RESEARCH ARTICLE

Reproductive biology and larval development of the temperate soft coral *Dendronephthya gigantea* (Alcyonacea: Nephtheidae)

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Abstract *Dendronephthya gigantea* (Verrill, 1864), the dominant species in the waters surrounding Jeju Island, Korea, is a gonochoric internal brooder that releases its planulae from July to September. The ratio of females to males in this azooxanthellate soft coral is 2:1. Oogenesis takes place for 12 months and spermatogenesis for 3–5 months. Gametes mature as seawater temperatures increase, suggesting a seasonal factor in the reproductive cycle. Planulae brooded in the gastrodermal canal were expelled around the times of the full moon and the new moon; a significant difference in numbers expelled was not found between day and night. Ciliated planulae had negative buoyancy after planulation, and showed rapid metamorphosis into a primary polyp stage within 2 days.

Introduction

Recent studies of sexual reproduction in soft corals show that the reproductive patterns are diverse in sexuality, reproductive mode, fecundity, seasonality, and periodicity. Soft corals belonging to the order Alcyonacea have three modes of reproduction: the broadcasting of gametes; internal brooding; and the external surface brooding of planulae (Benayahu et al. 1990; Benayahu 1991; Dahan and Benayahu 1997; Ben-David-Zaslow et al. 1999; Cordes et al. 2001). Generally, the spawning events of tropical soft corals are seasonal and synchronous, while temperate soft corals exhibit prolonged gametogenesis and brooding episodes (Benayahu 1991; Ben-David-Zaslow et al. 1999; Cordes et al. 2001).

Different taxa of soft corals have a tendency to employ different reproductive strategies (Sebens 1983; Farrant 1986; Benayahu et al. 1990; Benayahu 1991; Achituv et al. 1992; Dahan and Benayahu 1997; Cordes et al. 2001; McFadden et al. 2001; McFadden and Hochberg 2003; Schleyer et al. 2004). The sexual reproductive patterns of alcyonaceans are summarized in Table 1. Gonochorism is dominant in soft corals, although some exhibit hermaphroditism. Most species belonging to the families Alcyoniidae and Nephtheidae are gonochoric, whereas the 20% of the family Xeniidae are hermaphrodites (Table 1). All broadcasting soft corals are in the Alcyoniidae and the Nephtheidae; the reproductive mode is not found in the Xeniidae. All members of the family Xeniidae exhibit internal brooding except for one external surface brooder, *Efflatounaria* sp. (reference in Benayahu et al. 1990; Benayahu 1991). The mode of reproduction is almost identical within a given genus, except for the genus *Alcyonium* in which there are both brooding and broadcasting species (Benayahu et al. 1990; McFadden and Hochberg 2003).

Reproductive strategies of cnidarians in general and soft corals in particular, such as the development time of gametes and planulae, and the existence of a defined breeding season may be affected by environmental factors, especially the seawater temperature and lunar phases (Campbell 1974). Food availability and light conditions also trigger reproduction of Anthozoa (Ben-David-Zaslow et al. 1999; Harii et al. 2001; Neves and Pires 2002; Ryland and Westphalen 2004). In addition, a combination of factors mentioned above can induce the gametogenesis of coral species (Fritzenwanker and Technau 2002).

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Table 1 Reproductive patterns documented in the literature on Alcyonacea

Taxon	Number of species	Sexuality				Mode of reproduction				References
		G	H	G/H	P	BS	EB	IB	U	
Alcyoniidae	37	35	1	–	1	26	2	7	2	1,3,4,7,8,9,10
Alcyonium	11	9	1	–	1	5	1	4	1	1,3,8,9
Anthomastus	1	1	–	–	–	–	–	1	–	7
Cladiella	1	1	–	–	–	1	–	–	–	4
Discophyton	1	1	–	–	–	–	–	1	–	9
Lobophytum	7	7	–	–	–	7	–	–	–	3
Parerythropodium	1	1	–	–	–	–	1	–	–	3
Sarcophyton	4	4	–	–	–	4	–	–	–	3,10
Sinularia	9	9	–	–	–	9	–	–	–	3
Thrombophyton	2	2	–	–	–	–	–	1	1	9
Nephtheidae	3	3	–	–	–	1	1	1	–	2,3,6
Litophyton	1	1	–	–	–	–	–	1	–	3
Dendronephthya	1	1	–	–	–	1	–	–	–	6
Capnella	1	1	–	–	–	–	1	–	–	2
Xeniidae	24	18	5	1	–	–	1	16	7	3,4,5
Xenia	15	12	3	–	–	–	–	10	5	3,4,5
Heteroxenia	4	1	2	1	–	–	–	4	–	3
Anthelia	2	2	–	–	–	–	–	1	1	3,4
Sympodium	1	1	–	–	–	–	–	1	–	3,4
Efflatounaria	1	1	–	–	–	–	1	–	–	3
Cespitularia	1	1	–	–	–	–	–	–	1	4

References: (1) Sebens (1983), (2) Farrant (1986), (3) Benayahu et al. (1990), (4) Benayahu (1991), (5) Achituv et al. (1992), (6) Dahan and Benayahu (1997), (7) Cordes et al. (2001), (8) McFadden et al. (2001), (9) McFadden and Hochberg (2003), (10) Schleyer et al. (2004)

BS broadcast spawner; *EB* external brooder, *G* gonochoric, *H* hermaphroditic, *G/H* gonochoric or hermaphroditic, *IB* internal brooder, *P* parthenogenetic, *U* unknown

The patterns of gonadal development are similar in the Alcyonacea; namely, immature gonads develop along the mesenteries within the polyps, and then the gonads detach from the mesenteries during maturation (Benayahu 1991; McFadden and Hochberg 2003). In the genera *Anthomastus*, developing gonads are found within the siphonozooids, which are specially employed for reproduction (Cordes et al. 2001). Even though differences in gonad size are usually used in gametogenic studies of marine invertebrates, specifically which aspects of reproductive strategies cannot be determined by gonad size alone (Giese and Pearse 1974). Gonadal volume, the size and volume of yolk, and the ratio of yolk volume to cytoplasm provide more information on gametogenic development because the energy available from the yolk mass is associated directly with larval development and dispersal (Ryland and Westphalen 2004).

