Sexual reproduction of soft coral, Scleronephthya gracillimum, (Alcyonacea: Nephtheidae) based on long-term collection from Jejudo Island, Korea

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Abstract The soft coral Scleronephthya gracillimum (Alcyonacea: Octocorallia), a member of family Nephtheidae, is abundant in the southern part of Jejudo Island, Korea. To examine the sexual reproductive pattern, monthly collections and histological studies were conducted from June 2003 to October 2007. The sexual reproductive pattern of the genus Scleronephthya was described for the first time in the present study, which established that S. gracillimum is a gonochoric broadcaster, with the sex ratio of 1:1. The gametogenic cycle was annual, and a difference between female and male colonies was apparent, with biorhythms of 12 and 5–7 months respectively. Gametogenesis and spawning are related to seasonal factors such as seawater temperature and algal blooms. Actual spawning event occurred, and others may have taken place, when seawater temperature attained an annual peak between August and September. The actual spawning occurred in the field at 10 a.m. on September 3, 2006, 4 days before the full moon.

Keywords sexual reproduction, gametogenesis, Scleronephthya gracillimum, soft coral, Anthozoa

Introduction

Soft corals, of the order Alcyonacea, are ecologically important sessile members of coral reef communities, and are distributed from tropical to temperate regions. These corals have three modes of sexual reproduction, namely the broadcasting of gametes, internal brooding, and external surface brooding involving planulae (Farrant 1986; Benayahu et al. 1990; Benayahu 1991; Cordes et al. 2001). The reproductive patterns of alcyonaceans are summarized in Table 1. The reproductive mode of soft corals varies among different family groups; gonochorism is generally dominant in soft coral species, although a few species are hermaphroditic (Sebens 1983; Benayahu et al. 1990; Achituv et al. 1992; McFadden et al. 2001; McFadden and Hochberg 2003; Choi and Song 2007; Hwang and Song 2007). Most species belonging to the family Alcyoniidae employ the broadcast mode of reproduction. In the family Xenidae, by contrast, only brooding behavior has been described, although the examination about reproductive mode has not been finished yet.

Generally, the production and development of gametes is similar in most soft corals. Gametes are formed in the mesenteries, and then move into the gastrovascular cavity, where they mature (Benayahu 1991; McFadden and Hochberg 2003). Broadcast spawning events of corals are
usually synchronous and seasonal, although only a low level of synchrony is apparent in temperate species (Harrison et al. 1984; Benayahu and Loya 1986; Harrison and Wallace 1990; Benayahu 1991; Ben-David-Zaslow et al. 1999; Kapela and Lasker 1999; Cordes et al. 2001; Schleyer et al. 2004). Limited information is available regarding the sexual reproduction of Nephtheidae, compared with that on Alcyoniidae and Xeniidae. Also, most studies on reproduction have been conducted in only a few geographical regions, mainly the Red Sea, the Australian Great Barrier Reef, and the Northeastern Pacific Ocean (Benayahu et al. 1990; Benayahu 1991; McFadden et al. 2001; McFadden and Hochberg 2003). The genus Scleronephthya, a member of the family Nephtheidae, inhabits tropical-to-temperate regions (Fabricius and Alderslade 2001), and only one species, Scleronephthya gracillimum (Kükenthal, 1906), has been reported in Korean waters to date (Song 1976). In the present report, reproductive features such as sexuality, reproductive mode, gametogenesis, and the reproductive cycle, are described for S. gracillimum for the first time.

Materials and methods

Collection of specimens

Colonies of S. gracillimum are abundant on the south coast of Jejudo Island (Fig. 1) in Korea, located in the temperate zone but with a somewhat subtropical climate.

Table 1  Reproductive pattern reported in the literature on Alcyonacea

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Number of species</th>
<th>Sexuality</th>
<th>Mode of reproduction</th>
<th>References</th>
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<tr>
<td></td>
<td></td>
<td>G  H  G/H</td>
<td>P    BS  EB  IB  U</td>
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<tr>
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<td>35 1   -</td>
<td>1    26 2 7 2</td>
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<td>-    - 1 -   -</td>
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<tr>
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<td>-    - 1 -   -</td>
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<td>-    - 1 1 3,4</td>
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<td>1 -   -</td>
<td>-    - 1 - 3</td>
<td></td>
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<tr>
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<td>1</td>
<td>1 -   -</td>
<td>-    - 1 4</td>
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</table>


