REPRODUCTIVE BIOLOGY OF *Palythoa caribaeorum* AND *Protopalythoa variabilis* (CNIDARIA, ANTHOZOA, ZOANTHIDEA) FROM THE SOUTHEASTERN COAST OF BRAZIL

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(With 7 figures)

ABSTRACT

The reproductive biology of *Palythoa caribaeorum* (Duchassaing & Michelotti 1860) and *Protopalythoa variabilis* (Duerden 1898) was studied through monthly samples from tagged colonies from June 1996 to June 1997, in São Sebastião channel, São Paulo, Brazil (45º26'W, 23º50'S). The gametogenesis was similar to that of other zoanthids as shown by histological preparations. Oocyte diameters and maturation stages of testis vesicles were evaluated on squash preparations. Both species showed sequential protogynic hermaphroditism, with high frequency of fertile polyps (83% in *P. variabilis* and 72% in *P. caribaeorum*), high frequency of colonies in female sex condition (65.3% of *P. variabilis* and 41.7% of *P. caribaeorum*), and apparently continuous gametogenesis. In *P. caribaeorum*, egg release was continuous and sperm release took place during half of the analyzed period. In *P. variabilis*, egg and sperm release occurred in April-May and February-March 1997, respectively.

Key words: Anthozoa, Zoanthidea, Palythoa, Protopalythoa, sexual reproduction.

RESUMO

Biologia reprodutiva de *Palythoa caribaeorum* e *Protopalythoa variabilis* (Cnidaria: Anthozoa: Zoanthidea) da costa sudeste do Brasil

A biologia reprodutiva de *Palythoa caribaeorum* (Duchassaing & Michelotti 1860) e *Protopalythoa variabilis* (Duerden 1898) foi estudada por amostras mensais de colônias etiquetadas de junho de 1996 a junho de 1997, no canal de São Sebastião, São Paulo, Brasil (45º26'W, 23º50'S). A gametogênese apresentou-se semelhante à de outros zoantídeos, como evidenciado em preparações histológicas. O diâmetro dos oócitos e os estágios de maturação dos folículos testiculares foram avaliados por preparações do tipo "squash". Ambas as espécies mostraram hermafroditismo seqüencial protogínico, com alta freqüência de pólipos férteis (83% em *P. variabilis* e 72% em *P. caribaeorum*) e de colônias na condição sexual feminina (65,3% para *P. variabilis* e 41,7% para *P. caribaeorum*), e, aparentemente, gametogênese contínua. Em *P. caribaeorum*, a liberação de oócitos foi contínua e a liberação de espermatozóides ocorreu durante metade do período analisado. Para *P. variabilis*, a liberação de oócitos e de espermatozóides ocorreu em abril-maio e fevereiro-março de 1997, respectivamente.

Palavras-chave: Anthozoa, Zoanthidea, Palythoa, Protopalythoa, reprodução sexuada.

INTRODUCTION

The zoanthids are frequent organisms within shallow water communities along the coast of Brazil, in which *Zoanthus* spp. and *Palythoa* spp. are as common (Rohlfs de Macedo & Belém, 1994) as they are in other tropical reefs of the world (Ryland & Babcock, 1991). At the southeastern coast of Brazil there are numerous colonies of *Protopalythoa variabilis* (Duerden 1898) (*non Palythoa variabilis*).

The systematics of zoanthids is even today considered confusing. Both colonies and polyps are highly variable within species. Many published descriptions lack essential diagnostic information, which makes comparisons difficult. Recent work using molecular genetics (Burnett *et al.*, 1997) suggests that many nominate species really represent no more than synonyms.

Studies on reproductive biology are few (reviewed by Ryland, 1997) and mainly consider Brachycnemina (i.e., *Palythoa*, *Protopalythoa*, *Zoanthus*). Muirhead *et al.* (1986) studied two deep-sea species of Macrocnemina (*Epizoanthus* spp.), which might be expected to exhibit differences in comparison with the shallow water brachycnemic species.

The goal of this study is to clarify sexual reproduction of *Palythoa caribaeorum* (Duchassaing & Michelotti 1860) and *Protopalythoa variabilis* (Duerden 1898) by describing the gametogenesis and focusing on monthly variation of the gonad profile within colonies to determine the frequency of fertile polyps and the reproduction rate of polyps and colonies. This is the first study on sexual reproduction of Zoanthidea for the South Atlantic, in which the methods employed include quantitative evaluation of observed testis vesicles. In Brazil, other research on zoanthids species has centered on composition (Rohlfs de Macedo & Belém, 1994) and aspects of asexual reproduction of *Palythoa caribaeorum* (see Moreno, 1999).

MATERIAL AND METHODS

Colonies of *P. caribaeorum* and *P. variabilis* sampled were living in the shallow rocky bottom of the São Sebastião Channel (SSC), São Paulo, Brazil, in the vicinity of the Centro de Biologia Marinha, University of São Paulo (CEBIMar – USP) (45º26'W, 23º50'S). The colonies occur mainly on the rocky shores in from 1 to 3 m depths. The colonies of *P. caribaeorum* cover large areas and the orange-brownish polyps (~1 cm height x 0.5 cm diameter) interconnect by a thick coenenchyme out of which only the oral discs appear. The colonies of *P. variabilis* are brownish with polyps (~3 cm height x 1 cm diameter) interconnected by a mat of basal coenenchyme (Boscolo, 2000).

Samples from tagged colonies were obtained at five stations monthly between June 1996 and June 1997. The five stations were called: (A) located between Itacuçê and Guaecá point (one colony of *P. caribaeorum*); (B) Saco Grande bay (one colony of *P. caribaeorum*); (C) south side of Segredo beach (two colonies of *P. caribaeorum* and two colonies of *P. variabilis*) (C1 and C2, respectively); (D) north side of Segredo beach (two colonies of *P. caribaeorum*) (D1 and D2); and (E) Cabras island (two colonies of *P. variabilis*) (E1 and E2) (Fig. 1). Each sample consisted of 20 polyps, 10 from the center and 10 from the margin of each colony. Only half of the collected polyps were studied; the remaining samples have been deposited in the Cnidaria collection of Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ 3415 to 3666).

