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REPRODUCTIVE SEASONALITY OF THE REEF BUILDING CORAL *PLATYGYRA PINI* ON SINGAPORE'S REEFS

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ABSTRACT. — The gametogenic cycle of *Platygyra pini* was investigated at three sites around Singapore's southern islands from Mar.2001 to Apr.2002. Equatorial locations, such as Singapore, typically experience moderate annual environmental variation. This has lead to the suggestion that the amplitude of environmental variation at the equator is insufficient to provide reliable cues to synchronise reproduction in marine invertebrates. However, distinct and predictable seasonal patterns of sea surface temperature and rainfall occur in Singapore as a result of the Southeast Asian Monsoon system. *Platygyra pini* had a seasonal pattern of gametogenesis, with maturation of gametes and spawning occurring predominantly in April. A second, smaller peak in reproductive activity occurred in November suggesting that some colonies also spawn at this time. The major spawning for this species followed a period of rising sea surface temperatures and occurred after the period of heaviest rainfall. While a correlation between environmental fluctuations and spawning timing is not proof of a causal link, these data do indicate that the amplitude of change in environmental parameters such as temperature in Singapore is sufficient to provide a seasonal cue for reproduction and spawning synchrony.

KEY WORDS. — coral reef, coral reproduction, seasonality, spawning synchrony, *Platygyra pini*, Singapore

INTRODUCTION

Sexual reproduction is a fundamental process in the persistence and recovery of coral reef assemblages. The majority of scleractinian corals are hermaphroditic broadcast spawners and typically exhibit an annual gametogenic cycle, marked reproductive seasonality, and population spawning synchrony (Baird et al., 2009). In addition to high levels of population synchrony, in diverse coral assemblages, spawning times among species often overlap, leading to multi-species synchrony (Harrison et al., 1984). The timing and extent of synchrony within and among species varies both spatially and temporally; however, the factors that drive this variation are not well understood. Early studies indicated a latitudinal breakdown in reproductive seasonality (Oliver et al., 1988), however, more recent studies have shown that, even at the equator, coral populations and assemblages exhibit marked spawning synchrony (Guest et al., 2005a, 2008).

Knowledge about the timing of coral spawning has increased dramatically in recent years with studies emerging from previously under-represented geographical locations such as Southeast Asia (Vicentuan et al., 2008; Kongjandtre et al., 2010), the Red Sea (Hanafy et al., 2010; Bouwmeester et al., 2011), and the Western Indian Ocean (Mangubhai & Harrison, 2008a). Despite this, the role of different environmental factors in the regulation of spawning timing is not fully understood. The most common paradigm is that environmental cues work at progressively finer temporal scales to regulate spawning, each one acting like a 'gate' for different stages in the gametogenic cycle (Babcock et al., 1986). Sea temperature and/or insolation are considered to

be the most likely seasonal cues (van Woesik et al., 2006), while lunar cycles, tides and diel cycles may be involved in regulating the night and time of spawning (Babcock et al., 1986). Seasonal environmental variation may also lead to selection for a particular spawning timing, for example, during the warmest period of the year or outside of the months of heaviest rainfall (Mendes & Woodley, 2002).

Platygyra species including *P. lamellina*, *P. pini*, *P. sinensis*, *P. daedala*, *P. ryukyuensis*, and *P. contorta* have been documented participating in mass coral spawning events in several locations, including: the GBR (Willis et al., 1985, Babcock et al., 1986, Richmond & Hunter, 1990); Western Australia (Babcock et al., 1994); the Central Pacific (Heyward, 1988; Richmond & Hunter, 1990); Japan (Hayashibara et al., 1993); and the Red Sea (Shlesinger & Loya, 1984). The existing studies show all *Platygyra* species to be hermaphroditic broadcasters; however, there are few detailed descriptions of the length and pattern of gametogenic cycles of any *Platygyra* species. One such study investigated the reproductive patterns of two morphospecies of *P. daedalea* on equatorial lagoonal reefs in Mombasa, Kenya and found single annual gametogenic cycles in the majority of colonies, with a low proportion of the population spawning biannually (Mangubhai & Harrison, 2008b).

This paper focuses on the reproductive cycles of *Platygyra pini* from reefs around Singapore's southern islands. Six *Platygyra* species have been recorded on Singapore's reefs and this genus is one of the most common on the reef flats at most sites in the southern islands (Huang et al., 2009). The aims of this study were as follows: a) to describe the annual gametogenic cycles of *Platygyra pini* using histological techniques; b) to examine the extent of reproductive seasonality and spawning synchrony; and c) to examine the relationship between spawning timing and local seasonal environmental patterns.

MATERIAL AND METHODS

Study species. — Typically *Platygyra* colonies are massive and either dome shaped or flattened with meandroid corallites forming valleys of varying lengths. The genus is divided into several morphological species that have either very short or monocentric valleys, or longer meandroid valleys (Veron, 2000). In Singapore *P. pini* has short or mono-centric valleys (generally <10 mm in length), thick walls (~3–5 mm diameter) with rounded edges and is sufficiently abundant to conduct a 14-month study, without repeat sampling of colonies (Fig. 1). While morpho-species of *Platygyra* can be distinguished, there is a great deal of overlap in characteristics among morpho-species (Miller & Benzie, 1997). Due to the taxonomic uncertainties associated with this genus we cannot exclude the possibility that some samples were taken from other morpho-species (e.g., *P. verweyi*, *P. ryukyuensis*) during sampling occasions, particularly during periods of low underwater visibility. Voucher specimens were not collected during this study, thus making it impossible to confirm the identification of every colony that was sampled. Therefore, while we are confident that the majority of sampled colonies were *P. pini*, we recommend caution if making direct comparisons to the data presented in future studies of this species.

Study sites and sample collection. — Samples were collected each month, typically a few days prior to the full moon, for a period of 14 months, between Mar.2001 – Apr.2002, from three sites around Singapore's southern islands: Raffles Lighthouse (Pulau Satumu) (01°9'36.9"N, 103°44'24.7"E), Pulau Hantu (01°13'36.2"N, 103°44'43"E), and Little Sisters Island (Pulau Subar Darat) (01°12'56.3"N, 103°49'48.0"E). On each sampling occasion, four colonies were sampled at each of the three sites, so a total of 12 colonies were sampled each month (total n = 168). It was possible to tell if a colony had previously been sampled by the presence of a sample scar, thus the same colony was not sampled twice during the study period. Small samples of approximately 3–4 cm^2 were removed from colonies by a scuba diver using a hammer and chisel and placed in separate pre-labeled, re-sealable plastic bags at depths between 1–4 m.