BS broadcast spawner, EB external brooder, G gonochoric, H hermaphroditic, G/H gonochoric or hermaphroditic, IB internal brooder, P parthenogenetic, U unknown.
because the coast is affected by the Tsushima Warm Current branching from the Kuroshio Current. Colonies live mainly on the vertical surfaces of rocks in our study area, where seawater temperatures range from 14–26°C depending on season. Long (5–6 cm) fragments were collected from randomly selected colonies (height > 30 cm) located at depths of 10 m and 30 m by researchers wearing SCUBA apparatus, between June 2003 and October 2007. After collection, samples were anesthetized with menthol for 4–6 hours, and were then fixed in 4–5% (v/v) neutral formalin, diluted with seawater, for 24 hours. Finally, samples were transferred into 70% (v/v) ethanol for preservation.

Dissection and histology

Each preserved specimen was examined under a Zeiss (Semi SV-6) stereomicroscope to determine the sexuality of each colony and the external features of gametes. To investigate gametogenic cycles, the longest and shortest axes of about 30 gametes from each colony were measured using an image analyzer (Motic Image Plus 2.0 instrument) on a monthly basis, and an average axis length was calculated. Seawater temperature near the collection site was recorded monthly by Korea Hydrographic and Oceanographic Administration (KHOA) during the entire study period.

Histology was employed to identify gametogenic stages and the reproductive mode. Tissues 0.5–1.0 cm in both length and width were cut from preserved specimens, decalcified in 10% (w/v) EDTA solution for 5 days, and then dehydrated in a graded series of ethanol baths. Dehydrated tissues were cleared with a mixture of xylene and ethanol, embedded in paraffin, and cut into sections of 10 µm thick using a microtome. Serial sections were stained with Harris hematoxylin and eosin Y and mounted using Shandon Synthetic Mountant (Thermo). Sections were observed under an Olympus BH-2 microscope.

Images and analysis

Photographs of living colonies in water were taken with an Olympus 5060-WZ digital camera fitted with an Underwater Patima 7070 Housing, and all images of gametes and histological sections were obtained using the same camera attached to stereomicroscopes (Semi SV-6 and SV-11 instruments) or a light microscope (Olympus BH2 instrument). The sex ratio was determined by counting the numbers of female and male colonies from June to October throughout study periods, when oocytes and spermarys were observed together. The χ² goodness-of-fit test was used to test for deviation from a 1:1 sex ratio, using SPSS (version 12).
Results

Sexuality and sex ratio

All *S. gracillimum* colonies analyzed microscopically and histologically were determined to be gonochoric. Among the total of 269 colonies examined during the entire study period, 131 were female, 79 male, and 59 contained small or no gametes. Female colonies were significantly more abundant than male colonies, with a sex ratio of 1.76:1. However, male colonies were observed only from late spring or early summer to fall. On the other hand, female colonies were present throughout the year. Thus, only 85 female and 75 male colonies obtained between June and October were used to calculate the precise sex ratio, excluding possible male colonies with small or no gametes. This sex ratio was not significantly different from 1:1 ($\chi^2=90.625$, $df=1$, $P=0.429$).

Gametogenesis (gonadal development)

Development of gametes in *S. gracillimum* was similar to that in other alcyonaceans; gametes originated from gastrodermal mesenteries within the polyp cavity. Immature gametes gradually moved into the gastrovascular cavity, being initially connected to the mesenteries by pedicles, and then detached from the mesenteries as they matured.

**Oogenesis.** Oocyte was easily defined microscopically by the presence of a transparent follicular layer and a prominent nucleus with a single nucleolus. Spherical oocytes tended to change from transparent to cream in color, and then to vivid orange, as they matured. The development of oocytes was classified into five stages, depending on maturity, as shown in Table 2.