In the laboratory, the animals were anesthetized in a 1:1 solution of 7.5% magnesium chloride and seawater (until not reacting to touch), fixed in 4% formaldehyde solution in seawater, and dissected under a stereomicroscope to remove the complete mesenteries or macrosepta.

The mesenteries were dehydrated in an ethanol series, cleared in xylene, and wax-embedded. Serial sections of 7 µm were rehydrated with an ethanol series and stained with Weigert hematoxylin + Mallory trichromic (Mahoney, 1973), a suitable stain for basophilic and acidophilic oocytes. Six slides, 3 of *P. variabilis* and 3 of *P. caribaeorum*, were deposited in the Cnidaria collection, MNRJ 3667 to 3672. The histological treatment made the oocytes shrink to approximately 50%-60% of their size, so that preserved oocytes of *P. variabilis* up to 400 µm in diameter measured no more than 160 µm in the histological sections.

Squash preparations of the mesenteries were studied with a Wild M20 microscope so as to measure the oocytes and estimate the percentage of total vesicle area occupied by spermatozoa. From each sample the diameters of 50 oocytes having a visible nucleolus were measured using an ocular micrometer. Non-circular cells were measured by the average of the longest and shortest dimensions. Samples with fewer than

10 oocytes were characterized as sterile. Divisions between oocyte size classes were set at 45 μm intervals. The Kolmogorov-Smirnov 2-sample analyses (α = 5%) (Sokal & Rohlf, 1995) tested differences between sets of oocyte diameter measurements for different regions of the same colony. When the null hypothesis (H_o: $\delta^2_1 = \delta^2_2$) was rejected, the set of oocyte diameter measurements remained separated. The testis vesicles (n = 50 per sample) were studied and categorized by subjectively evaluating the percentage of total vesicle area occupied by spermatozoa (head and tail).

We also analyzed the percentage of fertile polyps in every reproductive state in relation to the total number of polyps, and the reproductive state of the polyps and colonies by the percentage found in female, male, hermaphroditic, or sterile condition.

RESULTS

Palythoa caribaeorum

The polyps of *P. caribaeorum* had from 6 to 19 perfect mesenteries with gonads (Table 1) and were 5 mm in length.

The oocytes appear as endodermal cells with nuclei near the gastrodermal layer (Fig. 2A and 2B), and having a maximum diameter of 51.25 μm in the histological sections (basophilic, 112.5 μm in squash); all were basophilic. The testis vesicles spread extended from the ciliary band to the aboral region of the mesenteries. These vesicles were clear, with the central lumen even more translucent. The histological sections showed three layers: spermatogonia-spermatocytes, spermatids, and spermatozoa (Fig. 2C and 2D).

From the beginning of the sample period, the oocytes in the colonies preceded the testis vesicles, which in turn preceded the recovery of oocyte production. For a short period, all colonies presented a hermaphroditic condition (Fig. 3).

In October 1996, only two colonies (stations B and C1) had oocytes larger than 90 μm in diameter (max. 112.5 μm) (Figs. 3B and 3C1). All the other colonies showed oocytes ranging up to 90 μm. The entire number 2 colony at station D had been sterile for over 3 months, while its edges had been sterile for over 6 months (Fig. 3D2).

Testis vesicles developed over approximately 6 months. From December 1996 to January 1997, testis vesicles had only spermatogonia, and until the following period (February to May 1997) they were filled with spermatozoa (diameter of ~ 480 μm, in squash). A generally large proportion of these germ cells showed production and liberation until May 1997.

Protopalythoa variabilis

The polyps of *P. variabilis*, which were up to 15 mm in length, had from 15 to 34 perfect mesenteries with gonads (Table 1).

In most fertile mesenteries, the oocytes and testis vesicles occupied all of the gonad area. In squash preparations, oocytes of up to 450 μm in diameter and without zooxanthellae were observed. In histological preparations, the largest diameter of the basophilic oocytes was 53.25 μm, with a nucleus 21.75 μm in diameter and a 6 μm-diameter nucleolus. The average diameter of acidophilic oocytes was over 150 μm, with a 40 μm nucleus, and a 10 μm nucleolus. During the maturation of the oocyte, the nucleus migrated to its edge, next to the gastrodermis; the surrounding mesoglea thinned out and the gastrodermis thickened (Fig. 2B). The testis vesicles were similar to those of *P. caribaeorum* (see above) (Fig. 2C and 2D) except in size (*P. variabilis* with ~ 280 μm in diameter).

The oocyte size of colonies of *P. variabilis* increased gradually throughout the study periods in all stations (Fig. 5). From March to May 1997, only the two colonies of station E spawned or were sterile. The testis vesicles were being produced from November 1996 on and apparently were released in February-March 1997 (Fig. 6).

The oocytes and testis vesicles occurred together during 4-5 months, whereas the timing for spawning was not totally simultaneous (Figs. 5 and 6).

Reproductive patterns

Oocytes and testis vesicles of both species can co-occur in the same mesentery and are distinguishable either by their coloration (sperm, white; eggs, yellow) or by a short margin between the regions. In the female gonad, the mesoglea and gastrodermis are slender next to the site in which the oocyte nucleus is located.

Both species had high percentages of fertile polyps, although in *P. caribaeorum* the polyps in the central region of the colonies were more fertile than those on the edges. The colonies of *P. caribaeorum* showed the highest percentages of fertile polyps in August-September 1996 and in March 1997. From June 1996 to February 1997, all polyps of the majority of the colonies of *P. variabilis* were fertile (Fig. 7).