Sample processing. — The sample processing procedure followed the method used by Szmant-Froelich et al. (1980) and Glynn et al. (1991). Coral samples were fixed immediately after collection in a seawater–Zenker's solution (50 g zinc

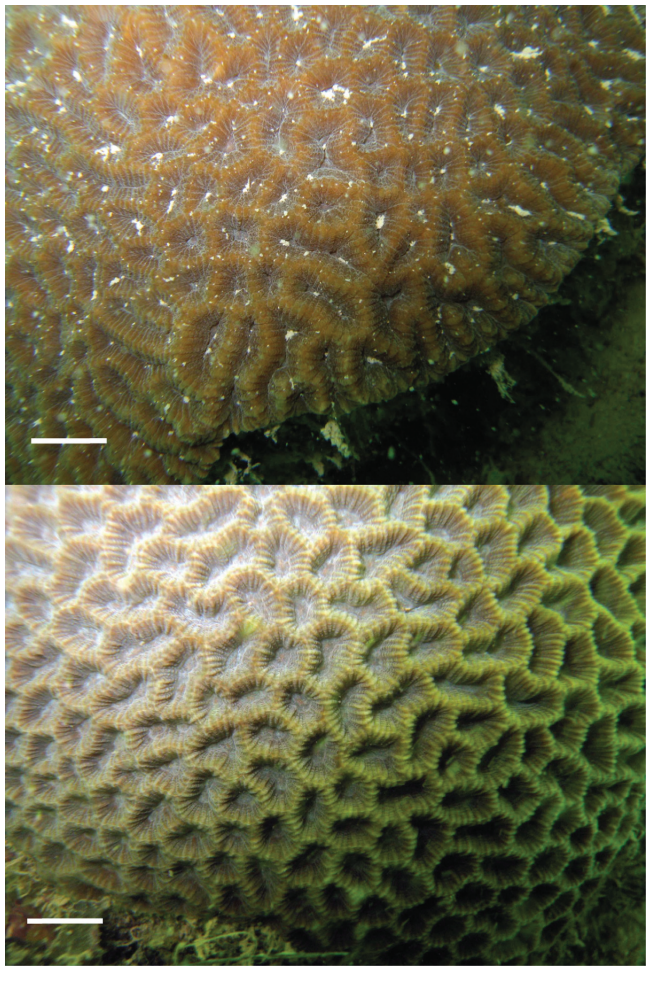

Fig. 1. *Platygyra pini* in Singapore. Scale bars = 1 cm. (Photographs by: Danwei Huang).

chloride, 25 g of potassium dichromate per liter of seawater) with 5% formaldehyde for 18–24 h. Samples were fixed in separate, labeled, disposable 200 ml plastic pots, filled with solution. Following fixation, samples were rinsed for 18–24 h in running tap water and stored in 70% ethanol. The ethanol was changed when necessary to remove dissolved pigments and other precipitates. Samples were decalcified in a 10% solution of HCl with 0.7 g EDTA, 0.008 g sodium potassium tartrate, and 0.14 g sodium tartrate per litre of solution. Decalcification generally took about one week, with regular changes of acid. Samples were rinsed in tap water and stored in 70% alcohol until histological processing.

Tissue samples with a surface area of around 1 cm^2 were processed for histological sectioning. Pieces of coral tissue were dehydrated in an alcohol series, cleared using Histoclear (National Diagnostics) and embedded in paraffin wax (BDH Laboratory Supplies) using a tissue processor (Reichert-Jung Histokinette 2000). Samples were orientated in cross section and cut with a microtome (Reichert-Jung 2030) at 6–8 µm thickness and stained with a modified Heidenhain's Aniline Blue (Luna, 1968) with azocarmine G (Glynn et al., 1991). Cover slips were mounted with DPX (BDH Laboratory Supplies). The approximate location of the gonads within the polyp was established, by taking cross sections at regular spatial intervals (approximately every 100 µm) through mature polyps and by examination of longitudinal sections of mature polyps. At least three slides were made from each sample at intervals of approximately 300 µm throughout the reproductive region of the polyp, with each slide consisting of three to four serial sections.

Analysis of histological slides. — Slides were analysed under a binocular compound microscope (Olympus BH2) at magnifications of ×40 to ×1000. A square of area 0.25 cm^2 was placed haphazardly over the slide and the geometric mean diameters and gametogenic stages of all gonads within the square were recorded. The maximum gonad diameter ($D1$) and a second measurement perpendicular to the maximum ($D2$) were measured with a calibrated ocular micrometer and the geometric mean diameter (GMD) of each gonad was calculated as follows:

$$GMD = \sqrt{(D1 \times D2)}$$

Gonad classification followed the widely used system developed by (Szmant-Froelich et al., 1985). Four gametogenic stages of oogenesis and spermatogenesis were identified and these are described in detail in the results section. The average GMD of each gonad stage was estimated from 100 randomly selected gonads.

Environmental parameters. — The seasonal patterns of SST (°C) and rainfall (mm mth^{-1}) were examined from Jan.2001 – Jun.2002. Monthly mean SSTs and standard deviations (°C) were estimated from measurements taken at least twice-monthly at the three study sites with a multi-parameter probe (YSI Inc.) at a depth of approximately 0.5 m. Monthly mean rainfall values (mm mth^{-1}) were obtained from the Singapore Meteorological Service from the rain station at Sentosa Island (1°15'N, 104°50'E).

Data analysis. — A chi-square analysis was performed to determine if the proportion of colonies with mature gametes (Stage IV) was independent of seasons. The year was divided into 4 seasons based on the Southeast Asian Monsoon as follows: Northeast (NE) Monsoon (Nov–Feb); SW Monsoon (May–Aug); first inter-monsoon (Mar–Apr); and second inter-monsoon (Sep–Oct). Data were pooled for each season and a contingency table was constructed with five rows (representing the five seasons covered during the study), and two columns (representing number of colonies with mature gametes and number colonies without mature gametes).

RESULTS

Gametogenic cycle and reproductive strategy. — All of the colonies sampled during the breeding season were simultaneous hermaphrodites. In mature colonies, oocytes and spermaries were intermingled on the same mesenteries (Figs. 2, 3) and gamete development occurred in the sub-oral region.

