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THE WIDELY-DISTRIBUTED INDO-PACIFIC ZOANTHID *PALYTHOA TUBERCULOSA:* A SEXUALLY CONSERVATIVE STRATEGIST

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ABSTRACT

Here we characterize the reproductive strategy of *Palythoa tuberculosa* (Esper, 1791) in the northern Gulf of Aqaba. Polyp sampling revealed that *P. tuberculosa* is a protandrous hermaphrodite over its life cycle, but has an annual protogynous hermaphroditic cycle. Fecundity was highest in the center of the colonies, with a mean (± SD) of 1196 ± 629 oocytes polyp^{-1}. Spawning coincided with a rise in seawater temperature, and occurred on the first week after full moon each month (June–August). Gamete release and fertilization success peaked 2 d after full moon. Larvae developed into zoanthellae 13 d post-fertilization. High fecundity and long larval developmental periods suggest high investment in sexual reproduction. Nonetheless, a low recruitment was noted on the reef. *Palythoa tuberculosa* appears to maintain its complex reproductive strategy throughout its wide geographic distribution.

Although extremely common in the world's oceans, relatively little is known about the reproduction and development of species of the order Zoanthidea (Cnidaria: Anthozoa, see Babcock and Ryland 1990). The tropical zoanthid *Palythoa tuberculosa* (Esper, 1791) of the family Sphenopidae has been shown to form extensive mats in the shallow inter- and infratidal zones. Its wide Indo-Pacific distribution includes the reefs of Japan, Saipan (Micronesia), Indonesia, Madagascar, and the Red Sea (Reimer et al. 2006). *Palythoa caesia* (Dana, 1846) from the Great Barrier Reef (GBR), Australia, is considered to be conspecific (Burnett et al. 1994, Ryland 1997). *Palythoa tuberculosa* has been intensively studied for its palytoxin, a potent hemolytic natural product (Wu 2009), and also for its mycosporine-like amino acids (MAAs) that function as UV absorbents and antioxidants (Dunlap and Shick 1998).

Most zoanthids are broadcast spawners, with the exception of the cold-water species, *Isozoanthus giganteus* (Carlgren, 1923), which is a brooder. *Palythoa caesia* from the GBR (Burnett et al. 1997) and *P. tuberculosa* from Okinawa, Japan (Yamazato et al. 1973, Shiroma and Reimer 2010), both exhibit a distinct annual gametogenic cycle and a well-defined spawning period. The reproductive strategy of *P. tuberculosa* was studied by Kimura et al. (1972), Yamazato et al. (1973), and Shiroma and Reimer (2010) in Okinawa, Japan, and found to display an annual protogynous cycle. Moreover, Kimura et al. (1972) and Yamazato et al. (1973) reported that the colonies featured both gonochoric and hermaphroditic zooids, with both male and female gonads co-occurring on the same septum or on different ones within a given zooid, as well as a variation in the location of sexual zooids within individual colonies. In Okinawa, this species is a broadcast spawner, releasing gametes during July–August within 5 d after the full moon (Kimura et al. 1972, Shiroma and Reimer 2010).

The gametes of zoanthids develop into larvae of one of two forms: zoanthina (suborder Macrocnemina), which are ovoid and possess a short ciliated median band; and zoanthella (suborder Brachycnemina), which features a distinct longitudinal cilia-band along the ventral axis (Ryland 1997). The latter form was found in *P. tuberculosa* (see Kimura et al. 1972). Reproductive strategies in Anthozoa may be related to taxa, as well as to environmental conditions. Sexuality (i.e., hermaphroditic vs gonochoric), for example, may be considered a taxonomic feature and is thought to be highly conserved in coral species with wide geographic distributions (Baird et al. 2009). On the other hand, reproductive mode (broadcasting vs brooding) may be relatively plastic in this group of animals (Baird et al. 2009). In the present study, we investigated the reproductive strategy of the widely distributed Indo-Pacific anthozoan *P. tuberculosa,* to ascertain whether and what aspects of reproductive strategy are conserved or plastic traits.

Materials and Methods

Study Sites.—Colonies from three sites along the western coast of the northern tip of the Gulf of Aqaba, Red Sea (29°32′85″N, 34°57′47″E), were sampled. Sampling was carried out from the reef table, 0.5–3 m deep, at the Eilat Underwater Observatory, Lighthouse, and Princess Hotel sites (Fig. 1) throughout 2001/2002. Sea surface temperature (SST) at Eilat was measured daily at the Eilat Underwater Observatory site, using a hand-held Hg thermometer (0.1 °C precision).

Sexuality, Annual Gonad Development, and Fecundity.—For this part of the study, a section measuring ~4–5 cm^2 was removed from either center or margin of each colony. In the laboratory, the polyps were cut using a scalpel into 5 mm thick serial slices (~6–8 slices per sample), from which gonad development and sexuality were determined under a dissecting microscope. Additionally, in each sample, gonad coloration and arrangement on the septa and in the polyps were recorded. In order to determine colony sexuality, 5–25 polyps were drawn from the center of colonies of various sizes, ranging from 4–200 cm^2 (n = 16 colonies in total), measured using a 2 × 2 cm^2 quadrate, and collected from the Lighthouse site (Fig. 1). The sexuality of polyps within colonies and annual gonad development was determined during July 2001–September 2002 by monthly sampling of large random colonies (> 9000 cm^2;

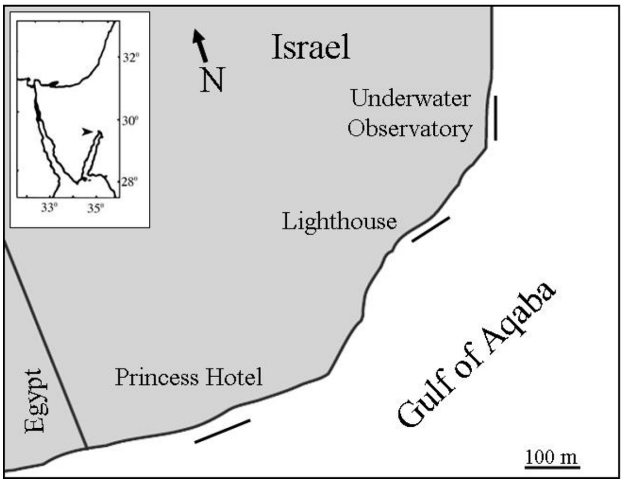

Figure 1. Map of the study sites in the northern Gulf of Aqaba. Collection sites indicated by lines along coast.

n = 8–13 colonies mo^{-1}). Samples were removed from the center and margins 10 d before the full moon of the month, and 9–52 polyps from each colony were examined as noted above. In order to compare between the reproductive features of polyps from the margins and center of the colonies, three large random colonies (> 9000 cm^2) were sampled. Five sections, 3–4 cm^2 each, were removed from different parts of these colonies and examined under a dissecting microscope while still alive.