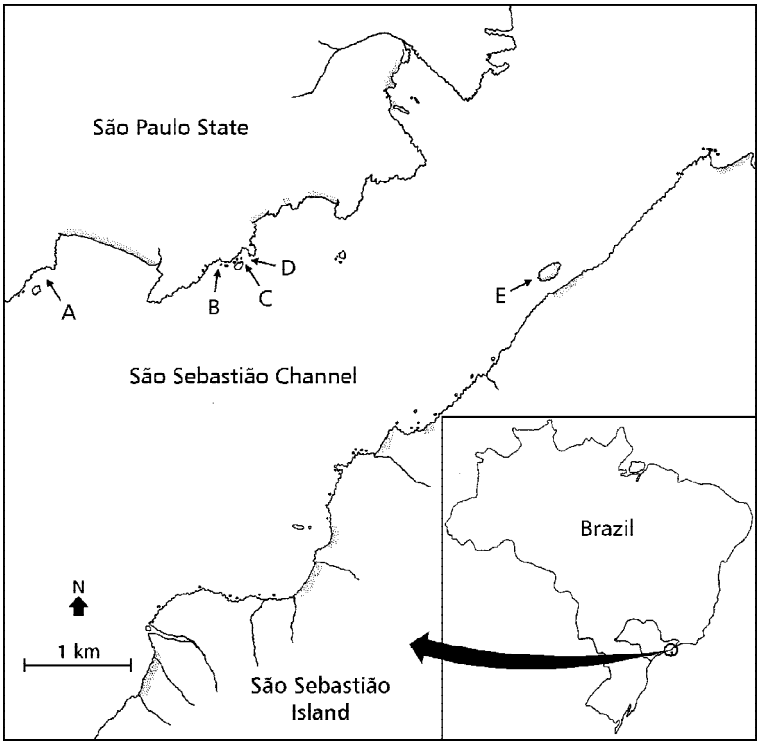

Fig. 1 — Map of São Sebastião Channel, São Paulo, Brazil. Stations are represented by letters: A – located between Itacuçê and Guaecá point; B – Saco Grande bay; C – South side of Segredo beach; D – North side of Segredo beach; and E – Cabras island.

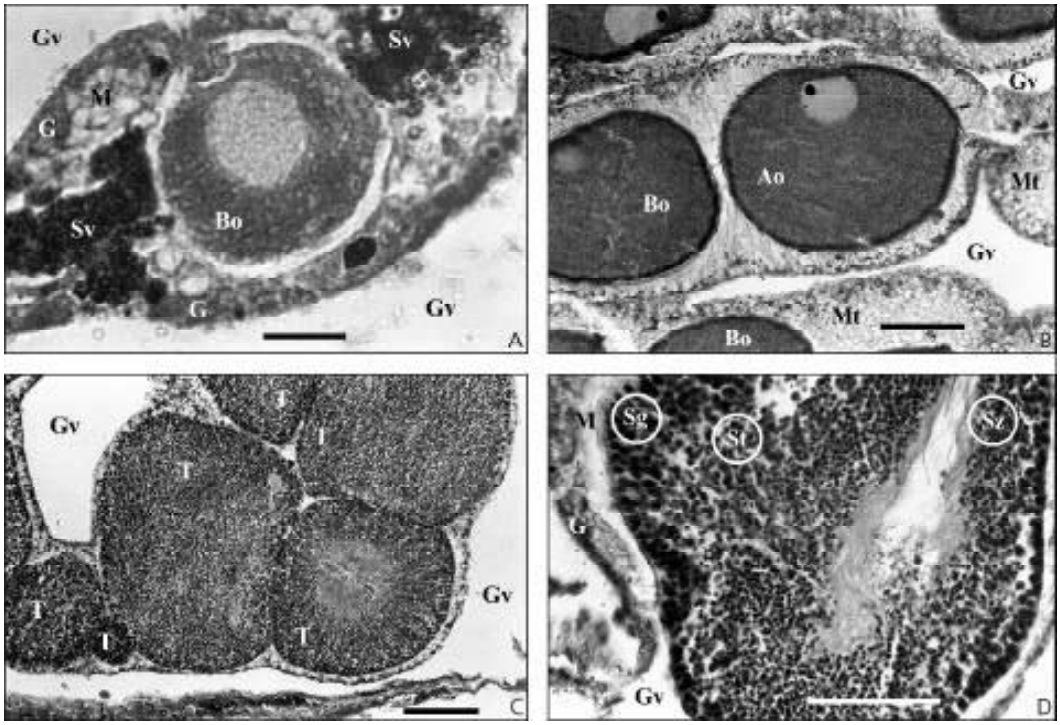

Fig. 2 — Microphotographies of sections of polyp mesenteries in *Palythoa caribaeorum* and *Protopalythoa variabilis*: (A) basophilic oocyte in *P. caribaeorum* (scale: 10 µm), arrows point nuclei of gastrodermis cells; (B) acidophilic oocyte in *P. variabilis* (scale: 50 µm); (C) testis vesicles in *P. caribaeorum* (scale: 25 µm); (D) test vesicle in *P. caribaeorum* (scale: 25 µm). Abbreviations: Ao = acidophilic oocyte; Bo = basophilic oocyte; G = gastrodermis; Gv = gastrovascular cavity; M = mesoglea; Mt = mesentery; Sg = spermatogonia; St = spermatids; Sv = secretion vacule; Sz = spermatozoa; T = testis vesicles.

For both species, the largest percentage of polyps were female, but with different frequencies of fertile polyps between central and peripheral areas in the colonies of *P. caribaeorum* (Table 1). Male polyps were common in *P. caribaeorum*, but not in *P. variabilis*. Hermaphroditic polyps constituted the smallest percentage in *P. caribaeorum*; in *P. variabilis* they were the second most common. Sterile polyps of *P. caribaeorum* occurred more on the edges than within the central area of the colonies (Table 1: (a) and (b)). For both species, the largest percentages of sterile colony areas were at the edge of the colonies (Table 1: (c) and (d)).

DISCUSSION

Gametogenesis seems to be comparable to that of several species of zoanthids, for example: *Palythoa tuberculosa* (see Kimura *et al*., 1972), *Epizoanthus abyssorum* and *Epizoanthus paguriphilus* (see Muirhead *et al.*, 1986), and *Protopalythoa* sp. (see Ryland & Babcock, 1991).

Male and female germinative products in *Palythoa tuberculosa*, and *Protopalythoa* sp. are different from those of *P. variabilis* and *P. caribaeorum* (Kimura *et al.*, 1972; Karlson, 1981; Muirhead *et al.*, 1986; Ryland & Babcock, 1991; Ryland, 1997). Gastrodermis cells may occur in the mesoglea area of oocytes of *P. caribaeorum*, allowing contact with the gastrodermis, the so-called trophonema (Dunn, 1975).