Oogenesis. — Mesoglea stained light blue and stage I oocytes were 34.4 ± 11.2 µm (mean GMD ± SD), located along the mesogleal lamella and characterised by a large oval purple-gray staining nucleus and bright red stained nucleolus, sometimes surrounded by a thin layer of lightly purple stained cytoplasm (Fig. 2a). Stage II oocytes were 77.6 ± 17.5 µm (mean GMD ± SD), were completely enveloped by mesoglea. Vitellogenesis increased the amount of cytoplasm around the nucleus and this appeared grainy and stained red or purple (Fig. 2b). Stage III oocytes were 164.1 ± 33.2 µm (mean GMD ± SD), had extensive dark red or purple staining grainy cytoplasm and a distinct, deep magenta staining vitelline membrane (Fig. 2c). Stage IV oocytes were 264.8 ± 48.0 µm (mean GMD ± SD), were large and oval or teardrop shaped. The nucleus was often saddle shaped and had migrated to the periphery of the oocyte and was sometimes adjacent to a small invagination (Fig. 2d). Cytoplasm appeared denser around the nucleus and there was often a dark purple stained patch within the nucleus. Oocytes did not contain zooxanthellae.

Spermatogenesis. — Stage I spermaries were rare and were typically 10–20 µm in diameter consisting of clusters of light to navy blue staining cells, each approx 4 µm in diameter, which were presumably interstitial cells (Giese & Pearse, 1974) (Fig. 3a). These clusters appeared to be migrating into, but were not enveloped by, mesoglea. Stage II spermaries were 48.0 ± 14.2 µm (mean GMD ± SD), were completely enveloped by mesoglea and were similar in staining properties to stage I but larger due to the proliferation of germ cells (Fig. 3b). Stage III spermaries were 114.9 ± 45.1 µm (mean GMD ± SD) and contained dark blue-purple staining spermatocysts. In most cases cells had migrated to the periphery of spermaries creating a lumen in the center of stage III spermaries (Fig. 3c). Stage IV spermaries were 98.0 ± 33.4 µm (mean GMD ± SD), spermatocyte heads had condensed, were very numerous with tails typically staining

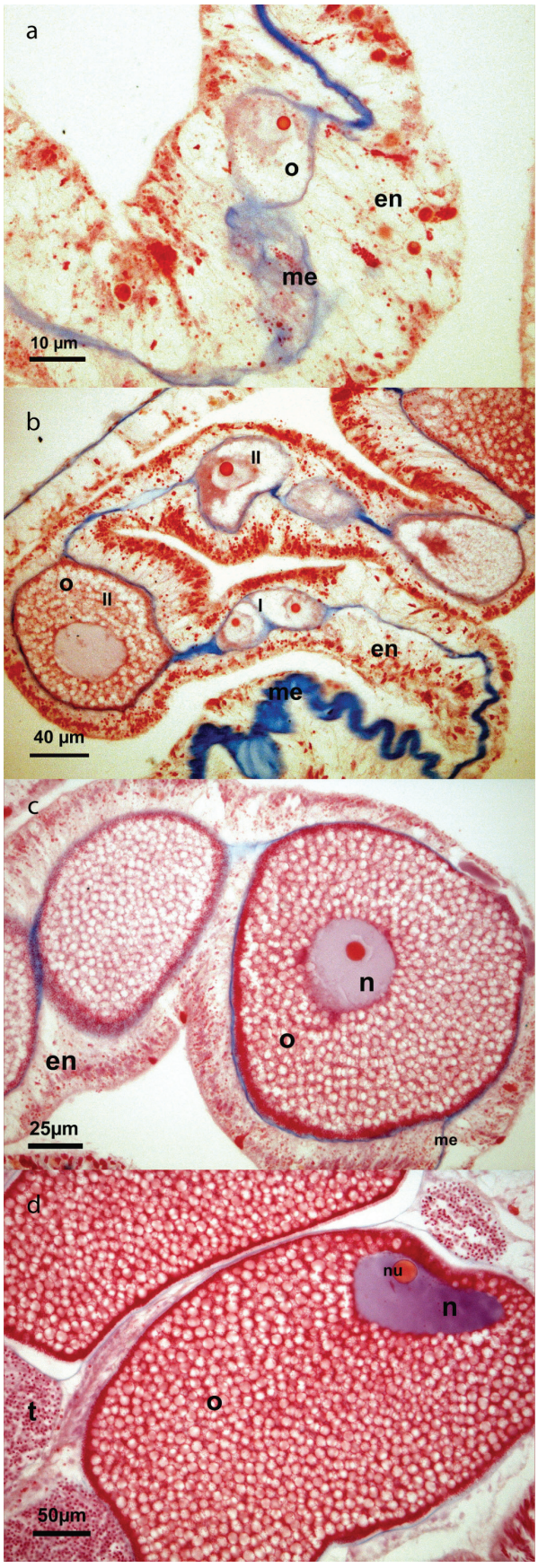

Fig. 2. Oogenesis in *Platygyra pini*. a) Stage I oocyte entering mesenterial mesoglea; b) stage I and II oocytes together in mesenterial mesoglea; c) stage III oocyte; d) stage IV oocyte, note the darker purple patch within the nucleus and the small invagination adajacent to the nucleus. en = endoderm, me = mesoglea, n = nucleus, nu = nucleolus, o = oocyte, t = spermaries.