In October 2001, when the sexuality of the colonies was difficult to determine under a dissecting microscope, a septum was removed from five randomly selected polyps for histological examination. The septa were removed from the polyp and fixed in 4% seawater-formaldehyde for 24 hrs, carefully rinsed with fresh water, placed in 70% ethanol, and later dried in 80%, 90%, and 100% butanol. They were embedded in paraffin and serially sectioned (8 μm thick), glued onto slides, and stained with Hematoxylin-Eosin. The sections were examined under a microscope (Nikon, Optiphot), and gonad diameter was measured using an eyepiece scale. Gametocyte size was recorded and corrected allowing a 20% tissue shrinkage (Ryland and Babcock 1991). To determine size distribution of the gonads, the oocytes and spermaries from 2–3 polyps of each of the monthly sampled colonies were removed and measured under a dissecting microscope.

To determine the fecundity of the polyps, as assessed by the number of oocytes per polyp (see below), an additional 23 polyps from the center of five colonies were sampled on 2 May, 2002, 1 mo prior to spawning (see Results). Samples were removed from the center of the colonies, where fecundity was assumed to be highest. Fecundity was calculated by multiplying the mean number of oocytes counted per septum (n = 23 polyps) by the mean number of oocyte-bearing septa per polyp (n = 10 polyps). Multiplication of mean was also followed by multiplication of the respective standard deviations.

Gamete Release.—During June–August 2002, spawning was observed and gametes were collected one night before the full moon and for the following 4–6 nights (for clarification purposes, the day of full moon was regarded as day 1). For this purpose, plankton nets (200-μm mesh) were placed over 8–16 colonies growing on protruding substrate at the Underwater Observatory and Lighthouse sites on the afternoon (17:00 hrs) prior to each night. The nets were cone-shaped with 50 cm base-diameter, and 80 cm high, with a 1-L PVC container attached to their top, designed to collect the released eggs (see Ryland and Westphalen 2004). Each morning, the nets were removed from the colonies and the fertilized eggs were immediately counted under a dissecting microscope and then placed in 3 L containers filled with filtered seawater (FSW, 20-μm GFF) for rearing the larvae (see later). The water was replaced daily for the first 2 wks and thereafter every 2–3 d (Ben-David-Zaslow and Benayahu 1996). To avoid potential abortion of gametes, as observed in colonies grown in tanks (Polak 2003), only colonies releasing > 50 eggs per night were used to calculate the spawning percentage of the population per night. The percentage of successfully fertilized eggs out of the total number of spawned ones was calculated 36 hrs post release (June and July 2002).

On 27 June, 2002, 2500 fresh eggs were transferred to 50-ml PVC vials, each with 50 zygotes (n = 50 vials). The larvae were reared in FSW in the laboratory at 25–26 °C and under a lighting regime of 12L:12D. FSW was replaced every 2–4 d for a 5-mo period, and the survivorship and activity of the larvae were monitored under a dissecting microscope. We recorded, using slow motion film shot under a dissecting microscope, the whole body ciliary-movement and the fin-band stroke-rate per second. Fifty embryos were retrieved at each of the following time points post spawning: 0.5, 1.5, 2, 3, 4, 6, 8, 10, 12, 15, and 26 hrs, and then on days 2, 4, 17, and 28. At each retrieval, embryos were fixed in 2.5% seawater glutaraldehyde, dehydrated through a graded series of alcohol, critically point dried, gold-coated, and examined with a Jeol JSM 840A scanning electron microscope (SEM) at 25 kV.

Data Analysis.—A comparison was made between polyps from the center and the margins of colonies using Mann-Whitney U test. Gamete size and the spawning of eggs per colony were assessed by ANOVA, and percentage of fertilized eggs was analyzed using Kruskal-Wallis test. For comparison of number of eggs and spermaries in polyps from the center and

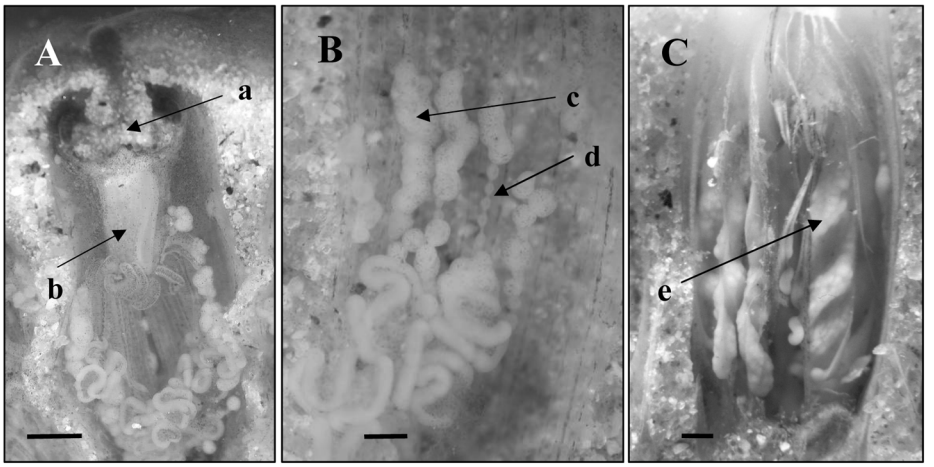

Figure 2. (A) Polyp configuration includes the oral end (a) and actinopharynx (b). Gonadal development of (B) oocytes and (C) spermaria reveals oocytes at late (c) and early (d) development, while mature spermaria (e) are observed on the complete septum. Scale = 1 mm.

margins of the same colony, Wilcoxon signed-ranks and Friedman's tests were applied. For comparison of size of female and male gonads, a t-test was performed.

Results

Annual Gonad Development, Sexuality, and Fecundity.—Wet preparations revealed that *P. tuberculosa* gonads had developed along the complete septa of the polyps and reached the actinopharynx (Fig. 2A). In the hermaphroditic polyps, male and female gonads were found together on the same septum and also on separate ones. In the former, the female gonads developed first in the middle part of the septum facing the polyp cavity, with the male gonads appearing only later as small transparent spheres near the base of the septum.