Broadcasting species generally have eggs with positive buoyancy which float near the surface of the water, and planulae with planktonic development; consequently, they have a long-competency period and the potential for wide dispersal (Arai et al. 1993; Nozawa and Harrison 2000). In contrast, brooding species release mature planulae with negative buoyancy, benthic larvae, suggesting a short-competency period and a narrow range of dispersal (Hariison and Wallace 1990; Sakai 1997). Larval dispersal is related to larval types and plays an important role in the distribution of coral species and in the maintenance of the natal population (Farrant 1986; Ben-David-Zaslow and Benayahu 1996; Harii and Kayanne 2003; Gutiérrez-Rodríguez and Lasker 2004).

Studies of the life history traits of soft corals have been focused mainly on the families Alcyoniidae and Xeniidae in the North Atlantic, the Red Sea, the Australian Great Barrier Reef, the South Seas and Northeastern Pacific Ocean (Sebens 1983; Benayahu et al. 1990; Benayahu 1991; MacFadden et al. 2001; MacFadden and Hochberg 2003). Despite their ecological importance and conspicuous live coverage and abundance, only a few results on the reproduction and larval development from the family Nephtheidae have been reported: these include *Litophyton arboreum*, a gonochoric internal brooder (Benayahu et al. 1992); *Dendronephthya hemprichi*, a gonochoric broadcaster (Dahan and Benayahu 1997); and *Capnella*

gaboensis, a gonochoric external brooder (Farrant 1986).

The goal of the present study is to examine in detail the sexuality, sex ratio, mode of reproduction, fecundity, gametogenesis, and larval development of *Dendronephthya gigantea*, a soft coral that is abundant in the most southern part of Korea. When compared with what is known from other congeneric species within *Dendronephthya*, these results provide valuable information about the reproduction patterns found within this poorly studied genus.

Materials and methods

Collection of specimens

Species of the genus *Dendronephthya* exhibits a wide range in zoogeographic distribution, from the tropical zone to the temperate zone (Utinomi 1952; Rho and Song 1977). *Dendronephthya gigantea* dominates off Jeju Island, the southernmost part of Korea. It is located between the temperate and subtropical regions, where water temperatures range from 14 to 26°C, and vary with the season. Most of the colonies are distributed there on vertical and horizontal rocky substrate from 2 to 35 m in depth, and show their peak abundance between depths of 10 and 20 m (researcher's observation).

All of the specimens used in this study were collected from Munseom (33° 22′ N, 126° 33′ E, Fig. 1), along the south coast of Jeju Island, which is designated as Natural Monument No. 420, according to Rho and Song (1977). Each month, from June 2003 to October 2004, 5–6 cm long cuttings were sampled from randomly selected colonies using SCUBA apparatus. Cuttings were taken at >30 cm colonies in height and between 10 and 20 m deep. After collection, they were fixed in 4–5% (v/v) formalin in seawater for 24 h and transferred into 70% (v/v) ethanol for preservation.

Dissection and histology

Preserved cuttings of polyp masses from each colony were dissected under a ZEISS (Stemi SV-6) stereomicroscope to determine the colony's sexuality and the external features of the gonads. Fresh gonads were examined under the stereomicroscope to determine the color difference of mature and immature gonads. The χ^2 goodness-of-fit was used to test for deviation from a 1:1 sex ratio with SAS (version 8.2). The number of total and mature oocytes from one polyp mass of each eight cuttings from female colonies in August 2004 was counted, and the number of polyps per polyp mass was also measured. The average fecundity was expressed as a percentage of mature oocytes to total oocytes per polyp.

The gametogenic cycles of *D. gigantea* were determined by measuring the longest and shortest axes of about 100–200 isolated oocytes or spermaries on a monthly basis. These data enabled us to present the monthly frequencies of gametogenic stages. To interpret the reproductive strategy of the soft coral, the volume of gonads, the diameter and volume of the yolk and the ratio of the volumes of yolk to oocyte were calculated. The dark colored yolk was easily distinguished from the whole oocyte because the layer between yolk and oocyte membrane was transparent under the light microscope. ANOVA and Duncan Grouping methods (SAS version 8.2) were applied to analyze the correlation between the groups classified by yolk characters and oogenic stages.

Histological sections were prepared to identify the gametogenic stages and the mode of reproduction. After pieces of tissue, 0.5–1.0 cm in length × 0.5–1.0 cm in width, from the cuttings were fixed in 70% ethanol and decalcified in 10% (w/v) EDTA for 5 days, they were dehydrated in a graded series of ethanol, cleared in ethanol/xylene mixtures and then

Fig. 1 Map of the study site. **a** Jeju Island is located in the most southern part of Korea. **b** Munseom (*dark circle* in a) is in the natural protected area
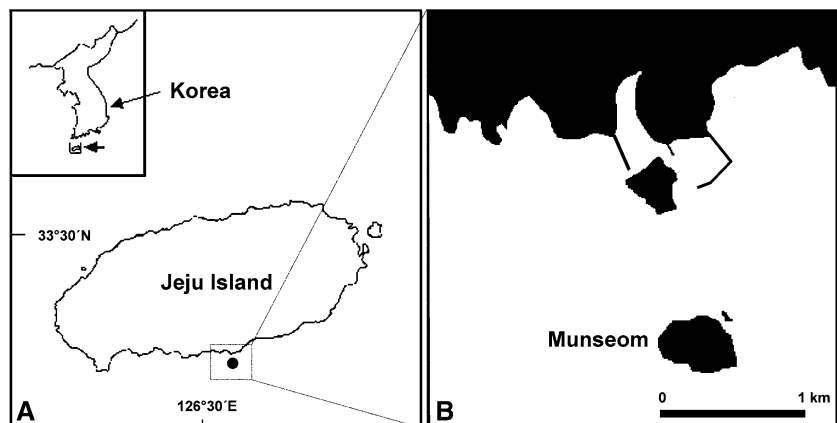
embedded into paraffin. Sections of 10 μm in thickness were stained with Harris hematoxylin and eosin Y and observed under an Olympus (BH-2) microscope.

Images were obtained by an Olympus (5060-WZ) digital camera attached to the stereomicroscope and the light microscope. Depending on the stage of development of oocytes and spermaries they were classified into five stages and four stages, respectively, according to their morphological characteristics.

Planulation and rearing of planulae

Planulation during various lunar phases (new moon, first quarter, full moon, and last quarter) from July to September 2003 and from July to August 2004 was examined in the field by SCUBA diving and in the laboratory. In the full moon of August 2003, the releasing of planulae was monitored in the field during day (0900–1600 hours) and night (1900–2100 hours).