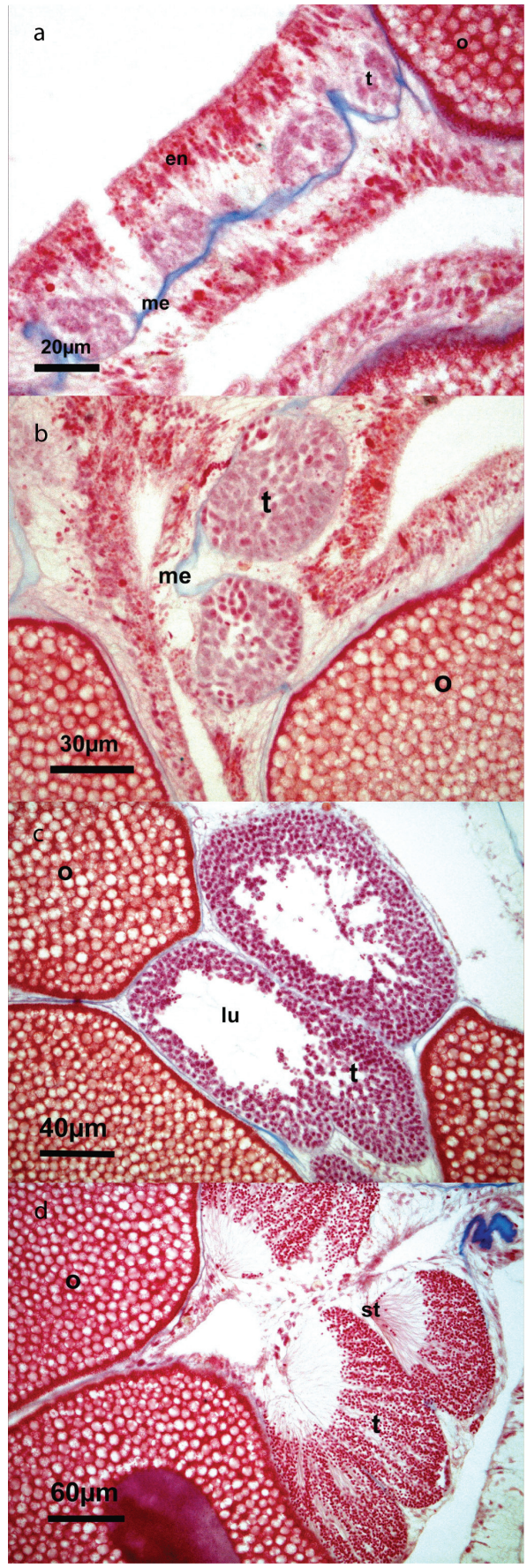

Fig. 3. Spermatogenesis in *Platygyra pini*. a) Stage I spermaries entering mesenterial mesoglea; b) stage II spermaries; c) stage III spermaries with lumen; d) stage IV spermaries arranged in bouquet. en = endoderm, lu = lumen, me = mesoglea, o = oocyte, st = spermatozoa tails, t = spermaries.

gold or light purple while spermatocyte heads stained dark blue or purple (Fig. 3d). Spermatozoa were arranged with tails pointing in one direction to give the impression of a bouquet (Fig. 3d).

Reproductive seasonality. — While gametes were present in almost all sampling months, reproduction was clearly seasonal. The highest proportion of colonies containing mature oocytes and spermaries (i.e., stage IV) occurred in Apr.2001 (100% and 83% respectively, n = 12) and 2002 (92% and 75% respectively, n = 12) (Fig. 4a,b). While colonies contained mature oocytes over several months, including two colonies in Nov.2001 (17%, n = 12), colonies only contained mature spermaries in Apr/May 2001, Nov/Dec.2001, and Mar/Apr.2002 (Fig. 4a,b). Chi-square analysis revealed a significant association between the proportion of the population containing mature gametes and seasons (df = 4, test statistic = 99.3, $p < 0.0001$; df = 4, test statistic = 41.4, $p < 0.001$) with the majority of stage IV gametes present during the first inter-monsoon season (Mar/Apr).

Oocyte development began in Aug.2001, indicating that the duration of the oogenic cycle is as long as 8 months (Fig. 5a). Spermatogenesis began in February and a high proportion of large mature spermaries were present in Apr.2002, indicating that the spermatogenic cycle was 2 to 3 in duration (Fig. 5). Mature (stage IV) oocytes were present during Mar–Jun.2001, Nov.2001, and Feb–Apr.2002 (Fig. 5a), whereas immature oocytes (stages I–III) were present in all sampling months except Jul.2001 (Fig. 5a). The greatest numbers of stage IV oocytes, i.e. mean number oocytes per 0.25 cm^2 of tissue cross-section area, were present in Apr.2001 (18.0 ± 1.7 oocytes 0.25 cm^{-2}, mean ± SE) and Apr.2002 (8.4 ± 1.9 oocytes 0.25 cm^{-2}, mean ± SE) (Fig. 5a). Stage IV spermaries were found predominantly in Apr.2001 (41.0 ± 7.7 spermaries 0.25 cm^{-2}, mean ± SE) and Apr.2002 (25.0 ± 5.2 spermaries 0.25cm^{-2}, mean ± SE). Comparatively fewer stage IV gametes also occurred in Feb, Mar, May, Jun, Nov, and Dec (Fig. 5). There was also seasonal pattern for gamete sizes (i.e., average gamete GMD [µm]; Fig. 6). During the period between Dec.2001 – Apr.2002, mean oocyte GMD (average of gravid colonies) increased from 64.4 ± 4.0 µm (mean GMD ± SE) to 256.7 ± 7.6 µm (mean GMD ± SE) (Table 1). In April of both years, the majority of colonies contained large gametes with colony mean oocyte GMD ranging from 202.9–289.8 µm (Fig. 6a). While large oocytes were found in colonies in other months, the range of mean colony oocyte GMD was much greater. For example, in Nov.2001, oocyte GMD ranged from 31.6–284.6 µm (Fig. 6a) suggesting the presence of both mature and immature colonies. The overall seasonal pattern for spermary size was less distinct (Fig. 6b); nonetheless, the greatest change occurred between January, when all colonies were empty, and Apr.2002 when mean spermary GMD was 89.8 ± 1.6 µm (mean GMD ± SE) (Table 1). The range of spermary

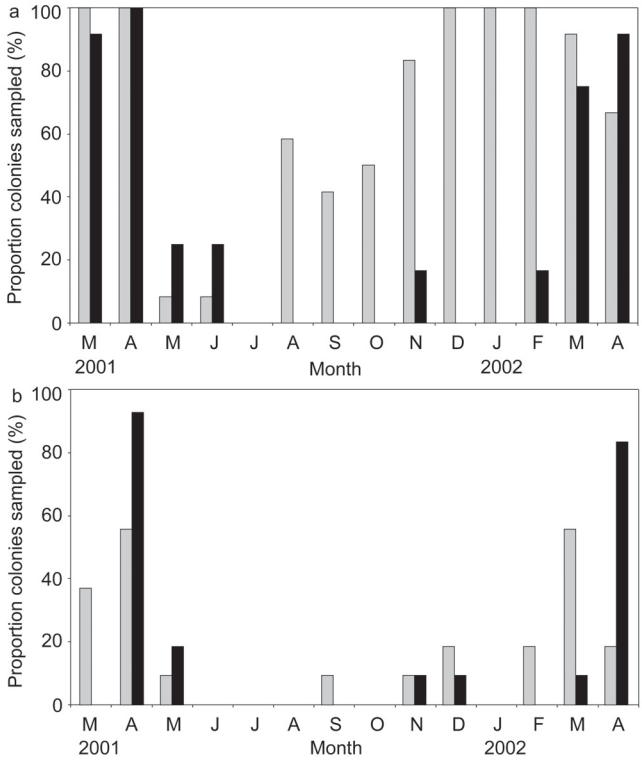

Fig. 4. Proportion of all sampled colonies (%) of *Platygyra pini* (n = 12) that contained mature (stage IV) oocytes (a) and spermaries (b) (black bars); and immature oocytes (a) and immature spermaries (b) (stages I–III) (grey bars) each month for 14 months. Totals are >100% in some months because colonies contained both mature and immature gametes simultaneously.