Maturation of the gonads was associated with changes in their arrangement along the septum. The oocytes that appeared in October 2001 were transparent-white, measuring 49 ± 4 μm diam (n = 21 oocytes). They gradually grew, measuring 479 ± 79 μm in June 2002 (n = 86), becoming irregularly shaped and deep orange in color. During October 2001–January 2002, the oocytes were arranged in a straight line along the septum, protruding on either side, and they subsequently emerged as pairs one on each side. During January–March 2002, a spiral arrangement of the oocytes became evident (Fig. 2B), and in the course of the breeding season (April–July), the large oocytes occupied almost the entire polyp cavity. The round spermaries remained transparent-white throughout their entire development (April–August). In March, they exhibited two size-groups, 59 ± 7 μm (mean ± SD, n = 30) and 227 ± 79 μm (n = 258), and at maturation (June–July) the septal edges acquired a curly appearance (Fig. 2C).

Sexuality of polyps in colonies of *P. tuberculosa* was determined in relation to their size (Fig. 3). Out of 16 colonies examined, 41% contained gonads. Colonies < 38 cm^2 in area (n = 7) did not possess gonads, colonies 38–46 cm^2 in size (n = 4) contained only male polyps, and those > 62 cm^2 (n = 5) contained male, female, and hermaphro-

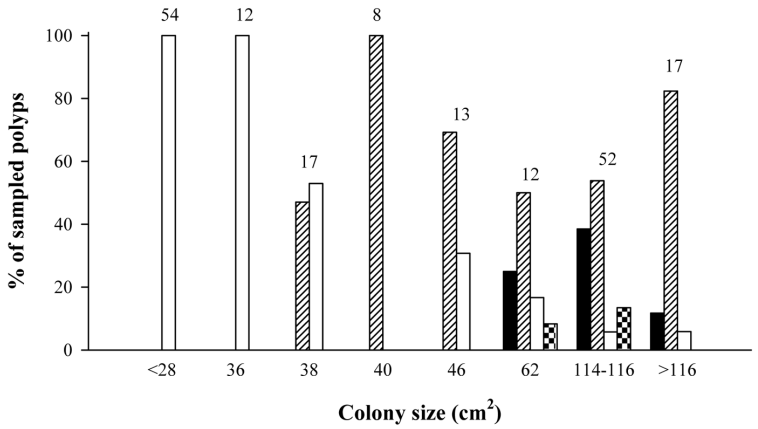

Figure 3. Presence of gonads in different-sized colonies of *Palythoa tuberculosa*. White = sterile, diagonal stripes = male, black = female, and checkered = hermaphrodite. Number of polyps sampled for each size class is indicated.

dite polyps. The vast majority of polyps (73%, n = 81) in the largest (> 62 cm^2) colonies possessed gonads. Further examination of this size group revealed an equal number of polyps with male or with female gonads (Wilcoxon signed-ranks test Z = −0.73, P > 0.4) that were significantly more abundant than the hermaphroditic or sterile polyps (Wilcoxon signed-ranks test: Z = –2.023, P < 0.05).

Palythoa tuberculosa exhibited an annual development of gonads that corresponded to seawater temperature at Eilat. This was evident in both the polyps derived from the margins of the colonies (Fig. 4A) and those from the center (Fig. 4B). Colonies exhibited an annual protogynous pattern, with the female gonads commencing development in November 2001 [mean SST (± SD) = 25.3 ± 0.3 °C] and the male gonads 4–5 mo later, in April 2002 [mean SST (± SD) = 22.0 ± 0.5 °C]. During April–August 2002, those fertile polyps that contained only male or female gonads, in both the margins and the center of the colonies, comprised 64% and 90% of the total number (n = 142 and 61 polyps, respectively; Fig. 4). In May 2002, hermaphroditic polyps were noted for the first time. They were in low abundance in both the center (< 22%, n = 84 polyps) and the margins (< 13%, n = 171 polyps) of the colonies. The highest percentage of polyps with oocytes was found in April 2002 (48%, n = 142 polyps from margins; 82%, n = 67 from center), and the highest percentage of spermaries were found the following June (61%, n = 122 polyps from margins; 72%, n = 161 from center). There were significantly more female polyps in the center of the colonies compared to their margins (Fig. 4, Mann-Whitney U test: U = 1553, N1 = 58, N2 = 75, P < 0.003), but no such pattern was found for the male and the hermaphroditic polyps (Mann-Whitney U test: U = 2170, N1 = 58, N2 = 75, P > 0.9; Mann-Whitney U test: U = 2018, N1 = 58, N2 = 75, P > 0.2, respectively). Development of the gonads coincided with the annual cycle of seawater temperature. Female gonads began to develop during the low temperature season whereas male gonad development commenced with the rise in water temperature and peaked in June 2002, 2 mo prior to the highest mean SST (± SD) of 26.5 ± 0.3 °C. Gamete release also coincided with temperature rise (see below). Similar results were obtained for the three additional largest colonies (> 9000 cm^2: containing 35, 64, and 72 sampled polyps per colony) prior to the

