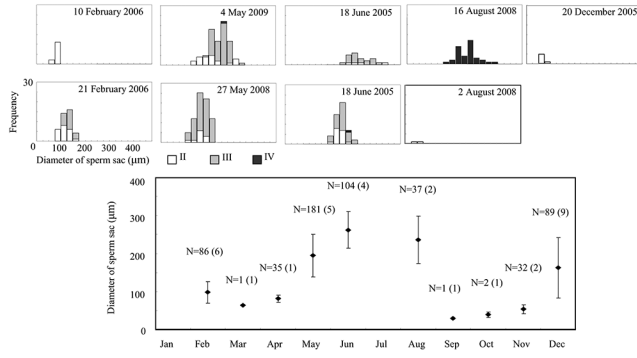


Figure 11. Annual cycle of diameter of sperm sacs (mean \pm S.D.) in *Paracorallium japonicum*. N: number of gonads (number of colonies). I, II, III, IV: stage of differentiation.

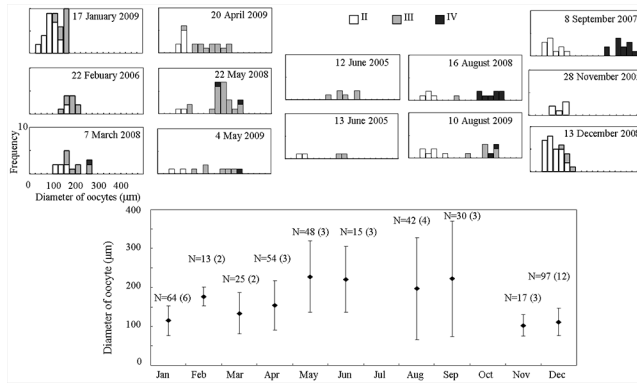


Figure 12. Annual cycle of diameter of oocytes (mean \pm S.D.) in *Paracorallium japonicum*. N: number of gonads (number of colonies). I, II, III, IwV: stage of differentiation.

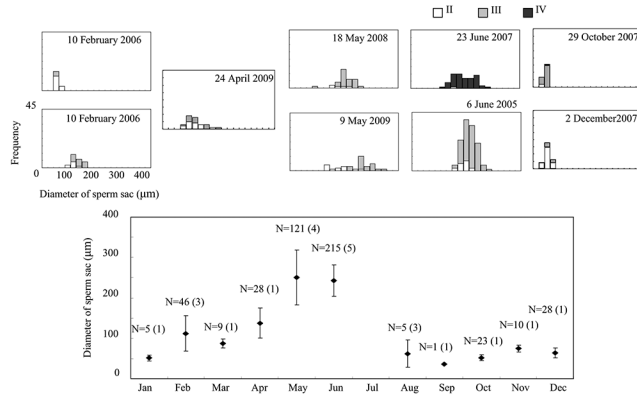


Figure 13. Annual cycle of diameter of sperm sacs (mean ± S.D.) in *Corallium elatius*. N: number of gonads (number of colonies). I, II, III, IV: stage of differentiation.

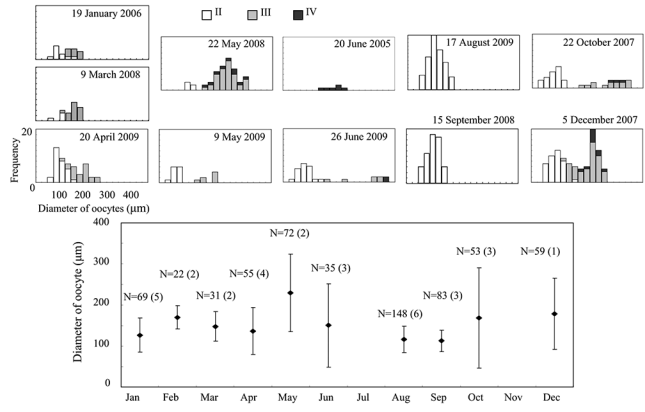


Figure 14. Annual cycle of diameter of oocytes (mean \pm S.D.) in *Corallium elatius*. N: number of gonads (number of colonies). I, II, III, IV: stage of differentiation.

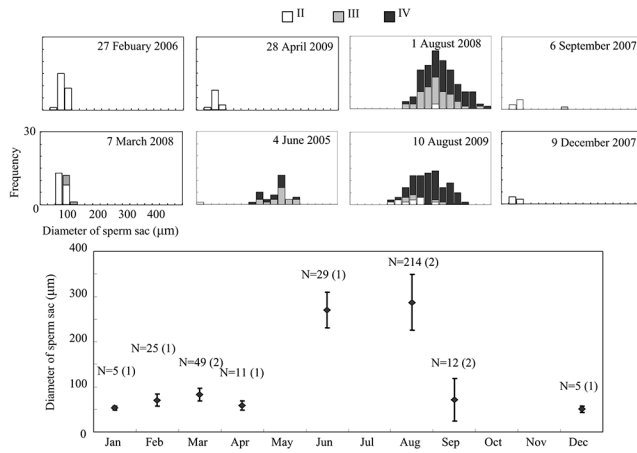


Figure 15. Annual cycle of diameter of sperm sacs (mean \pm S.D.) in *Corallium konojoi*. N: number of gonads (number of colonies). I, II, III, IV: stage of differentiation.

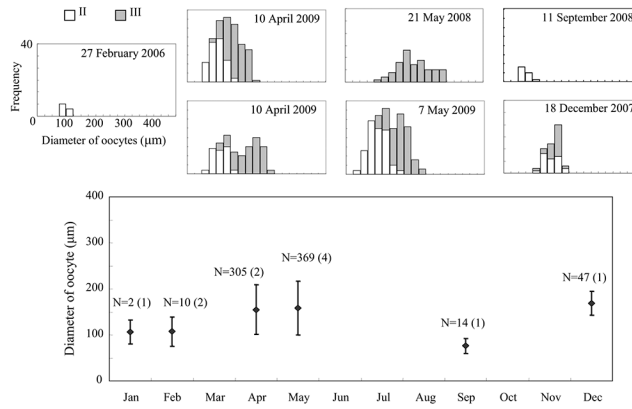


Figure 16. Annual cycle of diameter of oocytes (mean \pm S.D.) in *Corallium konojoi*. N: number of gonads (number of colonies). I, II, III, IV: stage of differentiation.