Cuttings from five to ten colonies were maintained in aerated aquariums containing 4 l of artificial seawater under the natural light condition in August 2003. The presence of and number of planulae released were monitored during the day and night. The paired t test was performed (paired samples test) to determine if there was any difference between number of planulae obtained by day and by night.

For the examination of larval development, 18 groups of three planulae were reared in 6-well tissue culture plates at 24°C, the ambient temperature of seawater, with each well containing 10 ml of filtered (Millipore; 0.45 μm pore size) seawater (FSW) during August to September 2003. Each day the development of early planula to primary polyp stage was examined under a stereomicroscope for 1 month.

Results

Sexuality, sex ratio, and fecundity

All of the *Dendronephthya gigantea* colonies examined were gonochoric, as determined by microscopic and histological examination. Among the total of 98 colonies analyzed, 46 were defined as female, 22 as male, and 30 had no gonads. The sex ratio was significantly different from 1:1, whether the inactive colonies were included or excluded (including, $\chi^2 = 9.1429$, $df = 2$, $P = 0.01$; excluding, $\chi^2 = 8.4706$, $df = 1$, $P = 0.003$ respectively).

In August 2004, the mean number of total oocytes and mature oocytes per polyp was 9.8 ± 2.0 and 4.0 ± 0.9 ($n = 8$ colonies), respectively, resulting in an average fecundity of $41.5(\pm 7.5)\%$ for female colonies.

Gametogenesis (gonadal development)

Gametes arose from the gastrodermis, and gradually moved into the gastrovascular cavity (polyp cavity) as they grew.

Oogenesis

Spherical oocytes were easily identified by a prominent nucleus together with a single nucleolus and cell layer under the light microscope. Oogenic development was divided into five stages as follows:

Stage I (oogonia)

The earliest oocytes, which had particularly large and centered nuclei, were transparent. Oocytes embedded in the mesoglea of mesenteries were clustered together (Fig. 2a). These primordial oocytes ranged from 10 to 50 μm in diameter, with an average of $40.70(\pm 9.84)$ μm (mean $\pm$ SD, $n = 197$).

Stage II (previtellogenic oocytes)

As they grew, oocytes were observed in the polyp cavity and were connected to mesenteries by pedicles (Fig. 2b). They became opaque and had a mean diameter of 78.03 (± 13.85) μm ($n = 671$).

Stage III (vitellogenic oocytes)

By Stage III, vitellogenesis had started and the oocytes had grown by active yolk synthesis to a mean diameter of $124.31(\pm 14.68)$ μm ($n = 446$). Nuclei were observed at the periphery of oocytes with yolk bodies around them (Fig. 2c). The maturing oocytes were detached from mesenteries into the polyp cavity.

Stage IV (late vitellogenic oocytes)

Additional yolk bodies were noted and dispersed throughout the whole oocyte, so that the oocytes became yellow (Fig. 2d). The nucleus was located at the periphery of the mature oocyte. The diameter of oocytes ranged from 160 to 300 μm, with an average of $239.30(\pm 41.61)$ μm ($n = 226$).

Stage V (mature oocytes or eggs)

Oocytes that had reached their full size changed to dark yellow; they were characterized by numerous yolk droplets, and were covered with follicular layers (Fig. 2e). The oocytes ranged in diameter from over 300 μm to a maximum of 480 μm. With the continuous maturation of eggs,
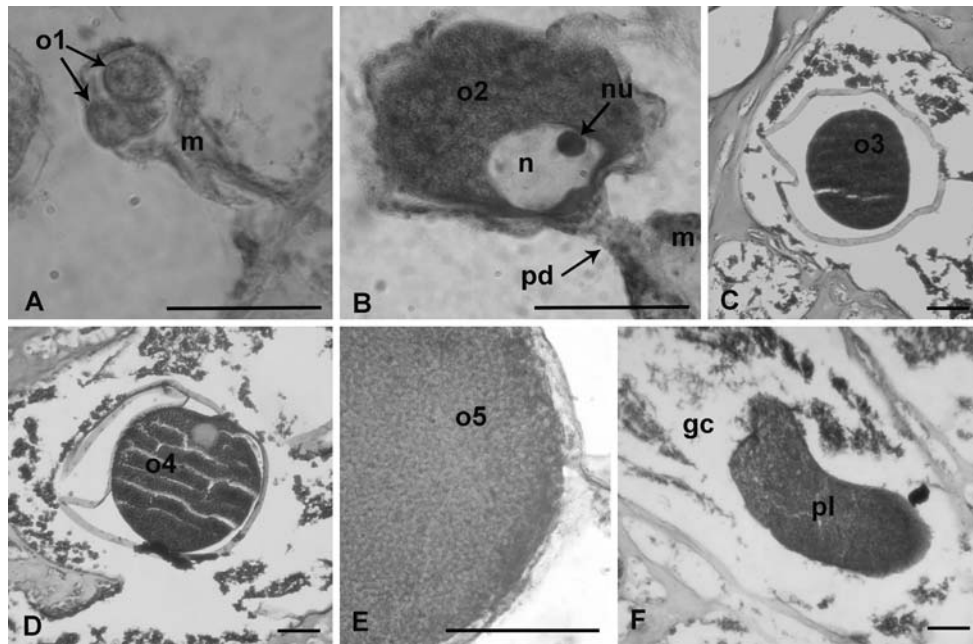
Fig. 2 *Dendronephthya gigantea*. Oogenic development. **a** Cluster of Stage I oocytes embedded in mesentery. **b** Stage II oocyte with a conspicuous nucleus connected to mesentery by a pedicle. **c** Stage III oocyte enveloped follicle layer. **d** Stage IV oocyte with nucleus at the periphery. **e** Stage V oocyte filled with numerous yolk bodies. **f** Planula brooded in the gastrodermal canal. The *scale bar* represents 50 µm (*m* mesentery, *n* nucleus, *nu* nucleolus, *o1* Stage I oocyte, *o2* Stage II oocyte, *o3* Stage III oocyte, *o4* Stage IV oocyte, *o5* Stage V oocyte, *gc* gastrodermal canal, *pd* pedicle, *pl* planula)

the oocytes were located within the bundle of polyps, and the branches and the stem (Fig. 8a).

Spermatogenesis

Stage I (spermatogonia)

The earliest spermaries had no conspicuous differences in nuclei, and clusters of spermatogonia were observed in the mesoglea of mesenteries (Fig. 3b). During this stage, the mean diameter of transparent spermaries was 42.89 ($\pm$7.88) µm ($n = 32$).