The size of testis vesicles filled with spermatozoa in *P. caribaeorum* is to date the largest recorded for any zoanthid. In *Palythoa vestitus* (see Cooke, 1976), *Zoanthus pacificus* (see Cooke, 1976), *Zoanthus pulchellus* (see Karlson, 1981), *Protopalythoa* sp. (see Ryland & Babcock, 1991), and *Zoanthus solanderi* (see Fadlallah *et al.*, 1984) smaller sizes were observed but there are no comments as to whether these measurements were made on testis vesicles containing spermatozoa. Ryland (2000) observed that in *Epizoanthus couchii,* testis vesicles were smaller and filled only with spermatocytes.

TABLE 1
Reproductive condition in *Palythoa caribaeorum* and *Protopalythoa variabilis.*

Number of perfect mesenteries (min.-max.)	***P. caribaeorum***	***P. variabilis***
	6-19	15-34
Frequency of fertile polyps	72% (a) (n = 559)	83% (n = 373)
Polyps in female reproductive state	36.1% (n = 280)	60.4% (n = 271)
Polyps in male reproductive state	30.2% (n = 234)	0.7% (n = 4)
Polyps in hermaphroditic reproductive state	5.7% (n = 45)	21.9% (n = 98)
Sterile polyps	28% (b) (n = 217)	17% (n = 76)
Colonies in female reproductive condition	41.7%	65.3%
Colonies in male reproductive condition	32%	–
Colonies in hermaphroditic reproductive condition	10.3%	24%
Sterile colonies	16% (c)	10.7% (d)
(a): 81.5% (n = 316) in center of colony and 62.6% (n = 243) on edge of colony		
(b): 18.5% (n = 71) in center of colony and 37.6% (n = 146) on edge of colony		
(c): 8% in center of colony and 24% on edge of colony		
(d): 7.7% in center of colony and 13.5% on edge of colony		

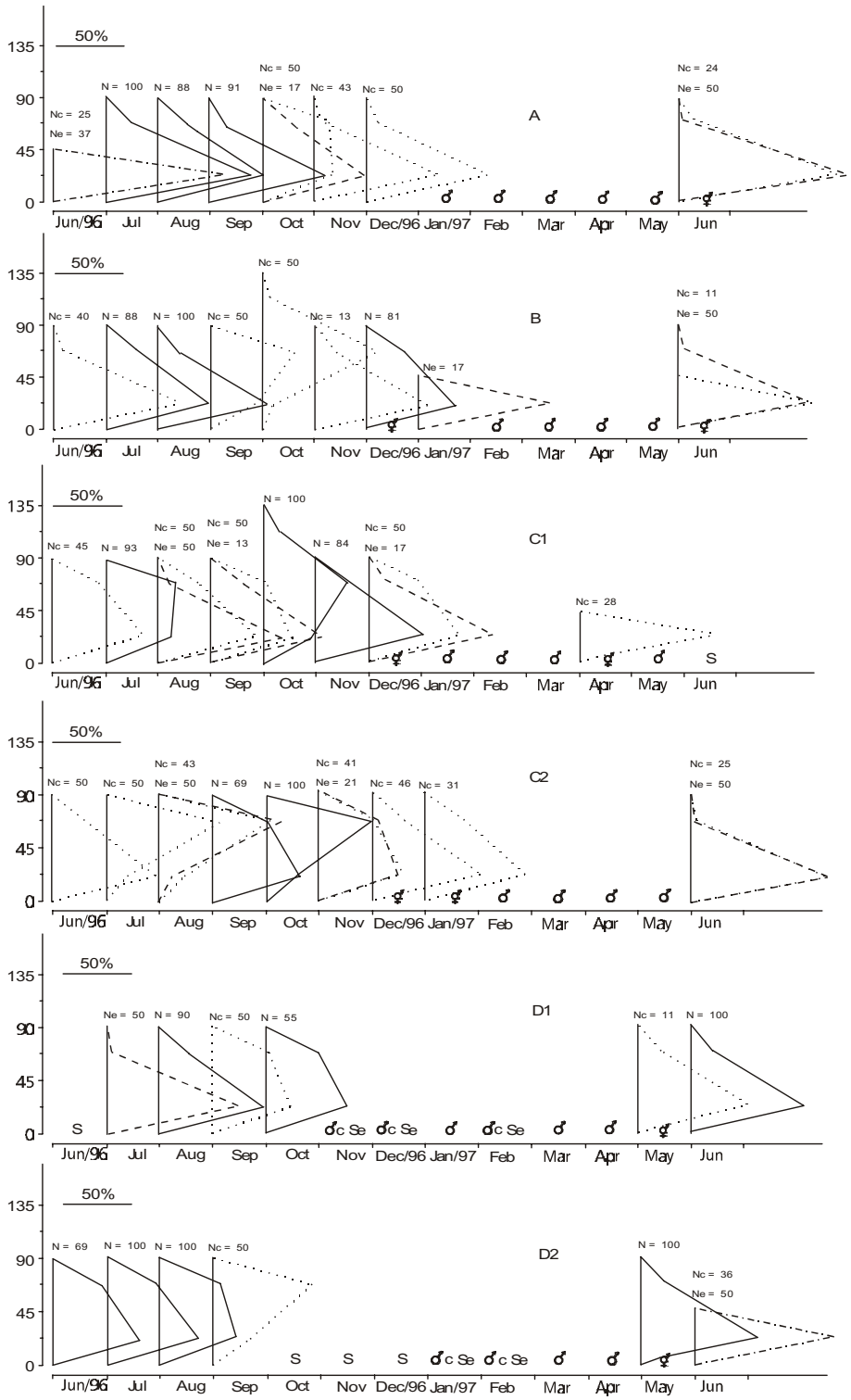

Fig. 3 — Size frequency polygons of oocytes in *Palythoa caribaeorum* from 7 June 1996 to 17 June 1997. Dotted polygons are representative from centre of the colony and dashed polygons are representative from edge of the colony (see Material and Methods). Abbreviations: A, B, C1, C2, D1 and D2 are stations explained in Material and Methods; S = sterile colony; Se = sterile in the edges of the colony; ♂= male sex condition; ⚥= hermafroditic sex condition; ♂c = male sex condition only in the centre of the colony; N = number of oocytes measured; Nc = number of oocytes measured in the centre of the colony; Ne = number of oocytes measured in the edges of the colony.