The earliest oocytes, in stage I, were in the mesoglea of mesenteries and were smaller than 50 µm in diameter, with an average size of $39.2 \pm 8.1$ µm (mean $\pm$ SD, $n=98$) (Fig. 2a). These oocytes contained large distinct nuclei, and were transparent because of a lack of cytoplasm. As the oocytes grew into stage II, the color changed to cream as a result of accumulation of cytoplasm, and one-sided nuclei were seen. At this stage, oocytes were observed in the gastrovascular cavity and were individually connected to mesenteries by pedicles (Fig. 2a). The diameter of oocytes ranged from 51–149 µm with a mean value of $112.2 \pm 24.5$ µm ($n=1,515$). At stage III, vitellogenesis (yolk synthesis) commenced, and yolk bodies accumulated around the nuclei of oocytes, resulting in a color change from cream to light orange. The maturing oocytes became detached from the mesenteries, entering into a cavity covered by follicular layers (Fig. 2b); the oocytes ranged in diameter from 150–199 µm, with a mean of $169.8 \pm 13.5$ µm ($n=590$). In stage IV, yolk bodies were continuously synthesized, and spread throughout the entire oocyte (Fig. 2c), so that the color of oocytes became gradually deeper. Late vitellogenic oocytes ranged from 200–299 µm in diameter, with a mean of $255.2 \pm 29.8$ µm ($n=464$). Fully mature oocytes, in stage V, were vivid dark orange in color and were over 300 µm in diameter, with a maximum of 473 µm and an average of $346.2 \pm 35.0$ µm ($n=370$) (Fig. 2d). These oocytes were covered with a well-developed follicular layer. Embryos and planulae were not observed when oocytes were at this stage within the cavity.

**Spermatogenesis.** The development of spermaries was classified into four stages depending on maturity, as shown in Table 2. Spermaries at the earliest stage were embedded.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Range (µm)</th>
<th>Oocytes</th>
<th>Spermaries</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean $\pm$ SD (µm)</td>
<td>n</td>
</tr>
<tr>
<td>I</td>
<td>≤50</td>
<td>39.2 $\pm$ 8.1</td>
<td>98</td>
</tr>
<tr>
<td>II</td>
<td>51–149</td>
<td>112.2 $\pm$ 24.5</td>
<td>1515</td>
</tr>
<tr>
<td>III</td>
<td>150–199</td>
<td>169.8 $\pm$ 13.5</td>
<td>590</td>
</tr>
<tr>
<td>IV</td>
<td>200–299</td>
<td>255.2 $\pm$ 29.8</td>
<td>464</td>
</tr>
<tr>
<td>V</td>
<td>≥300</td>
<td>346.2 $\pm$ 35.0</td>
<td>370</td>
</tr>
</tbody>
</table>

Table 2  Range and mean diameter of gametes, which depends of stages (n number of gametes at each stage)
Stage I spermaries were spherical and were less than 50 µm in diameter, with a mean of 43.2 ± 5.3 µm (n = 52). In stage II, spermaries were filled with developing spermatocytes and moved into the cavity, where they remain attached to the mesenteries by pedicles (Fig. 3b). These opaque spermaries had distinct boundaries and ranged in diameter from 51–99 µm, with a mean of 76.9 ± 14.1 µm (n = 350). In stage III, spermatocytes were arranged at the periphery of spermaries, resulting in hollow centers (Fig. 3b, c), and began to develop into spermatids. The spermaries changed from opaque to tinted cream in color as spermatids accumulated. The diameter of spermaries ranged from 100–174 µm, with a mean of 134.3 ± 20.2 µm (n = 1,047). In stage IV, spermaries attained their full size and were completely mature, with diameters in excess of 175 µm, with a maximum of 281 µm and a mean of 203.0 ± 23.4 µm (n = 281). Late in this stage, spermatids began to metamorphose into spermatozoa in these cream-colored spermaries. Tails began to be displayed, and the spermatozoa formed a parallel array. A convolution of spermatozoa at the on-side of spermaries was observed before sperm was released (Fig. 3d).

Gametogenic cycle and spawning

Annual reproductive cycle (oogenic and spermatogenic cycle). *S. gracillimum* showed a single annual reproductive cycle, with a clear difference between females and males. Whereas spermaries were detected only between June and December in 2003, June and October in 2004, April and August in 2005, June and October in 2006, and May and October in 2007, oocytes were observed throughout the year, except when collections were not possible.