Stage II (spermatocytes)

Spermaries with newly forming spermatocytes had distinct boundaries and were attached to mesenteries by pedicles (Fig. 3a, c). Transparent spermaries were round and ranged in diameter from 50 to 100 µm, with a mean of 84.31 ($\pm$13.54) µm ($n = 91$).

Stage III (spermatids)

Spermatocytes began to develop into spermatids, which were small and had condensed nuclei (Fig. 3a). At this stage, spermaries drastically increased to a mean diameter of 171.78($\pm$31.41) µm ($n = 543$). The spermaries contained numerous spermatids arranged at the periphery of the sperm sac; a change to opaque white as the result of the accumulation of spermatids was observed.

Stage IV (spermatozoa)

Spermaries with metamorphosed spermatozoa that had tails projecting toward the center of spermaries were recognized (Fig. 3d). The completely matured spermaries were cream-colored and had a mean diameter of 256.18($\pm$26.86) µm ($n = 129$).

Annual reproductive cycle

While spermaries were only detected from June to October in 2003 and from July to September in 2004, oocytes were found at all times of the year (Figs. 4, 5).

In females, oogonia (Stage I) were found throughout the study. Their frequency slightly increased during the period from October 2003 to May 2004, when the ratio averaged 14($\pm$8.0, $n = 8$)% and reached a peak value of 27% in April 2004. The frequency of oogonia decreased from June to September, when they averaged 5($\pm$5.1, $n = 4$)%. Stage II oocytes had a distinct rise and fall in frequency over the course of a year: between October 2003 and May 2004 their average ratio was 51($\pm$10.2, $n = 8$)%, with maximum frequencies in November 2003 and February 2004; from June

Fig. 3 *Dendronephthya gigantea*. Spermatogenic development. **a** Various spermatogenic stages in a polyp. **b** Spermary with spermatogonia in gastrodermis. **c** Stage II spermary connected to mesentery by a pedicle. **d** Stage IV spermary containing a large number of spermatozoa with tails. The *scale bar* represents 100 μm (*g* gastrodermis, *m* mesentery, *pc* polyp cavity, *pd* pedicle, *s1* Stage I spermary, *s2* Stage II spermary, *s3* Stage III spermary, *s4* Stage IV spermary, *sg* spermatogonia, *st* bundle of sperm tails)
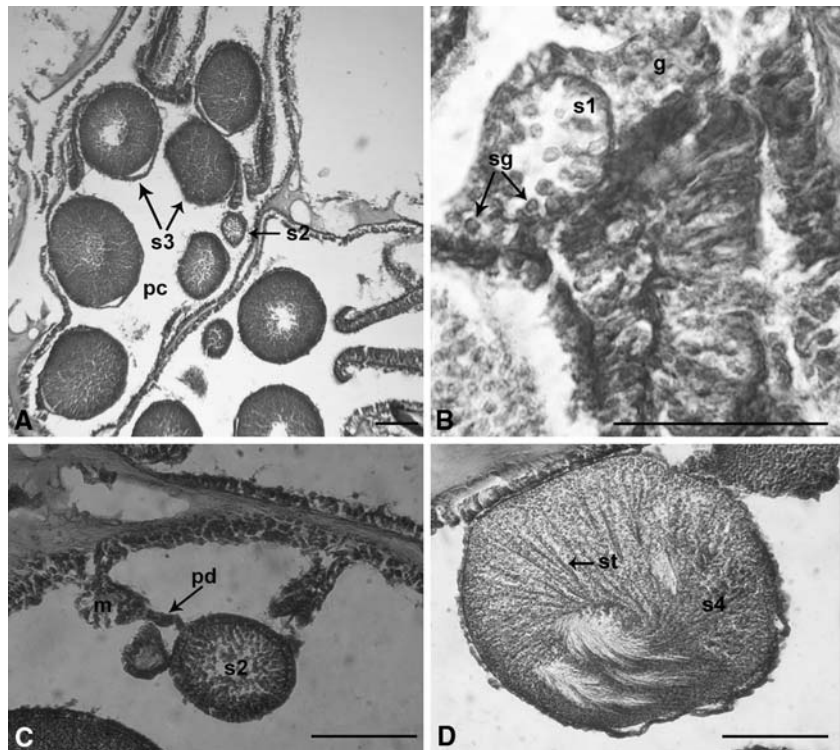

to September 2004 the value was average of 16(±11.7, $n = 4$)%. A similar pattern was observed for Stage III oocytes, which showed an average frequency of 27(±12.9, $n = 17$)% at all times. These vitellogenic oocytes increased in frequency, and had an average ratio of 33(±12.4, $n = 9$)% from October 2003 to June 2004, and 13(±5.4, $n = 3$)% from July 2004 to September 2004. Remarkably, mature oocytes, those of Stage IV and Stage V, were observed only between June and October, with an average ratio of 29(±16.6, $n = 10$)% and 25(±16.2, $n = 9$)%, respectively. Stage V oocytes of >300 μm in diameter were abundant from July to September, while Stage IV oocytes were found mainly between June and August.

Compared with oogenesis, spermatogenesis occurred during a short period of 3–5 months, with all stages of spermaries appearing simultaneously from June to October in 2003 and from July to September in 2004. Primordial spermaries, Stage I, were found at average of 3(±2.9, $n = 5$)% in 2003 and 8(±2.9, $n = 3$)% in 2004, averaging 5(±3.7, $n = 8$)% during the study periods. There was no significant difference in the frequency of Stage I spermaries between months. The mean percentage of Stage II spermaries was 11(±6.0, $n = 8$)%, with an average ratio of 10(±6.2, $n = 5$)% in 2003 and 13(±5.8, $n = 3$)% in 2004. Stage III spermaries were dominant, with an average ratio of 66(±16.2, $n = 8$)% and maximum ratio of 94% in September 2003. Mature spermaries (Stage IV) had the second highest mean ratio, 18(±11.3, $n = 8$)%; the average of 28(±9.3, $n = 3$)% for 2004 was higher than the 12(±7.7,

$n = 5$)% average for 2003. Stage III was largest, followed by Stage IV or sometimes Stage II, with Stage I as the lowest in all months.