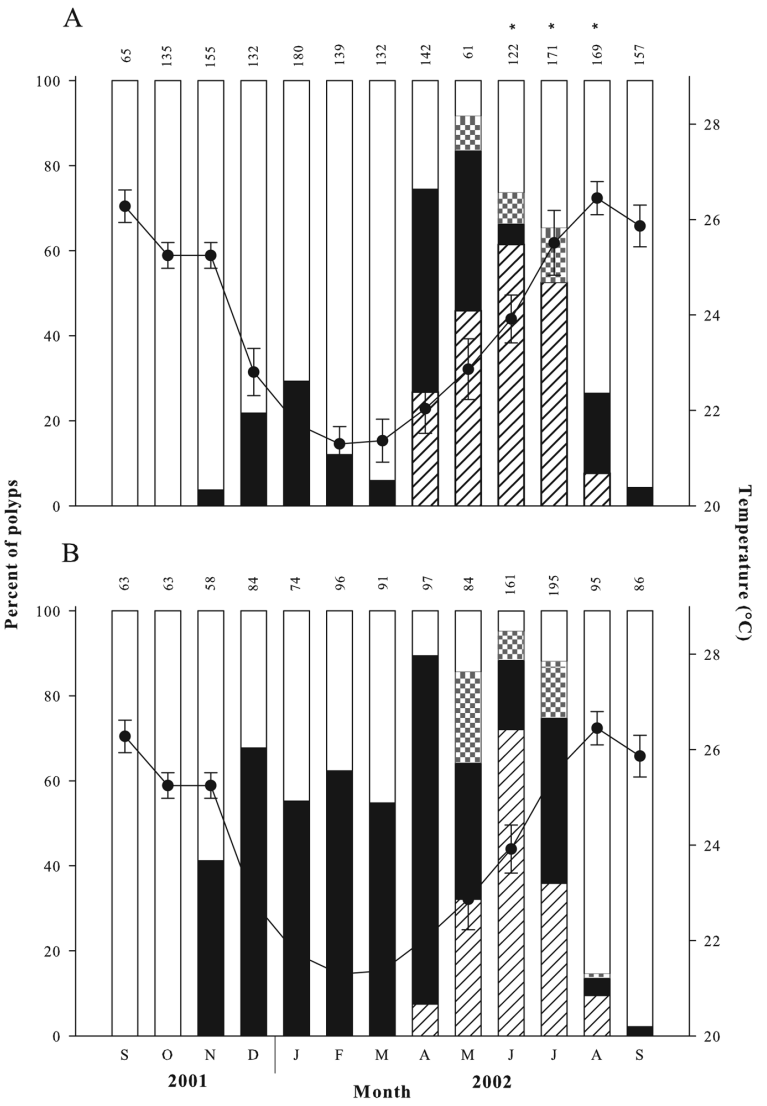

Figure 4. Distribution of sexual polyps at (A) margins and (B) center of *Palythoa tuberculosa* colonies at the reef flat (up to 1 m deep) from September 2001 to September 2002. White = sterile, diagonal = male, black = female, and checkered = hermaphrodite. Number of polyps sampled is indicated. Asterisks indicate months of gamete release. Sea surface temperature (°C) is given on the secondary axis.

expected spawning period. In general, 47% of the polyps contained male gonads, followed by 15% female, and 5% hermaphrodite (n = 171 polyps total in three colonies). The margins of these large colonies (n = 72 polyps) featured 35% male polyps and the rest were sterile. The polyps in the center contained 57% male, 27% female, and 9% hermaphrodite gonads, while 7% were sterile (n = 99 polyps). The mean percentage of male polyps in the center of the colonies did not differ significantly from that of female or hermaphrodite ones in the center (Friedman's test: Q1 = 3.0, P > 0.05), but

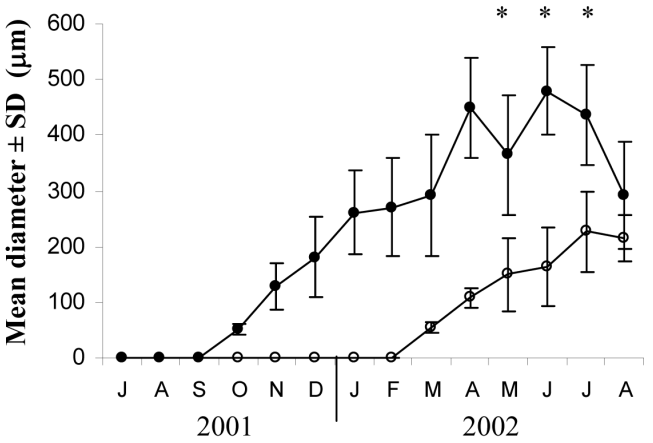

Figure 5. Temporal change in diameter of male (empty circles) and female (full circles) gonads of *Palythoa tuberculosa* (± SD). Asterisks indicate months of gamete release.

differed significantly from the percentage of male polyps in the margins (Wilcoxon sign-ranks test: Z = –2.2, P = 0.028).

Both the oocytes and spermaries of *P. tuberculosa* increased significantly in size during development (Fig. 5). The oocytes first appeared in histological sections in October 2001 and gradually grew, reaching a mean maximum diam (± SD) of 479 ± 79 µm (n = 86 oocytes) in June 2002, 2 mo prior to spawning, although similar sizes were already noted in some cases in April (one-way ANOVA: $F_{(10,1599)}$ = 392.7, post-hoc Scheffé: P > 0.8). The male gonads first appeared in March 2002, 5 mo after commencement of female gonad development, and attained a maximum diam (± SD) in July (227 ± 72 µm, n = 258), being significantly smaller than the oocytes (Fig. 5, t-test: t = 20.6, P < 0.0001). Female polyp fecundity (± SD) in May 2002, 1 mo prior to the first spawning event, reached 1196 ± 629 oocytes (n = 21 polyps, 5 colonies), and after the first spawning event (July) dropped significantly to 122 ± 76 (n = 37, 3 colonies, t-test: P << 0.001).

Gamete Release and Embryonic Development.—Spawning of *P. tuberculosa* colonies occurred at night during three consecutive monthly events (June, July, and August 2002). Gamete release in July was the highest of the three events (χ^2_5 = 29.13, P < 0.001 between June and July; χ^2_3 =5.58, P > 0.05 between July and August). Spawning always commenced at full moon and continued for five successive nights, peaking on the third night (Fig. 6A). The mean number of released eggs (± SD) during July was highest on nights two and three (3916 ± 5038, n = 13; and 3615 ± 5316, n = 12 colonies, respectively) during which there was also a high variability in the number of eggs released per netted colony. These values were more than 25 times greater and differed significantly from all other nights (t-test: t = –3.28, P < 0.005). There was no significant difference in fertilization success among colonies for any night (Kruskal-Wallis: H_6 = 6.0, P > 0.05), and therefore fertilization success for all the colonies was pooled. Fertilization success on night three of each monthly spawning event was the highest of all nights (χ^2_5= 55.96, P < 0.001), and ranged between 86% (total n = 2000 eggs) in June to 90% (total n = 2050 eggs) in July (Fig. 6B). Overall fertilization success was similar between June and July (Kruskal-Wallis: H_6 = 8.868, P > 0.05).