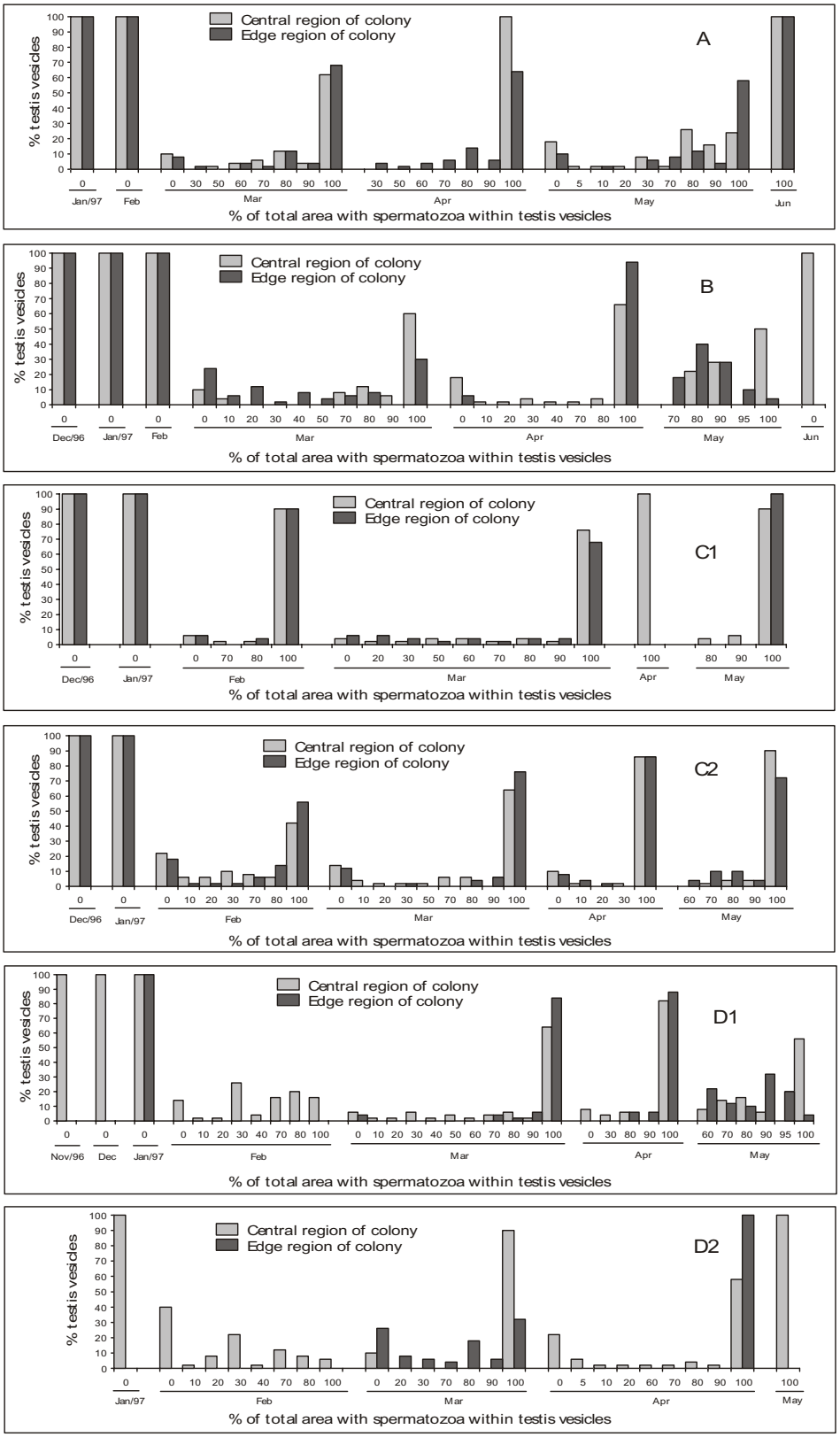

Fig. 4 — Diagrams showing male reproductive condition in all colonies (male or hermaphroditic) of *Palythoa caribaeorum* from 7 June 1996 to 17 June 1997. The axis 'x' has different scales. Abbreviations: A, B, C1, C2, D1 and D2 are stations explained in Material and Methods.