In females, oocytes of stages I and II were found throughout most of the study period, although their relative frequency changed with the season (Fig. 4). In June 2003, the sum of the relative frequencies of these two stages was comparatively high at 0.8, and this sum subsequently dropped sharply, to under 0.1, between July and August. By late September, the two stages of oocytes recorded the highest combined frequency of 1.0, and this high frequency was continuously maintained until the following May, with an average of 0.9 (±0.10, n = 9). A
similar pattern, with small fluctuations, appeared in successive years up to and including October 2007. Stage I and II oocytes showed a rise, followed by a fall, in frequency over the course of any year. The sum of the frequencies of stages I and II was low at 0.2±0.23 (mean±SD, n=16) between June and August, which lasted for 9 months until the following May, and then decreased again from June. Stage III showed a low frequency throughout the year, with a mean of 0.2±0.13 (n=40). No frequency fluctuation pattern was observed for this stage. Late vitellogenic oocytes, in stage IV, were found mainly between June and September, showing a mean frequency of 0.3±0.21 (n=21). Frequency peaks in stage IV oocytes in each year were recorded in July of 2003 and 2004, June of 2005 and 2006, and August 2007, with minimum and maximum frequencies of 0.3 and 0.8 in 2003 and 2007, respectively. Fully mature oocytes, in stage V, were observed from June to September, and were abundant in July and August at an average frequency of 0.3±0.20 (n=11). Stage V oocyte levels peaked in August 2003, July 2004, July 2005, August 2006, and June 2007, with a mean frequency of 0.5±0.14 (n=5). Generally, stage V oocytes tended to peak in frequency 1–2 months after the peak in stage IV oocytes, with fluctuation in each year.

In males, stages I and II were observed between April and December, with fluctuation in each year, throughout the entire study period (Fig. 5). The sum of the relative frequencies of these stages peaked every June between 2004 and 2007, but in 2003 the frequency peak was observed in November. However, only small numbers of spermaries were found between October and December 2003: 11 in October, 3 in November, and 30 in December. Thus, if the data from October to December 2003 are ignored, stage I and II spermaries showed the highest frequency in June, as occurred from 2004 to 2007. The mean frequency of stage I and II spermaries was notably elevated, at 0.7±0.25 (n=5), every June from 2004 to 2007, but from July of each year the frequency sharply decreased, to a mean of 0.1±0.12 (n=14). Stage III spermaries were usually apparent during the entire period when male colonies were present, but were particularly abundant from July to October, with a mean frequency of
0.7±0.23 (n=20). Mature spermaries, in stage IV, were found between June and October, with a mean frequency of 0.3±0.20 (n=15). Stage IV spermaries displayed distinct rise and fall patterns, suggesting that reproductive events were underway. In July and September 2003, the frequency of stage IV spermaries peaked, and then dropped to zero. Similar patterns were also evident in 2004 and 2005, with peaks in July followed by rapid declines. Such a rise and fall pattern was recorded twice in 2006, with peaks in June and late August. Also, an additional small peak was observed, together with a high frequency of stage III spermaries, in October 2006, but the number of spermaries rapidly declined from September, suggesting the termination of reproductive events. Rise-and-fall patterns were also noted between August and September 2007.

**Spawning of gametes (inferred and observed spawning events).** An actual spawning event was observed on September 3, 2006, in the field, and no embryos or planulae were found in histological sections or dissected colonies, suggesting that broadcast spawning is the reproductive mode of *S. gracillimum.*

In 2003, the number of mature oocytes (stage V) and the total number of oocytes fell abruptly between August and September (Fig. 6). Mature spermaries were observed only from July to September, and the number of spermaries declined between August and October (Fig. 7). Thus, it is reasonable to infer that a spawning event may have occurred between August and September 2003. In 2004, mature oocytes and spermaries were not found after September, and the numbers of oocytes and spermaries decreased from September, suggesting that spawning occurred in September. In 2005, the numbers of mature oocytes and spermaries fell rapidly between July and August, indicative of the spawning of gametes. However, the total numbers of oocytes and spermaries did not decrease, and oocytes of stage IV and spermaries of stage III were abundant, suggesting an additional spawning event in September even though sampling was not performed. The actual spawning occurred in the field at 10 a.m. on September 3, 2006, 4 days before the full moon, and several potential spawning events might be inferred from decreases in mature gametes between the middle of August and the end of September. Thus, declines in both
Fig. 6  Total number of mature and entire oocytes from June 2003 to October 2007. OS stage V oocytes, WO whole oocytes, * month with no collection.