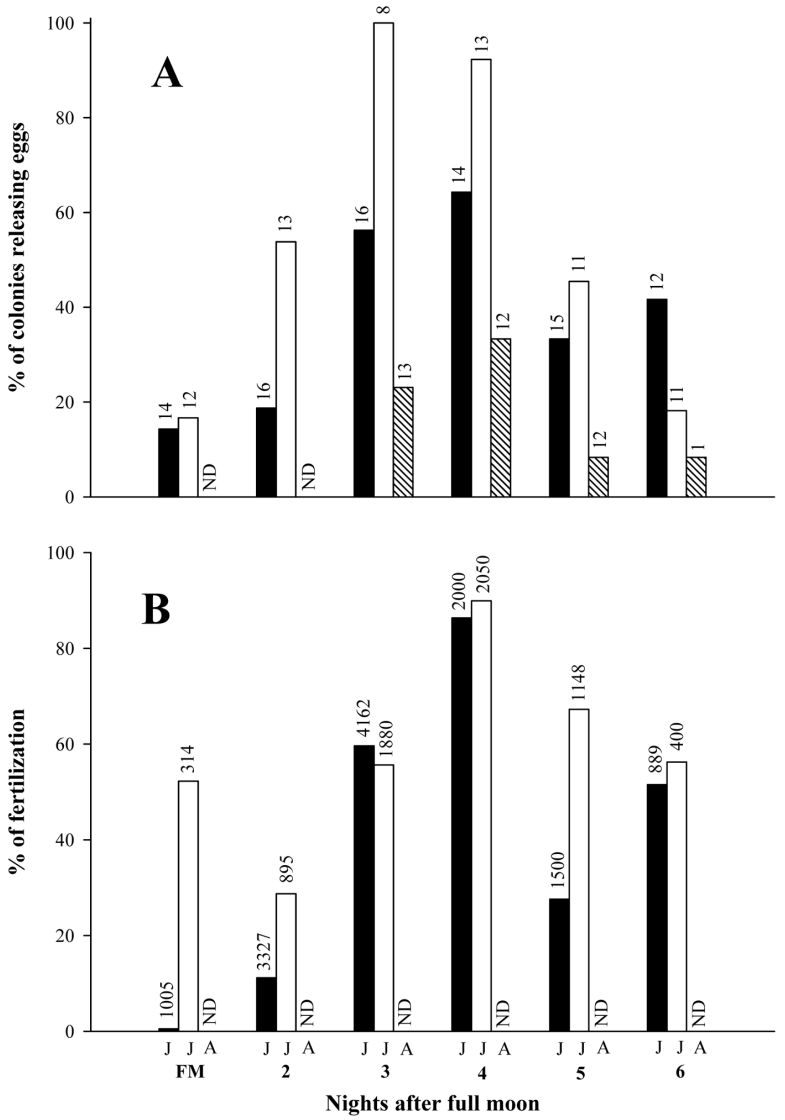

Figure 6. *Palythoa tuberculosa* gamete release during spawning season. (A) Percentage of colonies under plankton nets releasing eggs and (B) percentage of fertilization success (see text for fertilization success calculation; there were no data collection in August). White = June, black = July, and gray = August, 2002. Numbers above bars in (A) indicate number of plankton nets deployed and in (B) indicate number of eggs counted. ND = no data.

The eggs of *P. tuberculosa* were positively buoyant, were fertilized inside the net, and commenced first mitotic division within 30 min of release (Fig. 7A). After the first hour, most of the eggs were U-shaped and had undergone a furrow-elongation towards the two-cell stage. Initially, a transverse shallow furrow appeared, cleaving the egg vertically (Fig. 7B), and then widening (Fig. 7C). This was quickly followed by the holoblastic division resulting in two equal daughter cells (Fig. 7D). Already at this stage, lack of synchronization in the embryonic development was observed, as both

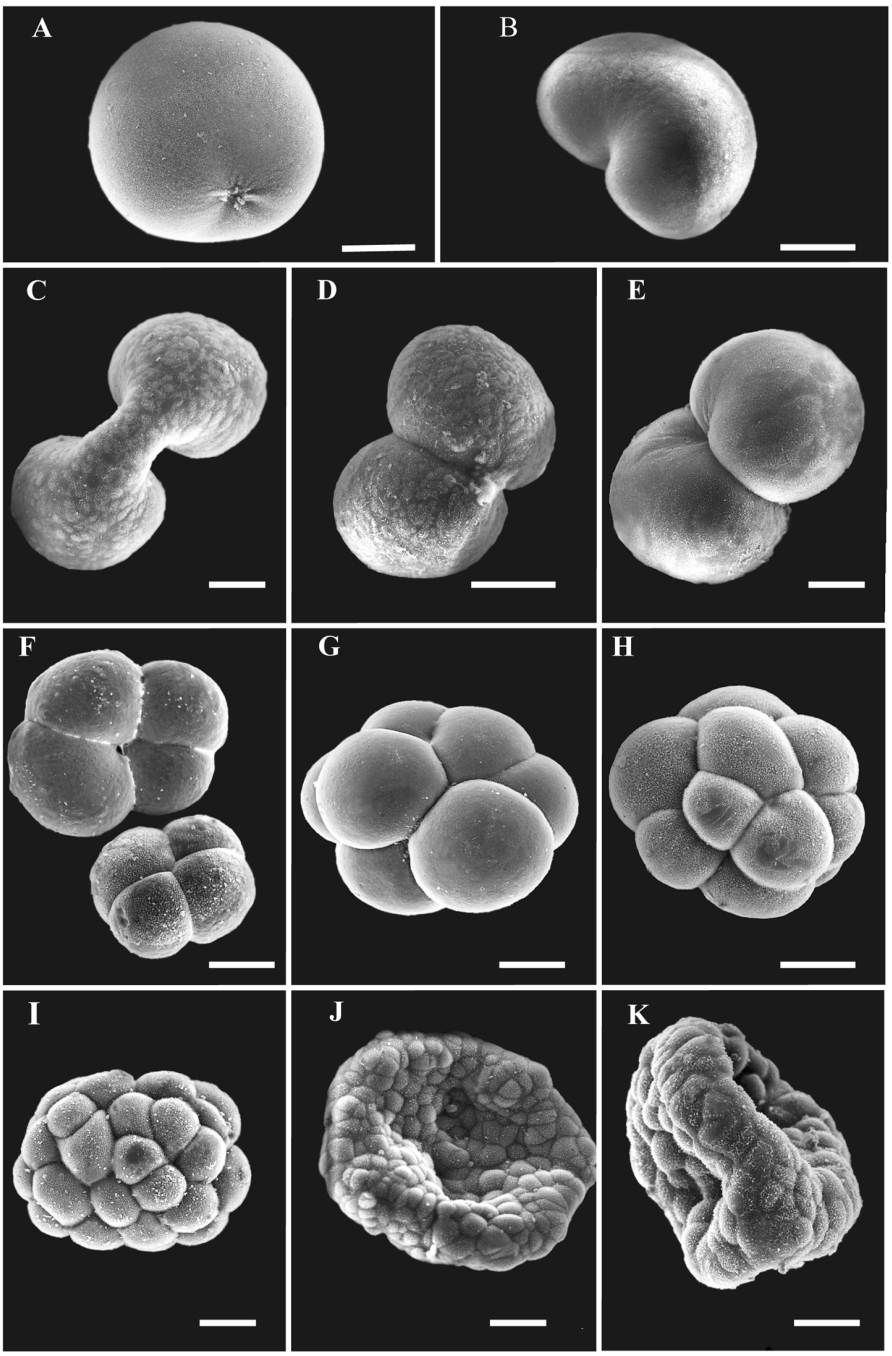

Figure 7. Larval development of *Palythoa tuberculosa* larva from egg to invagination 0–10 hrs post-fertilization. (A) Beginning of first division followed by (B) progression of furrow formation and (C) complete development of two cell units. (D) Beginning of second cleavage at 90° angle to first division and (F) complete 4-cell embryos. A 45° angle shift of the daughter cells forms (G) a pseudospiral 8-cell embryo, which continues to divide spirally to form (H,I) 16-and 64-celled embryos. (J) Flattening of the embryo and first invagination to form a gastrula. (K) A second invagination, forming irregular shaped embryos is about to appear. Scale = 100 µm.