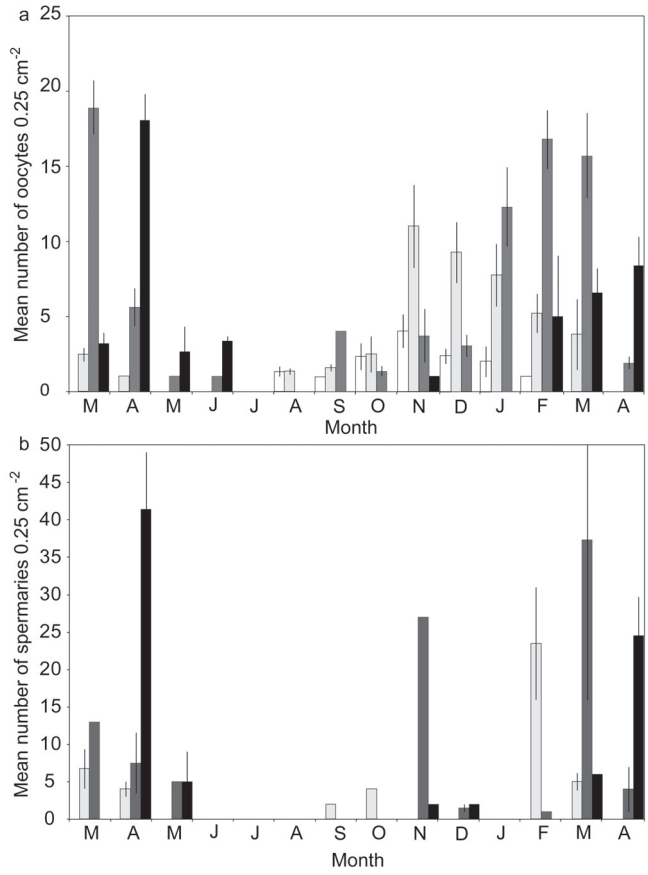

Fig. 5. Reproductive phenology (gamete stages) of *Platygyra pini* in Singapore between Mar.2001 and Apr.2002 (n = 12 colonies) showing a) mean number of oocytes 0.25 cm^{-2} tissue cross section; and b) mean number of spermaries 0.25 cm^{-2} tissue cross section showing proportions of each stage (stage 1 = white bars; stage 2 = light grey; stage 3 = dark grey bars; stage 4 = black bars).

Table 1. Mean gamete size (geometric mean diameter µm) during sampling period. SE = standard error, n = number of colonies containing gametes.

Month	Mar.2001	Apr	May	Jun	Aug	Sep	Oct	Nov	Dec	Jan.2002	Feb	Mar	Apr
Mean oocyte GMD (µm)	167.6	241.2	248.6	266.7	55.4	96.8	89.2	90	64.4	98.5	138.0	187.6	256.7
SE	6.8	4.8	10.5	6.8	4.3	8.7	16.3	24.0	4.0	6.1	7.2	7.9	7.6
n	12	12	8	3	7	5	7	11	12	12	12	11	11
Mean spermary GMD (µm)	45.5	98.9	114			42.5	45.2	71.3	61.2		50.9	58.6	89.8
SE	7.0	5.0	22.2					4.7	4.1		2.1	6.1	1.6
n	4	11	6	0	0	1	1	2	2	0	2	6	9

sizes among colonies was also relatively small during April with mean colony spermary GMD ranging from 73.3–131.4 µm (Fig. 6b).

Sea surface temperature (SST) and total monthly rainfall. — SST and to a lesser extent rainfall showed distinct seasonal patterns (Fig. 7). Mean monthly SSTs during the study period ranged between 27.22 ± 0.15 °C (mean ± SD) in Feb.2002 and 30.55 ± 0.35°C (mean ± SD) in May 2001, an annual variation of 3.33°C (Fig. 7). Mean monthly SSTs were lowest during Jan–Feb and there was a marked increase in SST from Feb–May, followed by a decline and a second smaller peak in November (Fig. 7). The pattern for rainfall was also seasonal although less distinct than for SSTs (Fig 7). Total monthly rainfall ranged from between 360.8–54.2 mm and was highest in Dec.2001 and lowest in May 2001, however high rainfall levels (309.3 mm) were also recorded in Mar.2001.

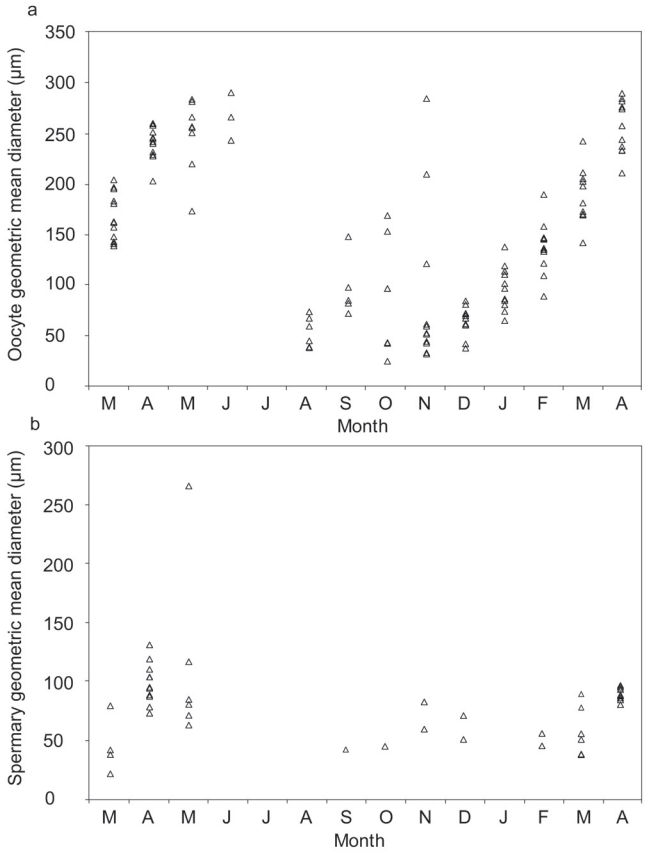

Fig. 6. Scatter plots of a) oocyte geometric mean diameter (µm) and b) spermary geometric mean diameter (µm) for *Platygyra pini* in Singapore between Mar.2001 and Apr.2002. Each point represents the mean gamete geometric mean diameter for one colony (n = 12 colonies per month).