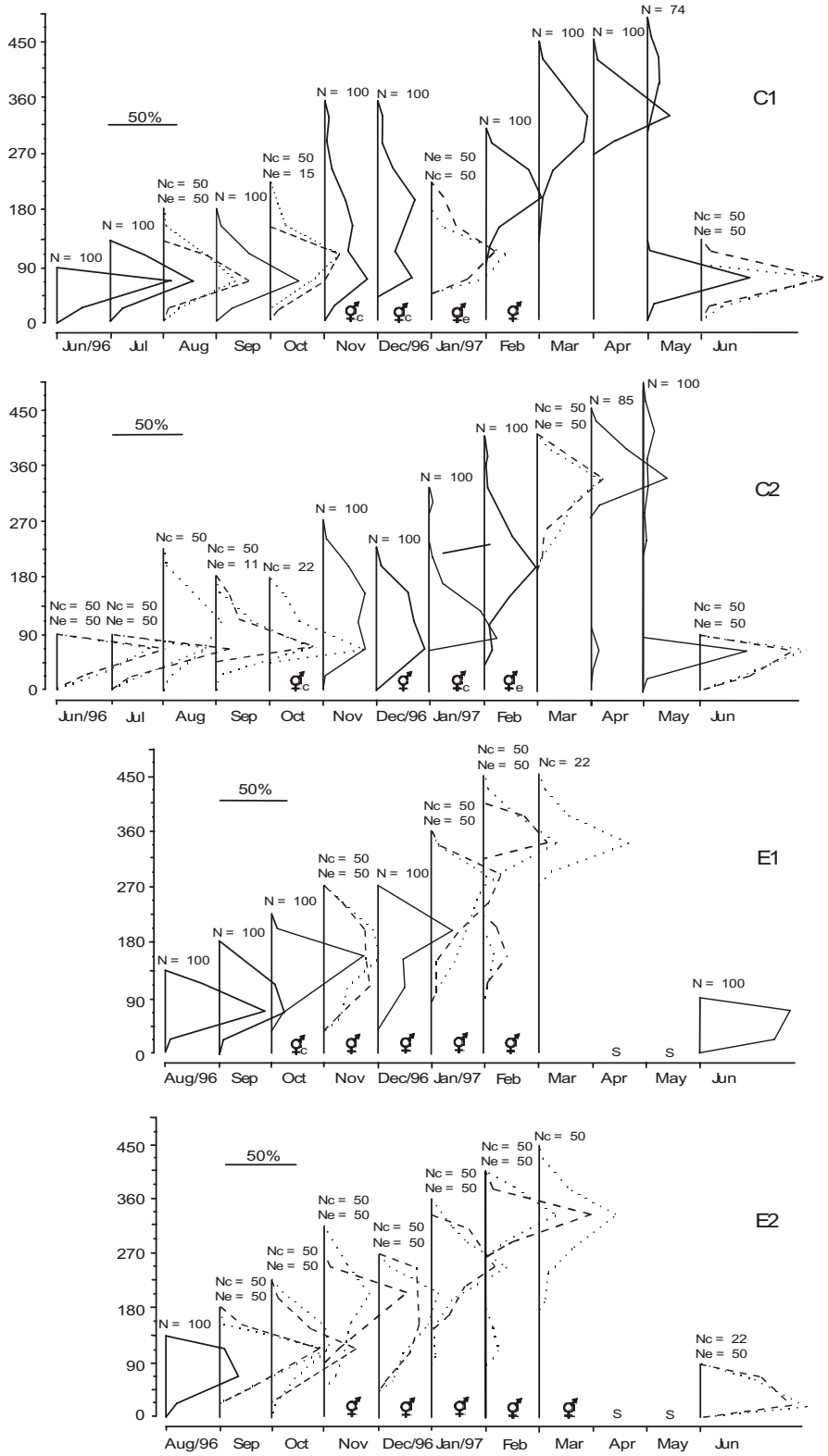

Fig. 5 — Size frequency polygons of oocytes in *Protopalythoa variabilis* from 7 June 1996 to 17 June 1997. Dotted polygons are representative from centre of the colony and dashed polygons are representative from edge of the colony (see Material and Methods). Abbreviations: C1, C2, E1 and E2 are stations explained in Material and Methods; S = sterile colony; ♂**c** = hermaphroditic only in the centre of the colony; ♂**e** = hermafroditic only in the edges of the colony; Nc = number of oocytes measured in the centre of the colony; Ne = number of oocytes measured in the edges of the colony.

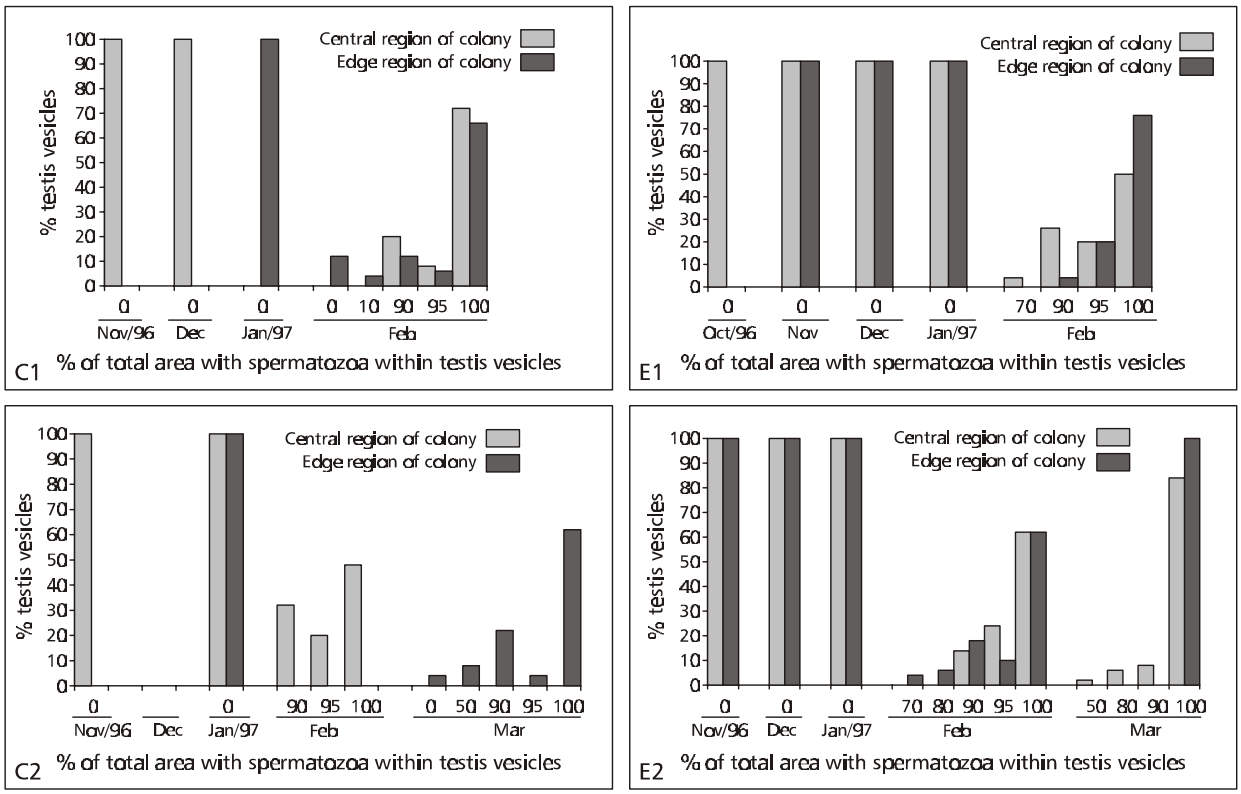

Fig. 6 — Diagrams showing male reproductive condition in all colonies (male or hermaphroditic) of *Protopalythoa variabilis* from 7 June 1996 to 17 June 1997. The axis 'x' has different scales. Abbreviations: C1, C2, E1 and E2 are stations explained in Material and Methods.