The monthly mean diameter and volume of gametes were related to seawater temperature (Figs. 6, 7). Oocyte (Stage I) formation began between October and November, when seawater temperatures were decreasing. From November to the following May, slight fluctuations were observed in the mean diameters and volumes of oocytes. The rapid increase of seawater temperature between May and July was correlated with abrupt oocyte growth. Oocytes became mature in summer, when water temperature reached a peak. As water temperature later dropped, mean diameters and volumes of oocytes decreased rapidly. The formation and maturation of spermaries occurred simultaneously from June to October in 2003 and from July to September in 2004, when seawater temperature was between 19 and 24°C. Spermaries were no longer observed after October, when water temperature fell too low. In 2004, the spermatogenic cycle was shorter than in 2003, since water temperature did not go above 19°C until July, rather than June. Whereas there were two temperature peaks in 2003, there was only one in 2004.
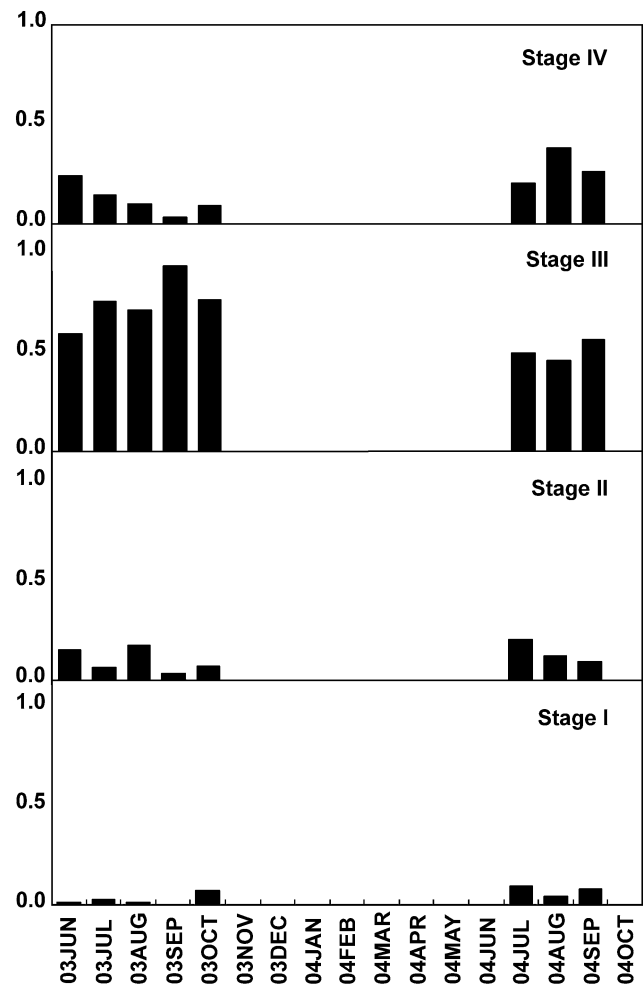
Differences in growth rate between gametogenic stages were noted by changes in gonad volume rather than diameter. Stage III oocytes were 1.59 times larger in diameter than Stage II oocytes, and Stage IV oocytes were 1.92 times larger than Stage III oocytes; therefore, volume increased 3.81-fold and 7.45-fold, respectively (Table 2). Spermaries
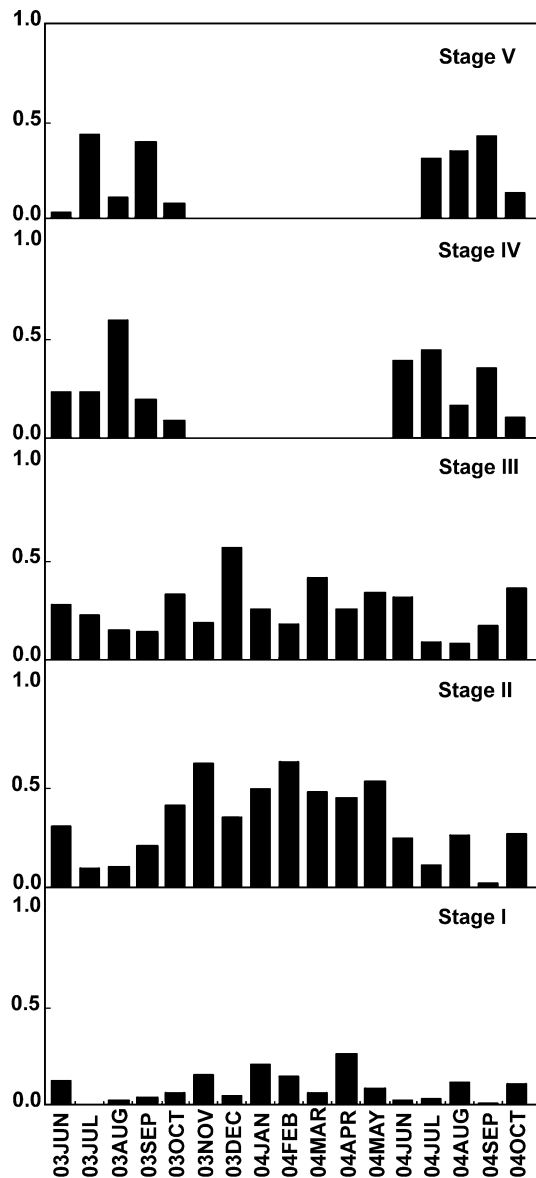
Fig. 4 *Dendronephthya gigantea*. Monthly frequency of oogenic stages in female polyps. Oocytes were found throughout the study period. All stages were observed commonly from June to October (*n* = 1732 oocytes)

Fig. 5 *Dendronephthya gigantea*. Monthly frequency of spermatogenic stages in male polyp. No spermaries were found from November 2003 to June 2004 or in October 2004 (*n* = 795 spermaries)

particularly increased in volume between Stage I and Stage III. The production of Stage I gonads was extended over several months, resulting in a low level of synchronization of maturation of gonads and planulation.

Vitellogenesis, or active yolk synthesis, is another index of maturation. The analysis of diameters and volumes of yolk indicated differences among the oogenic stages, particularly Stages III, IV, and V (ANOVA, *P* < 0.0001; Table 3). In addition, the ratios of the volume of yolk to oocyte were significantly different (*P* < 0.0001), especially between Stage V and the other stages. When matured completely, the oocytes contained 60% (v/v) ooplasm. Analysis by the Duncan grouping method revealed the usefulness of yolk in the study of gonadal development, because the groups distinguished by yolk were coincident with oogenic stages.

Planulation

Planulae internally brooded within the gastrodermal canals of the colonies were released from the colonies from July to September 2003 and in August 2004, when the maturation of gonads and the temperature of seawater reached a maximum (Fig. 2f, 6).