DISCUSSION

All colonies sampled during the spawning season were hermaphroditic, with oocytes and spermaries intermingled within the mesenteries (i.e., syngonic *sensu* [Policansky, 1982]). Planula larvae were never observed in histological sections and field observations confirm that *Platygyra pini* is a broadcast spawner. This reproductive strategy is shared by the majority of species studied to date within the family Faviidae (Baird et al., 2009) and broadcast spawning of *Platygyra* species has been documented in a number of locations (Richmond & Hunter, 1990). In the present study, oocytes appeared in some colonies as early as August, but were low in abundance until November, suggesting that the duration of the oogenic cycle is between five and eight months. The spermatogenic cycle appeared to be shorter in duration, lasting approximately two or three months. Spermaries were seen in colonies as early as Sep.2001; however no colonies contained spermaries in Jan.2002, suggesting that these may have been used during a minor November spawning rather than colonies having prolonged

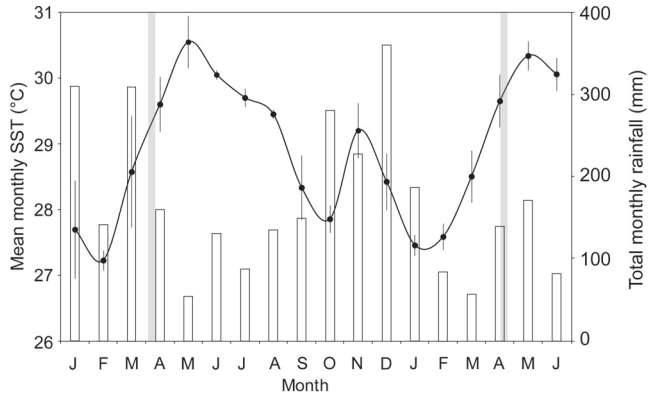

Fig 7. Monthly averages of sea surface temperature (°C) (black line) and total ranfall (mm) (white bars) from Jan.2001 to Jun.2002. Grey bars indicate the predicted main spawning period in April for *Platygyra pini*. Error bars are SD.

spermatogenic cycles. In Mombasa, *P. daedalea* colonies had oogenic and spermatogenic cycles lasting for up to seven and five months respectively (Mangubhai & Harrison, 2008b) and in general, members of the family Faviidae have gametogenic cycles ranging in duration from 5–9 mth for oogenesis and 1–3 mth for spermatogenesis (Harrison & Wallace, 1990).

There was a marked seasonal reproductive pattern, with the peak of activity occurring around the first inter-monsoon period from Mar–May; however, the greatest number of gametes, the largest gametes and the highest proportion of mature colonies were found in April in both years. Mature gametes, in much lower abundance, were also found in Feb, Mar, May, Jun, Nov, and Dec. Opposite sex gametes were only found to co-occur within colonies in Apr, May, and Nov.2001, and Mar and Apr.2002. These results suggest that a) spawning occurs predominantly during these four months of the year and b) the vast majority of reproductive activity occurs in only one month (April) each year. During night-time field observations carried out at one of the three study sites 3–5 nights after the full moon of Mar.2002 (on 29 Mar.2002), broadcast spawning of numerous scleractinian species was observed (Guest et al., 2002, 2005b) predominantly between 2000–2200 hours. Observations were not carried out following the April full moon in 2002 (on 27 Apr.2002), and while colonies of several *Platygyra* species were observed to spawn in March (Guest et al., 2005b), the presence of large mature gametes in most sampled colonies in April indicates that a higher proportion of the population of *Platygyra pini* spawned following the April full moon in 2002.

In Nov.2001, mature oocytes (stage IV) were present in two colonies at one site and these were accompanied by stages III and IV spermaries, indicating a smaller annual spawning period approximately six months after the major spawning season (i.e., Mar–May). Because colonies were not sequentially sampled in this study, we cannot confirm whether individual colonies underwent two gametogenic cycles or spawning within the population was split between seasons. Furthermore, due to the intrinsic taxonomic difficulties within this group, it is possible that the smaller number of colonies reproducing outside of the main spawning months belonged to other *Platygyra* morpho-species. Biannually spawning colonies have been found on other equatorial reefs, for example some colonies within populations of *Platygyra daedalea* spawn twice a year on reefs in Mombasa, Kenya (4°S) (Mangubhai & Harrison, 2008b) and Madang, Papua New Guinea (5°S) (Oliver et al., 1988). Furthermore, in Singapore, tagged colonies of *Porites lutea* and *Acropora humilis* have been found to contain mature gametes twice a year (Guest et al., 2005a, 2005b). Biannual spawning, however is not restricted to equatorial reefs. For example, colonies of *Montiopora digitata* are capable of spawning twice a year on parts of the Great Barrier Reef (GBR; Stobart et al., 1993). Similarly, a smaller secondary spawning, involving numerous coral species, also occurs on tropical reefs in Western Australia; however colonies have a single gametogenic cycle each year and spawning within populations is split between the austral spring (Oct/Nov) and autumn (Mar/Apr) spawning seasons (Rosser & Gilmour, 2008). Research is needed to establish whether secondary spawning seasons in Singapore are caused by multiple gametogenic cycles or population split spawning, and whether multiple gametogenic cycles are more common at lower latitudes. Individuals that spawn outside the main season are likely to be severely disadvantaged because fertilisation success is dependent on high sperm density in marine broadcast spawners (Oliver & Babcock, 1992); therefore, further research is also needed to better understand the adaptive implications of extended or split breeding seasons.

