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Research Article

Gametogenesis, Embryogenesis, and Fertilization Ecology of *Platygyra acuta* in Marginal Nonreefal Coral Communities in Hong Kong

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Understanding the reproductive biology of dominant coral species in subtropical nonreefal coral communities is critical in providing important information on the processes underlying the distribution limits of coral species and communities. This is the first study that investigates the reproduction cycle, gametogenesis, and fertilization ecology of *Platygyra acuta*. Results indicated that *P. acuta* is hermaphroditic and exhibits a single annual gametogenic cycle. Oogenic and spermatogenic cycle occurs for 6-7 months and for 2 months, respectively, prior to annual mass spawning event in May to June in Hong Kong. It took 18 hours for *P. acuta* to complete embryonic development, develop cilia, and start to rotate. High (>70%) fertilization success can be achieved under a broad range of sperm concentrations from 10^4 to 10^7 sperms mL^{-1}. Fertilization success remained consistently high 6 h after spawning, indicating a prolonged viability of its gametes that is much longer than that recorded for other coral species. Significantly higher percentage of fertilization success was recorded in the first of the two consecutive nights of spawning, suggesting differences in the quality of the eggs and/or sperms between days of spawning. These results serve as important baseline information for better understanding of corals in marginal communities.

1. Introduction

Corals show two aspects of sexual reproductive patterns. They can be hermaphroditic or gonochoric in their sexuality, and they can broadcast their gametes in mass/multispecific spawning (broadcaster) or brood their larvae (brooder) as a mode of larval development. Hermaphroditic corals have both male and female gonads developed within polyp or in different polyps within the same colony, whereas gonochoric corals have separate sexes at the colony level. Broadcast spawners carry out external fertilization in the water column, whereas brooders carry out internal fertilization within the maternal polyp and release planula larvae [1–5]. Overall, based on the information on sexuality and mode of development available worldwide, most scleractinian corals are hermaphroditic broadcasters [3]. The breeding season for broadcast spawning species is relatively short and discrete; each colony usually spawns only once per year on a seasonal cycle, with oogenic cycle typically ranging from 6 to 14 months [1, 3].

Since corals are sessile organisms incapable of aggregating for reproduction, synchronous release of gametes for external fertilization is crucial for reproductive success. High degree of synchronized spawning maximizes fertilization success by increasing the chance for gametes from conspecific individuals to meet in the water column or surface, preventing gametes from being drifted away by current before fertilization takes place, reducing sperm dilution effect, and avoiding predation [4, 6, 7]. Therefore, detailed information on the timing of gametogenesis is needed as a baseline for better understanding of coral spawning pattern as well as its reproductive strategy and implication on its sustainability in a region.

Mass spawning of scleractinian corals was first documented in the Great Barrier Reef, Australia, in the early 1980s [8]. Since then, research on sexual reproduction and mass- or multispecific spawning of corals has greatly increased in number and has expanded to different geographical regions over the last two decades [1–4, 9, 10]. Although there have been reviews on coral reproduction and development, descriptions of development profile of many coral species are necessary [11]. This is particularly true for coral species found in marginal communities. Marginal coral reefs and communities are those that exist near or beyond normal environmental limits of reef distribution, such as low temperature, low aragonite saturation state, and light limitation [12]. Studies on marginal communities can provide important information about the processes underlying the distribution limits of coral species and communities.

Hong Kong, located in a subtropical region, experiences large annual thermal variation with monthly mean SST that ranges from 14–16°C in winter to 30°C in summer [13]. This large temperature range makes it a marginal environment for reef building scleractinian corals. Yet, the coral diversity in Hong Kong is relatively high with at least 84 scleractinian coral species recorded [13]. Coastal subtropical environment, like Hong Kong, may become an important refuge for coral range expansion in the future should current pattern of climate change continues [14–17]. *Platygyra acuta* is one of the most dominant scleractinian corals in Hong Kong northeastern water [18]. Despite its key role in shaping the local coral community structures, its reproduction and gametogenesis have never been examined in detail neither locally nor elsewhere. An understanding of coral reproduction and development of *P. acuta* is therefore of particular importance if conservation of coral communities and coral reefs is considered in a global perspective.