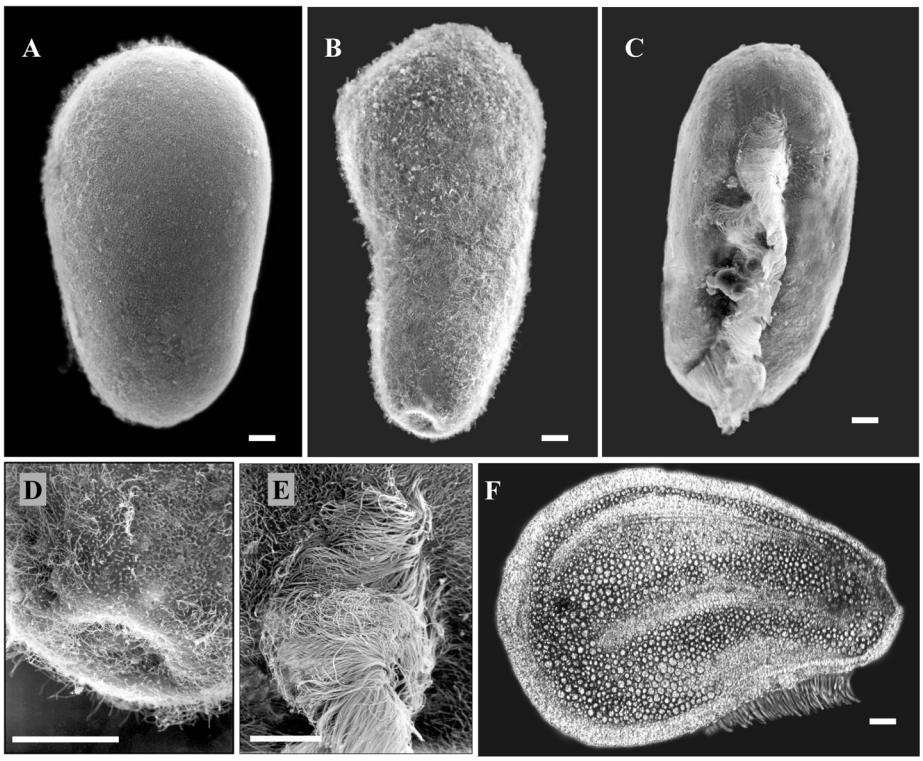

Figure 8. Development of *Palythoa tuberculosa* zoanthella from early to complete stage, 88 hrs until 17 and 30 d post-fertilization. (A) A fully ciliated pear-shaped embryo is seen 88 hrs post-fertilization. At 17 d post-release, the size of the developing zoanthella is unchanged, and (B) a cigar-shaped larva with a distinct mouth is evident, along with (C) a fully developed zoanthellae, with pronounced mouth and (D) their unique longitudinal band of long cilia. (E) A micrograph of 30-d-old zoanthella reveals internal division to mesenteries. Scale = 50 µm.

two- and four-celled embryos co-occurred. The second radial division was perpendicular to the previous one and commenced before completion of the first division (Fig. 7E). Consequently, four equal-celled embryos were present by the third hour (Fig. 7F). Eight and 16 cell-stages were observed 1 hr later, exhibiting a pseudo-spiral and unequal division-pattern (Fig. 7G,H). By the sixth hour, a variety of embryo stages was observed, mainly with 64 cells (Fig. 7I), featuring a prawn chip morphology (see Miller et al. 2000) with a distinct invagination (Fig. 7J). Irregular embryos and a second invagination on the opposite pole were evident by the 10th hour (Fig. 7K). At this stage, the development of the embryos was fully synchronized. Fifteen hrs after release, the embryos possessed a ciliated surface and exhibited slow movement (see below). Elongation of the embryo commenced at the 26th hour, and on day eight, typical oval larvae were observed (Fig. 8A,B). Mouth formation occurred at the end of day two (Fig. 8D). On day nine, there was the first evidence of ventral longitudinal elongated cilia, which became common by day 17, featuring the typical zoanthella shape (Fig. 8C,E). On day 30, developing septa were evident in the larva (Fig. 8F).

Larval Features.—By the 15th hour after egg release, cilia surrounded the spherical embryos of *P. tuberculosa,* and they exhibited a limited spiral movement that

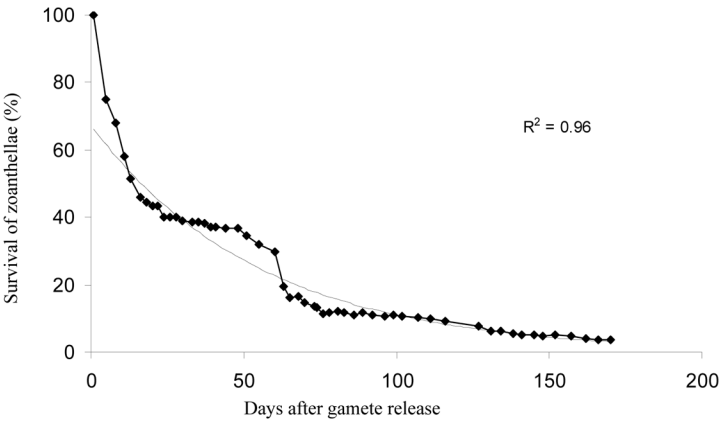

Figure 9. Percentage survivorship of *Palythoa tuberculosa* zoanthellae over time after spawning. Line is exponential regression fit to the data $F_{(0.05,52)} = 1337$, $P < 0.001$).

increased in frequency during the following 20 hrs. The cilia elicited a spiral movement of the larvae along their axis, from the aboral end towards the oral one. On day 13, a ventral longitudinal cilia band was observed, introducing two new movement types. The first was of medium speed, up to 3 mm s^{-1}, associated with ciliary-band movement at 4–5 strokes s^{-1}, as a consequence of which the larvae slowly quivered from side to side. The second movement was at higher speed, 25.3 ± 3.5 mm s^{-1} (n = 5 zoanthellae), revealing several tens of strokes per second, and the larval quivers disappeared. The spiral larval movement was attained concurrently with the appearance of activity of the ciliated band and featured an undulating pattern, running from the aboral to the oral end.