The predicted major spawning period in both years (i.e., during the week following the April full moon), followed the greatest positive changes in monthly mean SSTs and preceded the warmest month of the year (which was May in both years). Between Feb–Apr.2001, monthly mean SSTs increased by 2.38°C and between Jan–Apr.2002 by 2.2°C. In addition, the second peak in reproductive activity in November followed a period of increasing monthly mean SSTs (+1.35°C from Oct–Nov.2001) but preceded a period of cooler water during the north-east monsoon. These findings are consistent with patterns described from other regions, where coral spawning typically follows a period of marked change in SSTs (increasing and/or decreasing) and occurs close to the warmest period of the year (Mendes & Woodley, 2002). Though measurements of SST were only conducted twice each month during the study period, the SST pattern described in this paper is consistent with that previously found by Tham (1973), and with remotely sensed SST data during the same study period (Guest et al., 2012). Combined, these data indicate that changes in SST around Singapore's southern islands are predictable and markedly seasonal each year. Nonetheless, SSTs did not drop below 27°C, a temperature that is adequate for gamete maturation and spawning to occur in other locations (e.g., Hayashibara et al., 1993). These all year-round warm temperatures may explain the capability of some colonies to undergo a second gametogenic cycle, though further studies are required to investigate whether biannual spawning within colonies is more common at the equator. Even in equatorial locations such as Singapore, there is sufficient seasonal variation for corals to use factors such as sea surface temperatures as cues for reproduction. As no coastal location is truly aseasonal, reproductive synchrony within populations of broadcast spawners (at least in terms of gamete maturation) is just as likely to occur in an equatorial region with modest (but nonetheless distinct) annual environmental variation as it is in a high latitude location with large variations in environmental seasonality (Guest et al., 2005b).

Although temperature has long been considered one of the most important factors regulating gametogenic cycles in marine invertebrates (Giese & Pearse, 1974), global comparisons between coral spawning times and SSTs are not always consistent. For example, spawning occurs predominantly during different seasons for many species on the east and west coasts of Australia, despite these locations sharing a similar pattern for sea surface temperature (Babcock et al., 1994). Insolation (i.e., a measure of the amount of

solar radiation energy reaching the earth's surface) has been proposed as a better predictor of spawning times than SSTs for corals in the Western Pacific and Western Atlantic (Penland et al., 2004; van Woesik et al., 2006). Irradiance was not measured during the study period due to the technical challenges involved in gathering continuous measurement of light underwater in a highly turbid environment (Dunne & Brown, 2001). At the low latitudes however, insolation does vary seasonally as a consequence of the vernal and autumnal equinoxes (i.e., in March and September) when the geometric centre of the sun's disc crosses the equator. Therefore the two seasonal peaks for reproduction in Singapore occur close to the periods of maximum insolation (Penland et al., 2004). Considering that insolation and temperature are not independent, separating the effects of these environmental variables in regulating spawning timing and synchrony clearly poses an important challenge for reef scientists.

Although environmental conditions are suitable year round for breeding in Singapore, it is possible that there is selection for spawning when environmental conditions are optimal for fertilisation, larval survival, settlement and recruitment. In the present study it was found that the majority of spawning in *Platygyra pini* occurred just before the warmest month of the year and just after the period of heaviest rainfall. Larval developmental rates and pre-competency periods are shorter at higher temperatures (Heyward & Negri, 2010); therefore spawning at the warmest period of the year may confer a selective advantage by potentially reducing the period of time until settlement and metamorphosis. Similarly, spawning outside the period of heaviest rainfall may increase the chances of fertilisation success if it reduces the probability of spawning occurring during a rain shower. Meta-analysis of 19 other geographical locations revealed that the majority of coral spawning occurs in months without heavy rainfall when temperatures are warmest, suggesting an ultimate role for these factors in determining the optimal seasonal timing for spawning (Mendes & Woodley, 2002). That study also predicted that, in equatorial locations where temperature variations are moderate, spawning will occur before the months of heaviest rainfall. The possible advantage of this strategy is that increased rainfall may elevate nutrient levels due to increased river run-off, thus benefiting newly settled larvae. However the present study does not support this prediction, as coral spawning occurred after, rather than before the period when heaviest rainfall occurs.

While it is possible to infer relationships between reproductive patterns and environmental seasonality, it should be noted that any correlation does not prove a causal relationship. Although technically challenging, manipulative experiments to examine the effects environmental factors on spawning timing are required and should be combined with investigations into the underlying molecular and genetic basis for a causal relationship.

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LITERATURE CITED

Babcock, R. C., G. D. Bull, P. L. Harrison, A. J. Heyward, J. K. Oliver, C. C. Wallace & B. L. Willis, 1986. Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. *Marine Biology*, **90**: 379–394.

Babcock, R. C., B. L. Wills & C. J. Simpson, 1994. Mass spawning of corals on a high-latitude coral-reef. *Coral Reefs*, **13**: 161–169.

Baird, A. H., J. R. Guest & B. L. Willis, 2009. Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. *Annual Review of Ecology Evolution and Systematics*, **40**: 551–571.

Bouwmeester, J., M. T. Khalil, P. De La Torre & M. L. Berumen, 2011. Synchronous spawning of *Acropora* in the Red Sea. *Coral Reefs*, **30**: 1011–1011.

Dunne, R. P. & B. E. Brown, 2001. The influence of solar radiation on bleaching of shallow water reef corals in the Andaman Sea, 1993–1998. *Coral Reefs*, **20**: 201–210.

Giese, A. C. & J. S. Pearse, 1974. Introduction: General principles. In: Giese, A. C. & J. S. Pearse (eds.), *Reproduction of Marine Invertebrates*. Academic Press, New York and London. Pp. 2–49.

Glynn, P. W., N. J. Gassman, C. M. Eakin, J. Cortes, D. B. Smith & H. M. Guzman, 1991. Reef coral reproduction in the Eastern Pacific—Costa-Rica, Panama, and Galapagos-Islands (Ecuador). 1. Pocilloporidae. *Marine Biology*, **109**: 355–368.

Guest, J. R., A. H. Baird, B. P. L. Goh & L. M. Chou, 2002. Multi-specific, synchronous coral spawning in Singapore. *Coral Reefs*, **21**: 422–423.

Guest, J. R., A. H. Baird, B. P. L. Goh & L. M. Chou, 2005a. Reproductive seasonality in an equatorial assemblage of scleractinian corals. *Coral Reefs*, **24**: 112–116.

Guest, J. R., A. H. Baird, B. P. L. Goh & L. M. Chou, 2005b. Seasonal reproduction in equatorial reef corals. *Invertebrate Reproduction and Development*, **48**: 207–218.