In the field, planulation was observed only during the periods of the full moon and new moon in August 2003. Notably planulae were released continuously from colonies reared in the aquarium, exhibiting no relation to lunar phases. A significant difference in the number of planulae released in the laboratory was not found between day (0900–1700 hours) and night (1700–0900 hours) during 2003 (paired *t* test, *df* = 15, *P* = 0.234).

Fig. 6 *Dendronephthya gigantea*. Seasonal fluctuations in monthly mean diameter and volume of oocyte according to the temperature of seawater. Maturation of oocytes occurred with an increase of temperature and released planulae were observed at peak temperature (*dark data*). Temperatures were recorded when samples were collected. *Error bars* indicate 95% confidence intervals
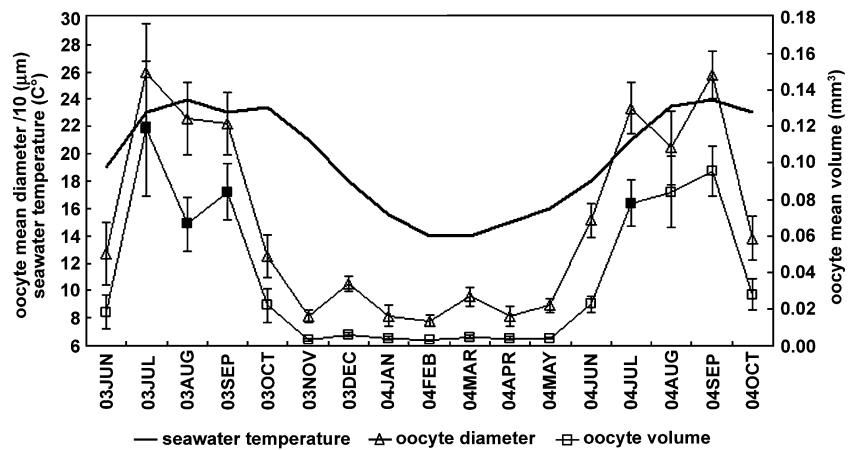
Fig. 7 *Dendronephthya gigantea*. Seasonal fluctuations in monthly mean diameter and volume of spermary according to the temperature of seawater. Spermaries appeared with an increase of temperature. Temperatures were recorded when samples were collected. Error bars indicate 95% confidence intervals
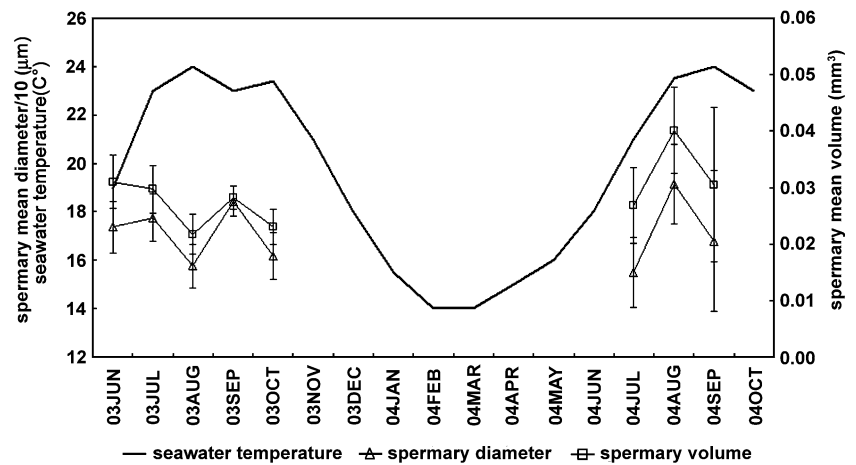
Table 2 *Dendronephthya gigantea*

Mean diameter and volume of gonads, which depends on gametogenic stages (*n* number of measured gonads at each stage)

Stage	Oocytes (mean ± SD)			Spermaries (mean ± SD)		
	Diameter (μm)	Volume (mm³)	*n*	Size (mm)	Volume (mm³)	*n*
I	40.70 ± 9.84	0.0003 ± 0.0002	197	42.89 ± 7.88	0.0004 ± 0.0002	32
II	78.03 ± 13.85	0.0022 ± 0.0011	671	84.31 ± 13.54	0.0027 ± 0.0012	91
III	124.31 ± 14.68	0.0084 ± 0.0031	446	171.82 ± 31.43	0.0233 ± 0.0113	542
IV	239.30 ± 41.60	0.0626 ± 0.0303	226	256.18 ± 26.86	0.0728 ± 0.0256	129
V	346.33 ± 30.32	0.1781 ± 0.0528	192	–	–	–

Larval and post-larval development

Various sizes and shapes of planulae, both contracted and elongated, were released into the water. The length and diameter of the orange and yellow planulae ranged from 600 to 1,500 μm and from 200 to 300 μm, respectively, and the rice-shaped planula became more round and cylindrical as the result of contraction and extension (Fig. 8b). Ciliated planulae had negative buoyancy immediately after release; they swam along the bottom of culture plates, rather than in the water column or near its surface.

The first settlement of planulae occurred within 2 days after the release of the larvae; metamorphosis was followed by the formation of oral part and tentacle lobes (Fig. 8c). On day 3 post-planulation, a polyp with tentacles appeared. The tentacles had pairs of pinnules, suggesting the onset of feeding, and eight mesenteries possessing septal filaments (Fig. 8d). Fully developed primary polyps bearing seven or eight pairs of pinnules were observed 7 days after planulation. The polyps were 2.2–2.3 mm in length and contained numerous white sclerites on their bodies and tentacles. As metamorphosis progressed, the polyps became lighter in color. The growth of the colony as a result of the budding of a new polyp (asexual reproduction) was not recorded until 1 month after planulae release.