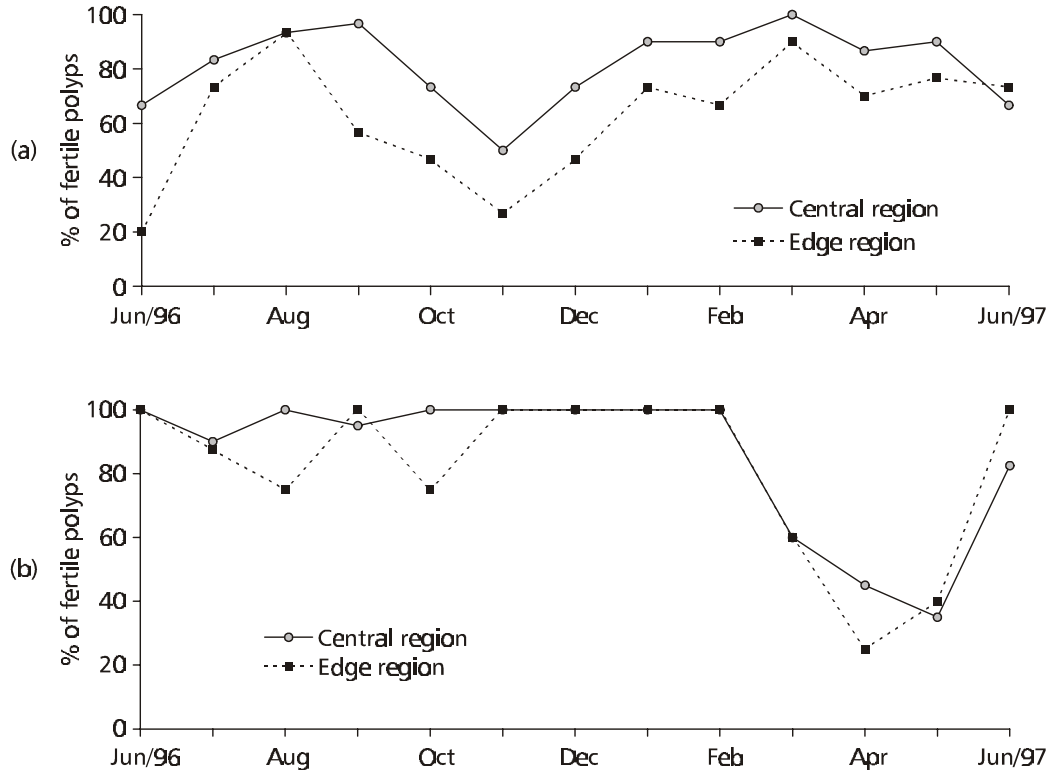

Fig. 7 — Variation in percentage of fertile polyps in all colonies for *Palythoa caribaeorum* (a) (n = 776) and *Protopalythoa variabilis* (b) (n = 449) from 7 June 1996 to 17 June 1997.

The colonies of *P. caribaeorum* and of *P. variabilis* showed high percentages of fertile polyps in relation to what was observed in *Protopalythoa* sp. (39%) on the Great Barrier Reef (Australia) (Ryland & Babcock, 1991), and in *Z. sociatus* (16.5%) and *Z. solanderi* (5.2%) in Jamaica (Karlson, 1981). The variable frequency of fertile polyps in zoanthids may depend on local abiotic factors (Cooke, 1976; Karlson, 1981; Fadlallah *et al.*, 1984; Ryland & Babcock, 1991). Karlson (1981) believed that the difference in fecundity was inversely proportional to polyp size in polyps of *Zoanthus pulchellus*, which are smaller than polyps of *Z. sociatus* and *Z. solanderi*. Although the polyps of *Z. pulchellus* are only half the size of polyps of *P. variabilis*, the percentage of fertile polyps is very similar (81% for *Z. pulchellus;* 83% for *P. variabilis*). Some colonies of *P. variabilis* had fewer fertile polyps, the same having previously been observed for *Z. pacificus* in Hawaii (Cooke, 1976).

In the hermaphroditic colonies of *Zoanthus* spp., polyps vary in reproductive activity (Karlson, 1981). This variability was also observed in polyps of *P. caribaeorum* and *P. variabilis* in the present study. Karlson (1981) argued that this variation in *Zoanthus* spp. could be a consequence of polyp mortality and colony regeneration.

The largest percentage of polyps were female, corroborating the hypothesis of Ryland (1997) that there are more polyps in the female state at any time, an expected trend because of the time needed to mature the eggs. Nevertheless, this hypothesis is not always valid because the reproductive state in polyps varies greatly. For *Palythoa tuberculosa,* Yamazato *et al.* (1973) reported a high percentage of sterile polyps, followed by polyps in the male reproductive condition.

Compared with the centers, the edge regions of colonies of *P. caribaeorum* showed a higher percentage of sterile polyps, something similar to that observed in *Palythoa tuberculosa* (see Yamazato *et al*., 1973). In addition, the edge region of colonies of both species showed a larger sterile-area percentage. As has been shown for interclonal border warrior anemones of *Anthopleura elegantissima* (Francis, 1976), some of the edge region areas for both species of zoanthids perhaps lack energy reserves for polyp gamete production. Moreno (1999) observed some strong antagonistic effects between border regions of colonies of *P. caribaeorum* in the same area as that of the present study.

When considering the sex condition of the species, it seems more appropriate to refer to the colony instead of the polyps (Ryland, 1997), although Kimura *et al.* (1972), Karlson (1981), and Fadlallah *et al.* (1984) considered only the polyps. The sex condition of a species is assessed by means of sequential sampling in tagged colonies, as has already been stressed by Yamazato *et al.* (1973). The colonies of *P. variabilis* and *P. caribaeorum* were fertile during the investigated period, with female, male, and hermaphroditic polyps. This characterizes a sequential hermaphroditism with an initial asynchronous oocyte production, followed by the development of testis vesicles. Hermaphroditic species are common within the brachycnemic zoanthids (Ryland, 1997). Protogynous hermaphroditism was also observed in colonies of *P. tuberculosa* and *Protopalythoa* sp. (Yamazato *et al.*, 1973; Ryland & Babcock, 1991).