Guest, J. R., A. H. Baird, K. E. Clifton & A. J. Heyward, 2008. From molecules to moonbeams: Spawning synchrony in coral reef organisms. *Invertebrate Reproduction & Development*, **51**: 145–149.

Guest, J. R., A. H. Baird, J. A. Maynard, E. Muttaqin, A. J. Edwards, S. J. Campbell, K. Yewdall, Y. A. Affendi & L. M. Chou, 2012. Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. *Plos One*, **7**: e33353.

Hanafy, M. H., M. A. Aamer, M. Habib, A. B. Rouphael & A. H. Baird, 2010. Synchronous reproduction of corals in the Red Sea. *Coral Reefs*, **29**: 119–124.

Harrison, P. & C. Wallace, 1990. Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky, Z. (ed), *Ecosytems of the World: Coral Reefs*. Elsevier, Amsterdam. Pp. 133–207.

Harrison, P. L., R. C. Babcock, G. D. Bull, J. K. Oliver, C. C. Wallace & B. L. Willis, 1984. Mass spawning in tropical reef corals. *Science*, **223**: 1186–1189.

Hayashibara, T., K. Shimoike, T. Kimura, S. Hosaka, A. Heyward, P. Harrison, K. Kudo & M. Omori, 1993. Patterns of coral spawning at Akajima Island, Okinawa, Japan. *Marine Ecology-Progress Series*, **101**: 253–262.

Heyward, A., 1988. Reproductive status of some Guam corals. *Micronesica*, **21**: 272–274.

Heyward, A. J. & A. P. Negri, 2010. Plasticity of larval pre-competency in response to temperature: Observations on multiple broadcast spawning coral species. *Coral Reefs*, **29**: 631–636.

Huang, D. W., K. P. P. Tun, L. M. Chou & P. A. Todd, 2009. An inventory of zooxanthellate scleractinian corals in Singapore, including 33 new records. *Raffles Bulletin of Zoology*, Supplement **22**: 69–80.

Kongjandtre, N., T. Ridgway, S. Ward & O. Hoegh-Guldberg, 2010. Broadcast spawning patterns of *Favia* species on the inshore reefs of Thailand. *Coral Reefs*, **29**: 227–234.

Luna, J. G., 1968. *Manual of Histological Staining Methods of the Armed Forces Institute of Pathology. 3rd Edition.* McGraw-Hill, New York. 258 pp.

Mangubhai, S. & P. L. Harrison, 2008a. Asynchronous coral spawning patterns on equatorial reefs in Kenya. *Marine Ecology-Progress Series*, **360**: 85–96.

Mangubhai, S. & P. L. Harrison, 2008b. Gametogenesis, spawning and fecundity of *Platygyra daedalea* (Scleractinia) on equatorial reefs in Kenya. *Coral Reefs*, **27**: 117–122.

Mendes, J. M. & J. D. Woodley, 2002. Timing of reproduction in *Montastraea annularis*: Relationship to environmental variables. *Marine Ecology-Progress Series*, **227**: 241–251.

Miller, K. J. & J. A. H. Benzie, 1997. No clear genetic distinction between morphological species within the coral genus *Platygyra*. *Bulletin of Marine Science*, **61**: 907–917.

Oliver, J. & R. Babcock, 1992. Aspects of the fertilization ecology of broadcast spawning corals—Sperm dilution effects and in situ measurements of fertilization. *Biological Bulletin*, **183**: 409–417.

Oliver, J. K., R. C. Babcock, P. L. Harrison & B. L. Willis, 1988. Geographic extent of mass coral spawning: Clues to ultimate causal factors. *Proceedings of the 6th International Coral Reef Symposium*. Contributed Papers, Townsville. Pp. 803–810.

Penland, L., J. Kloulechad, D. Idip & R. van Woesik, 2004. Coral spawning in the western Pacific Ocean is related to solar insolation: Evidence of multiple spawning events in Palau. *Coral Reefs*, **23**: 133–140.

Policansky, D., 1982. Sex change in plants and animals. *Annual Review of Ecology and Systematics*, **13**: 471–495.

Richmond, R. H. & C. L. Hunter, 1990. Reproduction and recruitment of corals: Comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. *Marine Ecology Progress Series*, **60**: 185–203.

Rosser, N. L. & J. P. Gilmour, 2008. New insights into patterns of coral spawning on Western Australian reefs. *Coral Reefs*, **27**: 345–349.

Shlesinger, Y. & Y. Loya, 1984. Coral community reproductive patterns: Red Sea versus the Great Barrier Reef. *Science*, **228**: 1333–1335.

Stobart, B., R. C. Babcock & B. L. Willis, 1993. Biannual spawning of three species of scleractinian corals from the Great Barrier Reef. *Proceedings of the 7th International Coral Reef Symposium*. Guam. Pp. 494–499.

Szmant-Froelich, A., M. Reutter & L. Riggs, 1985. Sexual reproduction of *Favia fragum* (Esper): Lunar patterns of gametogenesis, embryogenesis and planulation in Puerto Rico. *Bulletin of Marine Science*, **37**: 880–892.

Szmant-Froelich, A., P. Yevich & M. E. Q. Pilson, 1980. Gametogenesis and early development of the temperate coral *Astrangia danae* (Anthozoa: Scleractinia). *Biological Bulletin*, **158**: 257–269.

Tham, A. K., 1973. The Sea. In: Chuang, S. H. (ed.), *Animal Life and Nature in Singapore*. Singapore University Press. Pp. 140–149.

van Woesik, R., F. Lacharmoise & S. Koksal, 2006. Annual cycles of solar insolation predict spawning times of Caribbean corals. *Ecology Letters*, **9**: 390–398.

Veron, J. E. N., 2000. *Corals of the World*. Australian Institute of Marine Science, Townsville, Australia. 463 pp.

Vicentuan, K. C., J. R. Guest, M. V. Baria, P. C. Cabaitan, R. M. Dizon, R. D. Villanueva, P. M. Alino, A. J. Edwards, E. D. Gomez & A. J. Heyward, 2008. Multi-species spawning of corals in north-western Philippines. *Coral Reefs*, **27**: 83–83.

Willis, B. L., R. C. Babcock, P. L. Harrison, J. K. Oliver & C. C. Wallace, 1985. Patterns in the mass spawning of corals on the Great Barrier Reef from 1981 to 1984. *Proceedings of the 5th International Coral Reef Symposium*, Tahiti. Pp. 343–348.