Table 3 *Dendronephthya gigantea*. Comparison of yolks depending on oogenic stages analyzed by ANOVA

Morphological character	Mean				
	Stage III (*n:* 142)		Stage IV (*n:* 175)		Stage V (*n:* 243)
Yolk diameter (μm)	93.436	<	185.771	<	286.144
Yolk volume (mm³)	0.003835	<	0.031201	<	0.101489
Yolk : Oocyte volume	0.42395	<	0.49069	<	0.59206

Significant differences ($P < 0.0001$) among stages are indicated with a directional sign

Fig. 8 *Dendronephthya gigantea*. a Orange–yellow mature oocytes in the gastrodermal canals of the coenenchyme of a female colony. b Free-swimming planula. c Polyp with tentacle lobes undergoing metamorphosis. d Primary polyp with pinnate tentacles and well-developed oral part (*gc*, gastrodermal canal; *mo*, mature oocyte; *pt*, pinnate tentacle; *tl*, tentacle lobe)
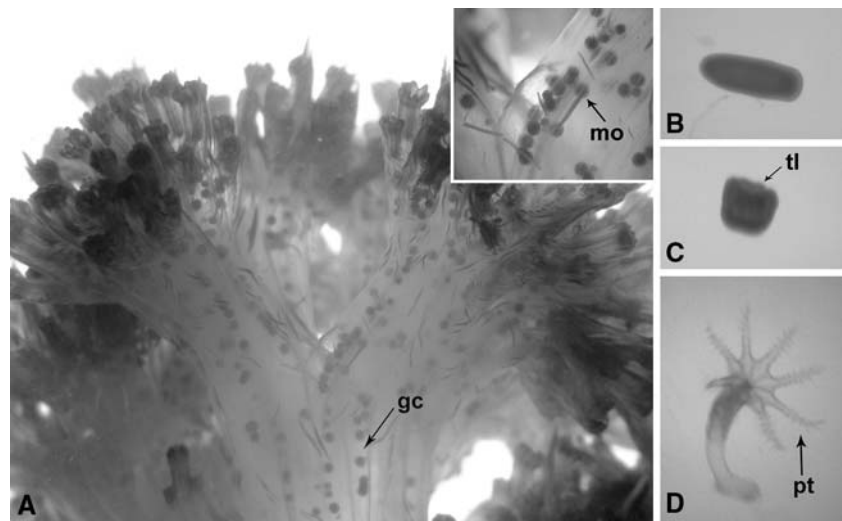
Discussion

Sexuality, sex ratio, and fecundity

The diversity of reproductive patterns in the genus *Dendronephthya* is represented by *Dendronephthya gigantea*, a gonochoric internal brooder, and *Dendronephthya hemprichi*, a gonochoric broadcaster (Dahan and Benayahu 1997). Gonochorism is known to be the dominant reproductive pattern in most soft corals, although a few Alcyoniidae and Xeniidae species have been shown to be hermaphroditic (Sebens 1983; Benayahu et al. 1990; Benayahu 1991). The sexuality of soft corals varies geographically; for example, the same species, *Heteroxenia elizabethae*, was gonochoric in the Great Barrier Reef, but hermaphroditic in the Red Sea. A low level of hermaphroditism was found in the gonochoric species *Sarcophyton glaucum* on KwaZulu-Natal, South Africa, but the mixed sexual pattern was not reported in the Red Sea (Benayahu et al. 1990; Schleyer et al. 2004). In some stony corals, the mode of reproduction changes over regions, e.g. *Pocillopora damicornis* and *Goniastrea aspera* are broadcasters or brooders, depending on location (Ward 1992; Sakai 1997; Shlesinger et al. 1998). The variation of reproductive mode between broadcasting and brooding in the same species depending on regions has not previously been described in soft corals.

For optimal fertilization and consequently reproductive success in marine broadcasting species, spawning is synchronized in and between colonies. Spawning occurs during periods of slow currents, which minimizes the concentrations of sperm by dilution (Kapela and Lasker 1999; Neves and Pires 2002; Penland et al. 2004). In brooding species, reproductive success and fecundity depends on the density of female colonies in the population. So the ratio of females to males may be different according to the mode of reproduction. For example, among soft corals the synchronous broadcaster, *S. glaucum*, exhibited a female to male ratio of 1:1, but the brooders *D. gigantea* and *Acabaria biserialis* have sex ratios of 2:1 and 4:3, respectively. (Zeevi Ben-Yosef and Benayahu 1999; Schleyer et al. 2004). Although a different sex ratio (female to male = 3:2) was found in the broadcaster, *D. hemprichi*, the ratio may be due to the species asynchronous reproductive period (Dahan and Benayahu 1997).

A single female polyp of *D. gigantea* contains an average of 9.8($\pm$2.0) oocytes, which shows higher fecundity than the *Anthomastus ritteri* (average of 5.3 ± 2.7 oocytes and planulae) and scleractinian brooding species (range of 1–4 oocytes) (Shlesinger et al. 1998; Cordes et al. 2001). Coral fecundity can be affected by several factors, such as polyp size, coenenchyme thickness, the number of gametogenic cycles, and the presence of additional compartments

for storage of mature oocytes and brooding (Benayahu 1991; Achituv et al. 1992; Dahan and Benayahu 1997; Kruger et al. 1998; Shlesinger et al. 1998). Multiple gametogenic cycles each year and year-round gametogenesis had been found in *Xenia umbellata*, *H. fuscescens*, and *D. hemprichi* (Benayahu 1991; Dahan and Benayahu 1997). The additional compartments for storing of mature oocytes and for brooding may give rise to the empty polyp cavities, allowing a simultaneous increase in the number of oocytes and planulae per polyp. This factor enhances the fecundity of a colony as is evident in *D. gigantea*, which utilizes gastrodermal canals for the storage of mature oocytes and to increase brooding capability. Premature planulae passed into siphonozooids from the gastrovascular cavities in *H. coheni* and *H. fuscescens*, and brooding pouches were made during the reproductive period between the coenenchymes of *X. macrospiculata* (Benayahu 1991; Achituv et al. 1992). Embryos and larvae of *Anthelia glauca* were brooded in the pharyngeal pouch formed by the expansion of the pharynx, with constrictions proximal and distal to the brood (Kruger et al. 1998). In *D. gigantea*, the thickness of the coenenchyme and the efficient use of space made by scattering small oocytes between large mature oocytes may increase of the species fecundity.

Gametogenesis and larval development

The formation and arrangement of gonads in *D. gigantea* resemble those of other soft corals (Benayahu 1991; Dahan and Benayahu 1997; Cordes et al. 2001; McFadden and Hochberg 2003). The histological sections indicate that gonads are produced in the mesenteries and then migrate into the polyp cavities, where they mature. Similar to the some soft or stony corals, oocytes of *D. gigantea* exhibit a distinct color change throughout maturation by yolk synthesis (Dahan and Benayahu 1997; Shlesinger et al. 1998; Schleyer et al. 2004), and the orange planulae are visible through the external surface, which may be a useful tool to predict planulation in the field.

The annual production of one seasonal gametogenic cycle per polyp from each female and male colony was present in *D. gigantea*. Oocytes were found throughout the year, but spermaries were observed only from summer to early fall (June to October). The full oogenic and spermatogenic development of *D. gigantea* took 12 and 3–5 months, respectively. In general, spermatogenesis occurs during a short period compared with oogenesis, similar to *D. gigantea* (Dahan and Benayahu 1997; Harii et al. 2001).