Fig. 7  Total number of mature and entire spermaries from June 2003 to October 2007. S4 stage IV spermaries, WS whole spermaries, * month with no collection.
Discussion

The sexual reproductive pattern of the genus *Scleronephthya* has been identified for the first time in the present study, which established that *S. gracillimum* is a gonochoric broadcaster. To date, very little information on sexual reproduction in the family Nephtheidae has been available, compared to that for other soft corals in the families Alcyoniidae and Xeniidae (Table 1). Gonochorism is known to be the dominant reproductive pattern in soft corals and also in most gorgonians (Grigg 1977; Benayahu et al. 1990; Brazaeu and Lasker 1990; Benayahu 1991; Achituv et al. 1992; Zeevi and Benayahu 1999; Lasker et al. 1996; Schleyer et al. 2004; Orejas et al. 2007; Seo et al. 2008). Whereas hermaphroditism is observed in several species of Alcyoniidae and Xeniidae, only gonochorism is found in Nephtheidae (Sebens 1983; Farrant 1986; Benayahu et al. 1990; Benayahu 1991; Dahan and Benayahu 1997; Choi and Song 2007; Hwang and Song 2007).

In soft corals, sexuality varies according to geographical region. For example, *Heteroxenia elizabethae* is a gonochoric coral in the Great Barrier Reef, Australia, but is hermaphroditic in the Red Sea. Also, *Sarcophyton glaucum* shows a low level of hermaphroditism in South Africa, but gonochorism in the Red Sea (Benayahu et al. 1990; Schleyer et al. 2004). The distribution of *S. gracillimum* is fairly wide, from tropical to temperate regions (Song 1976, researcher’s observation), which suggests that the sexuality of this species may differ geographically.

To optimize fertilization, and consequently to increase the success of reproduction in broadcasting species, spawning events usually occur synchronously (Benayahu and Loya 1986; Harrison and Wallace 1990; Kapela and Lasker 1999; Schleyer et al. 2004). Especially, reef-building corals show a high synchrony of spawning episodes, termed mass spawning, in the Indo-Pacific and Great Barrier Reef regions (Harrison et al. 1984; Babcock et al. 1986; Alino and Coll 1989). During mass spawning, vast numbers of colonies of many species release huge quantities of eggs and sperm over just 1 night. However, temperate corals have a tendency to exhibit a somewhat low level of synchrony in spawning, and display prolonged gametogenesis, whereas spawning events in tropical corals are synchronous (Benayahu 1991; Ben-David-Zaslow et al. 1999; Cordes et al. 2001). *S. gracillimum* contained mature gametes continuously during the months of summer and early fall, and gametes of different developmental stages were observed together within the same polyp during reproductive periods. These observations suggest relatively asynchronous spawning in our study population, perhaps reflecting the continuous release of gametes. *Dendronephthya suensoni* and *Anthopleura dimorpha* (respectively a soft coral and a gorgonian) are sympatric with *S. gracillimum*, and show similar spawning patterns during reproductive periods (Choi and Song 2007; Seo et al. 2008). Also, *Dendronephthya gigantea*, another sympatric species, released planulae continuously from summer to early fall (Hwang and Song 2007).

Thus, corals with asynchronous spawning need to employ a supplementary strategy to increase the possible low level of fertilization resulting from asynchrony. Control of sperm density may efficiently optimize fertilization rate, and sperm density can be affected both by dilution and the distance between female and male colonies. Actual spawning of *S. gracillimum* was observed in the field when the current was weak, and spawning also occurs during periods of slow current in other corals; this minimizes dilution of sperm in water (Kapela and Lasker 1999; Neves and Pires 2002; Penland et al. 2004). In the natural habitat, the colonies of *S. gracillimum* completely cover the surface of the rock, thus prohibiting establishment of other coral species. This high population density in a restricted space may result from asexual reproduction, which may increase the frequency of fertilization, as has been noted in another broadcast-spawning gorgonian, *Plexaura kuna* (Coffroth and Lasker 1998). However, detailed enumeration of female and male colony numbers in the clonal population is necessary for further study of
the reproductive strategy of \textit{S. gracillimum}.

The spawning time of \textit{S. gracillimum} approximately coincides with the full moon, occurring 4 days before a full moon in 2006 and in the interval 1 day after full moon to 1 day before the new moon in 2007, which is in line with the behavior of other spawning corals (Harrison et al. 1984; Schleyer et al. 2004; Carroll et al. 2006). However, it is unclear why \textit{S. gracillimum} spawned eggs in the morning on September 3, 2006, as most other spawning corals release eggs and sperm after sunset (Harrison et al. 1984; Lasker et al. 1996; Dahan and Benayahu 1997; Ryland and Westphalen 2004).