The longevity of the larvae decreased exponentially with time (Fig. 9; $y = 67.3e^{-0.0181x}$, r = 0.96), and 50% survivorship attained by day four. By day 60, the larvae had become round, lost their typical ventral cilia, and the septa had disappeared. By day 170, only 3.7% of the zoanthellae still survived, their motility had decreased to 0.1–1 mm s^{-1}, and they exhibited a spiral movement.

Discussion

Several studies have dealt with the reproductive cycle of zoanthids (Ryland and Babcock 1991, Ryland 1997, Ryland and Westphalen 2004, Ono et al. 2005). In the present study, we describe life-history features of the zoanthid *P. tuberculosa* from Eilat, northern Red Sea, and compare them to what is known for this species and its congeners in other geographic regions. Growing colonies of *P. tuberculosa* were found to be protandrous, beginning as males, but developing into hermaphrodites as the colonies grew. In addition, *P. tuberculosa* from Eilat displayed a "breeding system" similar to that reported for this species from Okinawa, Japan (Kimura et al. 1972), featuring a mixture of gonochoric and hermaphroditic polyps within a single colony.

The hermaphroditic polyps of *P. tuberculosa* contained both oocytes and spermaries along the same septum, and also on separate ones, as recorded for *P. tuberculosa* in Okinawa (Yamazato et al. 1973) and in other zoanthids (Ryland and Babcock 1991).

Table 1. Comparison of distinct sexual attributes of three species of zoanthids of the geus *Palythoa*. *Palythoa caesia* from the Great Barrier Reef (GBR) and *Palythoa tuberculosa* from both Israel (Eilat) and Japan (Okinawa). *TF = teardrop formation, PBP = polyp ball production, PCLO = pseudo-colony life-off.

	P. caesia (GBR)	*P. tuberculosa* (Okinawa)	*P. tuberculosa* (Eilat)
Sexual reproduction			
Sexuality		Mainly gonochoric polyps	Mainly gonochroric polyps
Reproductive pattern	Broadcasting	Broadcasting	Broadcasting
Mature oocyte size (μm, live sample)	≥ 500	300–500	~480
Fecundity (# eggs polyp^{-1})			1,208 ± 629
Breeding period	Early summer	Summer	Summer
# of spawnings	2	Possibly 2	3
Larva type	Probably zoanthella	Zoanthella	Zoanthella
Occurrence of zooxanthellae in larva	No	No	No
Larval longevity			190 d
Recruitment	Low		Low
Reproductive timing	During mass spawning event		Reproductive isolation, during July–August
Asexual reproduction			
Fission	Edge fission	Edge fission	Edge fission
Fragmentation	Yes	Yes	TF, PBP, PCLO*
Spatial distribution	Clumped	Clumped	Clumped
Reference	Burnett et al. 1994, Ryland 1997, Tanner 1997	Kimura et al. 1972, Yamazato et al. 1973, Yamazato and Isa 1986, Shiroma and Reimer 2010	Polak 2003, present study

Similar to what has been reported for many stony corals (Harrison and Wallace 1990), soft corals (Benayahu 1997), and sea anemones (Scott and Harrison 2009), oocyte development of *P. tuberculosa* is accompanied by a color change, as observed both in Okinawa (Shiroma and Reimer 2010) and in the present study.

Within the colony, the position of polyps of different sexual states may relate to energetic constraints, depending on budding and growth occurring at the edge of the colony (Boscolo and Silveira 2005), or as a result of the polyps at the margins being in competitive interactions with adjacent benthic organisms. Such constraints have been discussed for stony corals, where there are differences in the location of fertile polyps within colonies (Soong and Lang 1992), with polyps at the margins or tip often being sterile or only male. Similar patterns were noted for the zoanthids *P. tuberculosa* from Okinawa (Kimura et al. 1972, present study) and *P. caribaeorum* from the Caribbean (Acosta and Asbahr 2000, Boscolo and Silveira 2005). This may strengthen the premise that the trade-off between the available energy resources required for growth, budding, and gonad production may determine reproductive patterns and fecundity (see Soong and Lang 1992) of the polyps in these colonies.

Interestingly, the colony size at first reproduction of *P. tuberculosa* in Eilat was 32 cm^2; a size that is much larger than that of its congener *P. caribaeorum* from the Caribbean (6 cm^2, see Acosta and Asbahr 2000). It is possible that the development of small sterile or only male colonies may reflect the energetic conservation occurring in young polyps, allowing somatic growth prior to achieving full sexual development. As the colony matures and amasses more energetic reserves, it can then

develop female gonads as well, again strengthening the concept of energetic trade-off between growth and sexual reproduction (Soong and Lang 1992).

Sexually mature hermaphroditic colonies of *P. tuberculosa* exhibit an annual protogynous cycle, with fertile polyps developing female gonads 5 mo prior to male gonads. This protogynous cycle is characteristic of both the Okinawa and Red Sea populations, and may be a result of the higher energy requirement for the development of female gonads (Hall and Hughes 1996). Richmond and Hunter (1990) argued that in many coral species egg production may last > 10 mo, whereas sperm production takes only a few weeks. Similarly, Benayahu (1997) demonstrated that several soft coral species have a prolonged oogenesis of 22–23 mo, whereas spermatogenesis in those species is much shorter. It is therefore suggested that in *P. tuberculosa,* the higher energy expenditure required for development of female gonads (i.e., because it commences 5 mo earlier) leads to a prolonged maturation period. Additionally, average oocyte size at maturity was large (~300–500 μm) and similar in both regions (present study and Kimura et al. 1972), further stressing the high energy expenditure during gametogenesis.

Among corals, high gamete production suggests a remarkably high parental investment in sexual reproduction (Hall and Hughes 1996). Polyp fecundity of *P. tuberculosa* features values of 1195 ± 629 (n = 21 polyps) oocytes polyp^{-1}, resembling those recorded in *P. caesia* (Ryland 1997). This investment not only enables high fecundity (though not recruitment), but may also play a role in promoting larval longevity. Comparison of *P. tuberculosa* and *P. caribaeorum* suggests a close similarity in their life-history features. The similarity in gonad size, fecundity, annual reproductive pattern, timing, and larval development suggests a conservative reproductive strategy shared by these congeners.