The reproduction of corals in a temperate region was triggered by exogenous factors related to season. Water temperature is a direct cue for coral reproduction, including gonad maturation, the release of gametes, and planulation (Harii et al 2001; Neves and Pires 2002; Ryland and Westphalen

2004; Schleyer et al. 2004; Vermeij et al. 2004). Similarly, seawater temperature is clearly related to gametogenesis and the development of gonads in *D. gigantea*. The volume of oocytes increased rapidly and spermaries were formed when the temperature of seawater was over 18–19°C in June to July (Figs. 6, 7). Planulae were released after the temperature of seawater attained the annual peak in July to September (Fig. 6). Elevated seawater temperatures and an increased rate of larval metamorphosis were documented for *H. fuscescens* (Ben-David-Zaslow and Benayahu 1996) and *Platygyra daedalea* (Nozawa and Harrison 2000), suggesting that the reproduction of corals in the warmer months may maximize the survivability of their offspring.

Reproduction can be affected by seasonal fluctuations in nutrient availability (Ben-David-Zaslow et al. 1999). Carbon fixed photosynthetically by zooxanthellae, dissolved organic materials, and plankton are the main energetic resources for the growth and reproduction of corals. Azooxanthellate soft corals like *D. gigantea* absorb nutrients from the uptake of phytoplankton in the water (Fabricius et al. 1995a, 1995b). In Munseom, two blooms of chlorophyll, from a concentration of phytoplankton and standing crops, were recorded in May and September (Choa and Lee 2000). The energy budget for rapid maturation of oocytes and the production of spermaries may have benefited directly from the major algal bloom in May. The minor bloom in September is likely to be a main food source for growth of the colony after sexual reproduction and post-larval development of released planulae. Similarly, the azooxanthellate coral, *A. biserialis*, a phytoplankton feeder, released planulae after a major algal bloom in the Red Sea (Zeevi Ben-Yosef and Benayahu 1999).

Several studies revealed that the relationship between oocyte size and reproductive mode is not clear in soft or stony corals (Kruger et al. 1998; Shlesinger et al. 1998). The maximum diameter of oocytes in *D. gigantea* (brooder) and *D. hemprichi* (broadcaster) ranged from 480 to 500 μm, with no difference in length (Dahan and Benayahu 1997). It has been suggested that large oocytes are associated with a longer oogenic cycle. However, the oogenic period of *S. glaucum* in South African reefs was 4–7 months shorter than in the Red Sea, although oocyte diameter appeared to be the same (Benayahu and Loya 1986; Schleyer et al. 2004). In addition, soft corals produce lecithotrophic, non-feeding, planulae. It thus appears that the size of oocytes may reflect the larval energy budget for settlement, metamorphosis and dispersal rather than mode of reproduction (Dahan and Benayahu 1998; Cordes et al. 2001). A detailed understanding of the energy budget related to larval biology may not be manifested by a single oocyte size and therefore, the actual amount of nutrition needs to be calculated. In this study, yolk characteristics were employed

to examine the degree of nutritional amount between oogenic stages. Features of the oocytes including diameter, volume, and yolk-to-cytoplasm volume ratio may provide significant indicators for investigating the relationship between yolk supply and reproductive strategy (Crawford et al. 1999). The Duncan grouping method demonstrated that the groups classified by yolk were coincident with oogenic stages, and these may appear to be a useful tool for measuring the relationship between energy budget and type of larvae.

In general, the timing patterns of larval release in many of the marine invertebrate are regulated by lunar and tidal amplitude cycles (Morgan 1995). The timing of planulae release of *D. gigantea* around the full moon and new moon in the field indicates the regulation of planulation by lunar periodicity. Similar results have been reported for soft coral species; e.g. *S. glaucum* (Schleyer et al. 2004) and *A. glauca* (Kruger et al. 1998). However, no correlation between the release of planulae and lunar cycles was reported for *H. fuscescnes* (Benayahu 1991). Planulation of *A. biseralis* in the laboratory also had no correlation with lunar phases (Zeevi Ben-Yosef and Benayahu 1999). In the present study, the continuous release of planulae in the laboratory during various lunar phases may imply: (1) a loose connection between planulation and lunar phases, and (2) a continuous supply of environmental stimulus in the aquarium. The circumstance of the aquarium is similar to high or low tide without the flow of water, so the stimulus for planulae release may therefore be tidal amplitude cycles.

The benthic planulae of *D. gigantea* are able to settle within 2 days after planulation; metamorphosis and the later processes were then started and continued for the next 1–5 days, although the settlement of few planulae is delayed until 14 days. The rapid development and the short competency period, the length of time before settlement of the brooded benthic planulae are presumed to indicate a narrow dispersal range of planulae, suggesting a limitation in the natal population (Sebens 1983; Farrant 1986; Ben-David-Zaslow and Benayahu 1996; Harii and Kayanne 2003). Several previous studies on planulae of brooding species, however, described a long competency period of several months implying the long distance dispersal (Ben-David-Zaslow and Benayahu 1998; Dahan and Benayahu 1998; Cordes et al. 2001). It have been questioned that planulae have the long competency period regardless of their lecithotrophic level. The valuable study of biochemical composition in planulae of *H. fuscescens* demonstrated the direct absorption of the dissolved organic material (DOM) from the water, supporting the possible extension of competency periods (Ben-David-Zaslow and Benayahu 2000).

The potential dispersal distance of larvae is apt to be determined by current velocity as well as competency time

(Sebens 1983; Nozawa and Harrison 2000; Cordes et al. 2001; Miller and Mundy 2003; Harii and Kayanne 2003). During the summer season, the maximal current flow was 80 cm/s in the experimental site, suggesting the possibility of increasing the dispersal capability of *D. gigantea*.

Conclusion

The reproductive pattern of *Dendronephthya gigantea* is different from that of *D. hemprich* (Dahan and Benayahu 1997) in reproductive mode, and gametogenic cycle, and shows the variable reproductive strategies used by different congeneric species. Our results are summarized as follows:

– *Dendronephthya gigantea* is a gonochoric internal brooder.
– The spermatogenic cycle is shorter than the oogenic cycle.
– Gametogenesis is related to seasonal factors such as the temperature of seawater and algal blooms.
– Planulation occurs around the time of the full moon and the new moon.
– Rapid settlement and metamorphosis of benthic planulae suggest lecithotrophic development.

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