Some colonies of *P. caribaeorum* and *P. variabilis* have been proven sterile at certain times. Ryland (1997, 2000) has observed "non-reproductive" colonies among breeding zoanthids. Yamazato *et al.* (1973) believed that at certain times all colonies show great numbers of sterile polyps. Nevertheless, we consider that *P. caribaeorum* and *P. variabilis* showed continuous gametogenesis (*sensu* Giese & Pearse, 1974), with interruptions in gametogenesis among colonies. We interpret the two colonies of *P. caribaeorum* that had oocytes in a size class larger than 90 µm as demonstrating reproductive peaks that reflect subtle environmental fluctuations (Giese & Pearse, 1974).

The oocytes of *P. variabilis* were larger in size than those of any of the other species of zoanthids (see Yamazato *et al*., 1973; Cooke, 1976; Karlson, 1981; Fadlallah *et al.*, 1984). They had no zooxanthellae such as those observed in *Protopalythoa* sp. before spawning (Ryland & Babcock, 1991). In *P. caribaeorum*, the largest oocyte observed was smaller than the oocytes observed by Fadlallah *et al.* (1984) in Panama.

For the investigative period, the hypothesis that *P. caribaeorum* continuously spawns is based on the fact that the largest oocyte ever observed measured 112.5 µm in diameter (squash), or 51.25 µm (histological sections). The largest size observed by Fadlallah *et al.* (1984) was 430 µm (squash). Considering that size reduction of preserved oocytes reaches around 60% after embedding (as observed for *P. variabilis*), in serial sections one expects the maximum diameter observed for *P. caribaeorum* to

be ~ 172 µm. Therefore, the oocytes of *P. caribaeorum* may mature very rapidly into the acidophilus state and would then be quickly released, which is likely either in a space-limited mesentery and gastrovascular cavity or crowding caused by numerous developing eggs. Many broadcasting marine invertebrates are said to spawn primary oocytes (the expected acidophilus state of cells in this work), which are diploid cells that complete meiosis in the surrounding water or following fertilization (Giese & Pearse, 1974).

In this way, *P. caribaeorum* may attain more reproductive success by continuous egg shedding, which may be a strategy to minimize egg depletion and a protection against the effects of possible heavy predation in a single breeding season.

Contrary to *P. caribaeorum,* the colonies of *P. variabilis* showed a specific and apparently annual time for spawning, which occurs in April-May.

The spermatogenic cycle in *P. caribaeorum* presumably occurred over a period of ~ 6 months. The continuous production and liberation of testis vesicles observed possibly resulted from lack of space within both the mesentery and the gastrovascular cavity or by the crowded conditions caused by many developing testis vesicles. In *P. variabilis*, the spermatogenic cycle lasted approximately 5 months, with sperm release over 2 months. Spermatogenesis in both species is well delimited, suggesting seasonality.

In colonies of *P. variabilis*, the spermatogenic cycle was shorter than that of the egg cycle, which is similar to what was observed with species of *Protopalythoa* sp., and occurs because the testis vesicles mature more rapidly than the oocytes (Ryland & Babcock, 1991). The colonies of *P. variabilis* showed some synchrony between release of eggs and sperm.

In *P. caribaeorum,* oocyte production was generally interrupted during spermatogenesis. This may result from the great number of vesicles observed in the mesenteries, a feature that increases the chances of eggs released by other colonies of the local population to be fertilized (Babcock *et al.*, 1992).

The reproduction of marine animals is influenced by various factors, e.g., temperature, salinity, and food availability, but it is unclear how these factors may interact in a series of complex events leading to gametogenesis and spawning (Pearse *et al.*, 1991). Cooke (1976) believed that one, or a few of the factors above, triggers, but does not control, reproduction in *P. vestitus* and *Z. pacificus*. Fadlallah *et al.* (1984) suggested that repeated exposure to air during low tide, and consequent exposure of the colonies of *P. caribaeorum* and *Z. sociatus* to high temperatures, might be a cue to spawning. In the case of spawning in marine invertebrates, food availability, which in the rich upwelling waters near Cabo Frio, Brazil, favors just-released planktotrophic larvae, must also be taken into account (Ventura *et al.*, 1997). Temperature seems to be the ultimate factor influencing the reproduction of *Protopalythoa* sp. in the Great Barrier Reef (GBR) in Australia, because no reproductive activity was observed while seawater temperature was either high or declining (Ryland & Babcock, 1991). But temperature is only one of many factors that determine the best breeding season (Grahame & Branch, 1985).

Unsettled issues involving the timing of gamete production in different invertebrate animals as well as continuous *versus* seasonal reproduction remain. The traditional assumption has been that under stable environmental conditions many invertebrates would present continuous reproduction (Giese & Pearse, 1974; Barnes, 1975). However, whereas the broadcasting stony corals of the GBR tend to have an annual gametogenic cycle with a definite spawning period (Harrison & Wallace, 1990), brooding stony corals at the same site tend to have multiple gametogenic cycles without a definite spawning period within each colony (Harrison & Wallace, 1990). But Harrison & Wallace (1990: 162) noted that "Reproductive cycles of some corals in which gametogenesis has been studied cannot be clearly interpreted". They also stated that occurrence of mature gametes or planulae could be a result of "…multiple gametogenic cycles within each coral, or asynchronous reproduction among members of the population".

The average monthly mean of the temperature for surface seawater in SSC (Table 2) showed the largest variation for the period November-December 1996. Maybe such a variation triggered the onset of spermatogenesis, as observed in four colonies of *P. caribaeorum*. A possibility also exists of a correlation between the higher temperatures in December-May (see Table 2) and that in the same period both species in all colonies were developing testis vesicles.

The local populations of *P. caribaeorum* and *P. variabilis* seem to follow the reproductive patterns already observed for some tropical species, with continuous annual gametogenesis coupled with a defined spawning period.

TABLE 2
Monthly means temperatures of superficial surface seawater (°C) in São Sebastião.

	Years			
	1980-1994	**1995**	**1996**	**1997**
January	26.1	27.7	27.4	24.9*
February	26.2	26.9	26.6	24*
March	26.3	27.1	26	23.6*
April	25.7	25.3	25.5	24.4*
May	24.5	24.3	21.8	23.1*
June	22.4	21.8	21.6*	21.9*
July	21.3	21.5	19.6*	
August	20.9	21.7	19.8*	
September	21	21.9	20.9*	
October	22.6	22.4	22.1*	
November	23.8	23.9	21.9*	
December	24.8	25	26.1*	

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