Among Anthozoa, environmental parameters, including tidal range, salinity fluctuations, solar insulation, length of the day, and temperature have been shown to affect gametogenic cycles of stony corals (Harrison and Wallace 1990, van Woesik et al. 2006), soft corals (Benayahu 1991), sea anemones (Scott and Harrison 2009), and zoanthids (Cooke 1976, Fadlallah et al. 1984, Ryland and Westphalen 2004, Ono et al. 2005). In the present study, gonad development and gamete release in *P. tuberculosa*, similar to the vast majority of the corals in Eilat, coincided with the rise in seawater temperature occurring during the late spring–early summer (see also Shlesinger and Loya 1985, Benayahu 1991). Such environmental parameters appear to allow synchronization of sexual reproduction, as demonstrated for stony corals (Richmond and Hunter 1990).

During the peak spawning season of *P. tuberculosa* in the hermaphroditic colonies, most of the polyps were males, with relatively few female or hermaphroditic polyps. Similar results were obtained by Kimura et al. (1972), who argued that there is a transformation from female-to-male state via a temporary hermaphroditic state. Alternatively, the oocytes could have been reabsorbed, as was found in the stony coral *Stylophora pistillata* (Esper, 1797) from Eilat (Rinkevich and Loya 1979) and the zoanthid *Zoanthus sansibaricus* (Carlgren, 1900) at Kagoshima, Japan (Ono et al. 2005).

In the northern Gulf of Aqaba, the reproductive season of *P. tuberculosa* occurred during the summer (June–August), and gamete release was highest three nights after the full moon. Correlation of spawning with high temperature is common for both Eilat stony and soft corals, as well as for zoanthids in numerous geographic locations

(Shlesinger and Loya 1985, Reimer et al. 2008). In Japan, this species exhibits either one or two spawning events, as inferred from the disappearance of gonads from the polyps (Kimura et al. 1972), whereas in Eilat, two major spawning events and a subsequent minor event were directly observed. Interestingly, a high percentage of egg fertilization coincided with the timing of maximum gamete release. It is thus suggested that the highly synchronized spawning episodes lead to the high fertilization success, which, in turn, increases the colony's reproductive potential.

In general, the embryonic development of *P. tuberculosa* resembled that of the zoanthella larval type found for example in *Palythoa heliodiscus* (Reimer, 2006) (Babcock and Ryland 1990). Differences are apparent in the first longitudinal division of the fertilized egg, which occurred earlier after egg release (0.5 vs 2 hrs), and in the appearance of the prawn chip stage, which is related to the differentiation of ectoderm and endoderm in the coral *Acropora millepora* (Ehrenberg, 1834) (Miller and Ball 2000). In *P. tuberculosa,* this stage displayed a second irregular invagination, a morphological phenomenon that was also found in the octocoral *Dendronephthya hemprichi* (Kluzinger, 1877) from the Red Sea (Dahan and Benayahu 1998). Motility in embryos of *P. tuberculosa* was noted at the 15th hr post-fertilization, earlier than that recorded for embryos of the congener *P. heliodiscus* (Babcock and Ryland 1990). Despite the precocious development in *P. tuberculosa,* the timing of development of the ventral longitudinal ciliary band was similar in the larvae of the two species, resulting in highly motile larvae at this stage (over a period of 2 wks post-fertilization).

Two motility patterns were evident in the zoanthella larvae. For slow movement the larva utilized a typical spiral movement, and for faster movement it utilized the ciliated ventral band. It is possible that the different modes of movement are utilized for dispersal and settlement search purposes (Scheltema 1986). Contrary to our findings, Babcock and Ryland (1990) did not find any differences in swimming modes in the zoanthella larvae. In *P. heliodiscus,* settlement and metamorphosis of zoanthella could be delayed to 21 d post-fertilization (Babcock and Ryland 1990), and no further morphogenesis occurred during this period. In the present study, *P. tuberculosa* larvae were not observed to metamorphose, but on day 30, those that survived (but did not settle) developed a pharynx and well developed septa. This developmental period in *P. tuberculosa* is longer than in most coral larvae (Harrison and Wallace 1990). Under laboratory conditions, the larvae of *P. tuberculosa* developed ventral cilia, were considered mature on day 13, and exhibited 50% survivorship at this time. They survived for long periods in the water containers and exhibited a negative exponential mortality (Fig. 9). Since the larvae lost their characteristic shape and became degraded only after ~170 d, it is suggested that such long developmental and larval viability periods reflect their potential for dispersal (Ryland 1997) to new or denuded coral reefs. Moreover, Reimer et al. (2007), using ITS-rDNA, showed that *P. tuberculosa* demonstrates no geographic pattern over large distances, suggesting high gene flow. This long-distance dispersal may explain the wide Indo-Pacific distribution of *P. tuberculosa*.

Settlement and recruitment are important stages in the life-history of benthic organisms (Vandermeer and Goldberg 2007). Laboratory and in situ experiments using a variety of substrates like dead corals, granite stones, calcareous algae, tiles, glass, and plastic resulted in < 3.6% settlement of *P. tuberculosa* larvae, and no ensuing metamorphosis (Polak 2003). This is similar to the low metamorphosis rate observed in zoanthellae of *P. heliodiscus* (Babcock and Ryland 1990). This low rate corresponds

to similar rates for *P. caesia* from the GBR (Tanner 1997) and may be reflected in the low recruitment rate of *P. tuberculosa* observed on the Eilat reefs (O Polak, pers obs), and may be due to the lack of appropriate cues or substrates for settlement.

Establishment of populations of *P. tuberculosa* in Eilat may depend more on the rates of asexual reproduction than on settlement of sexual recruits. In this species, the rate of asexual reproduction was ~7.6% (n = 25 colonies, see Polak 2003), a rate that was similar to the 6.9% found for *P. caribaeorum* from Brazil (Acosta et al. 2001). The formation of asexually-produced ramets as new clonal entities seems to be the main means of local population growth, and is reflected in the patchy appearance of *P. tuberculosa* in Eilat (Polak 2003), similar to observations in other zoanthids (Karlson 1988, Bastidas and Bone 1996, Tanner 1997).

In the northern Gulf of Aqaba at Eilat, *P. tuberculosa* exhibits an extensive sexual reproductive effort, and a prolonged period of larval development allowing long distance dispersal. In contrast, the low settlement, metamorphosis, and recruitment rates observed in the present study may reflect the limits of sexual reproduction in this species. Therefore, it is likely that local populations rely more on the extensive modes of asexual reproduction exhibited by this species, and their long-distance dispersal is accomplished via the high longevity and competence of its pelagic larvae.

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