The pattern of gonadal formation and arrangement in \textit{S. gracillimum} is similar to those of other soft corals (Benayahu 1991; Cordes et al. 2001; McFadden and Hochberg 2003; Hwang and Song 2007). Gametes are produced in the mesenteries, and enter the gastrovascular cavity but remain attached to the mesenteries by pedicles. After migration into the cavity, gametes rapidly mature, and the connections between gametes and mesenteries are eliminated as the gametes grow.

The gametogenic cycle was seasonal, and a difference between female and male colonies was apparent, with biorhythms of 12 and 5–7 months respectively. Oocytes developed throughout the year, whereas spermarys formed only during a particular interval that was principally summer-to-fall (June to October) in \textit{S. gracillimum}. The cycle of gonadal development was thus similar to that of other corals, with a short period of spermatogenesis compared to that of oogenesis (Goldberg and Hamilton 1974; Benayahu and Loya 1984; Alino and Coll 1989; Brazaeu and Lasker 1990; Harri et al. 2001), although spermarys and oocytes are present together all year round in some soft corals (Kruger et al. 1998; Fan et al. 2005). Oogenesis usually takes over 1 year, and the duration varies among soft corals and with respect to geographical region in the same species. For example, oogenesis takes 11–12 months in \textit{Parerythropodium f. fulvum}, but 18–24 months in \textit{Sinularia humesi}, \textit{S. leptocladus}, \textit{S. mayi}, and \textit{Litophyton arboreum}. In \textit{S. glaucum}, oogenesis requires 16–18 months in Africa and 22–23 months in the Red Sea. All of \textit{Lobophytum paciflorum}, \textit{D. gigantea}, and \textit{D. suensonii} require 12 months for oogenesis (Benayahu et al. 1990; Schleyer et al. 2004; Fan et al. 2005; Choi and Song 2007; Hwang and Song 2007). However, no relationship between duration of gonadal development and reproductive mode is apparent.

In temperate regions, sexual reproduction, including gametogenesis and spawning, is directly correlated with seasonal factors such as seawater temperature (Harii et al. 2001; Neves and Pires 2002; Vermeiji et al. 2004). The monthly mean diameter of oocytes and spermarys in \textit{S. gracillimum} increased and decreased in line with fluctuations in seawater temperature throughout our entire study period (Fig. 8). Especially, spermarys were observed only during the warmer seasons, and mature gametes were found only after elevation of seawater temperature to 19–21°C in June and July. Actual spawning event occurred, and others may have taken place, when seawater temperature attained an annual peak between August and September. In the sympatric soft corals \textit{D. gigantea}, \textit{D. suensonii}, and \textit{A. dimorpha}, a similar reproductive cycle and release pattern of planulae or gametes over the same period correlated with rises and falls in seawater temperature (Choi and Song 2007; Hwang and Song 2007; Seo et al. 2008). Also, the survival and settlement of planulae were enhanced in warmer water, resulting in increased reproductive success (Ben-David-Zaslow and Benayahu 1996; Nozawa and Harrison 2000; Fan et al. 2005).

In addition, \textit{S. gracillimum} is an azooxanthellate coral which requires food resources for survival, growth, and reproduction, and thus feeds on phyto- and zooplankton in water (Fabricius et al. 1995a, b). \textit{Acabaria biserialis}, the azooxanthellate gorgonian of the Red Sea, released planulae after an algal bloom, which suggests that planula survival is increased by such episodes (Zeevi and Benayahu 1999). Major and minor algal blooms occurred in May and September during reproductive periods at our study site (Choa and Lee 2000). These blooms may form a direct nutrient resource encouraging gametogenesis in adult colonies of \textit{S. gracillimum}, and also facilitating survival and growth of offspring.

\section*{Conclusion}

The sexual reproductive pattern of \textit{Scleronephthya}
gracillimum has been identified for the first time in the present study, focused in sexuality, reproductive mode, gametogenesis, and the reproductive cycle.

- *Scleronephthya gracillimum* is a gonochoric broadcaster, with the sex ratio of 1:1.
- The oogenic cycle is longer than the spermatogenic cycle, with biorhythms of 12 and 5–7 months respectively.
- Gametogenesis and spawning is related to seasonal factors such as seawater temperature and algal blooms.
- Spawning approximately occurs around the time of full moon.

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