Coral assemblages in Hong Kong are usually small and isolated. This is true as well in many other high latitude regions [19]. The total amount of spawned gametes is much limited when compared to that in tropical reef areas. Therefore, it is important to understand the adaptive strategies, if any, being employed by Hong Kong corals, as exemplified by *P. acuta*, to ensure or promote fertilization success. These strategies may include the capability to fertilize under lower sperm concentration or increase gamete longevity to enhance the chance of sperm-egg encounters [6]. Manipulative experiments on sperm density and gamete ageing effects were therefore conducted in this study to test if such reproductive strategies are exhibited by *P. acuta*. In addition, given the growing awareness of research in marginal coral areas due to their potential importance under climate change and limited description of coral developmental profile available, this study also looked into detailed embryogenesis of *P. acuta*. All these pieces of information will serve as important baseline for future experiments on other aspects of coral biology in marginal environment. The objectives of this study are therefore to document the reproductive cycle and gametogenesis of *P. acuta* in Hong Kong and to examine its fertilization ecology, including optimal sperm concentration for fertilization and gamete ageing effects that influence its fertilization success.

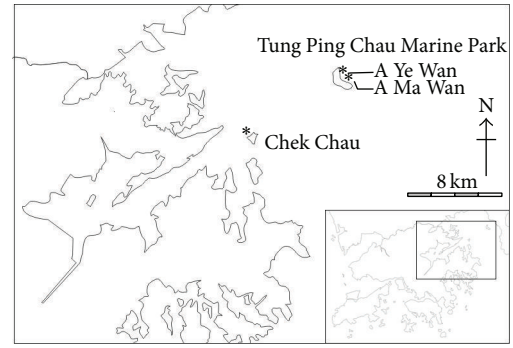

Figure 1: Map of Hong Kong showing the location of the study sites. Chek Chau and A Ma Wan are sites where samples for gametogenetic studies were collected. A Ye Wan was the site where coral spawning was monitored and hence where sperm-egg bundles of *Platygyra acuta* were collected.

2. Materials and Methods

2.1. Gametogenetic Study. Samples of *Platygyra acuta* were collected monthly, within 5 days prior to the full moon and within 1–3 m water depth range from A Ma Wan (AMW), Tung Ping Chau (22°54′N, 114°44′E) for a period of 15 months between May 1998 and July 1999, and again from Chek Chau (22°50′N, 114°36′E) for a period of 14 months between August 2010 and September 2011 (Figure 1). Both sites are within Tai Pang Wan (Mirs Bay), NE Hong Kong. On each sampling occasion, five visibly healthy colonies were haphazardly sampled, with the criteria that they were already of reproductive size >30 cm in diameter [20]. Small fragments of approximately 2 × 4 cm in size were removed from the colonies using hammer and chisel. Resampling of the same colony was avoided during the study period to eliminate, if any, stress or injury induced response which may affect the ability of corals to reproduce properly.

Coral samples were fixed in 10% formalin in seawater immediately after collection and kept for one week. Following fixation, samples were decalcified in 10% hydrochloric acid for two days with active ingredients including 100 mL concentrated hydrochloric acid (32%), 0.7 g ethylenediaminetetraacetic acid, 0.008 g sodium potassium tartrate, and 0.14 g sodium tartrate dehydrate per liter of solution. Samples were subsequently immersed in running tap water overnight and preserved in 70% ethanol until histological processing. Single polyp was randomly selected and cut out from each sample for dehydration processing in an ethanol series in Automated Vacuum Tissue Processor (Leica TP 1050, Leica Instruments GmbH) and embedded in paraffin wax. Samples were oriented in longitudinal positions for serial cut at a thickness of 7 μm at intervals of 400 μm and stained with haematoxylin and eosin. Slides were examined under a light microscope for oocytes or spermaries. The maximum length and perpendicular width of the six largest oocytes or spermaries were measured by the computer program Image-Pro Plus 6.0©. The geometric mean diameter (GMD) for each oocyte or spermary was obtained by calculating the square root of the product of the length and width measurements.

2.2. Embryonic Development and Fertilization Ecology of *Platygyra acuta*

2.2.1. Gametes Collection. Gametes of *Platygyra acuta* were collected in June 2010 and 2012 from A Ye Wan (AYW), Tung Ping Chau Marine Park (TPCMP) (22°32′N, 114°25′E) (Figure 1). Ten individual colonies of *Platygyra acuta* were tagged at depths of −0.5 to −1.5 m chart datum (CD). During spawning event, egg-sperm bundles were collected separately from different colonies and transferred on shore for further processing. Bundles collected in June 2010 were targeted for embryonic development monitoring. Bundles from different colonies were therefore mixed together in a culture tank and gently stirred to ensure fertilization. The culture tank was located semi-indoor and was equipped with HOBO Temperature Pendant data loggers (Onset) to record the water temperature every 30 min. The subsequent culturing procedures of the developing embryos followed those described by Hatta et al. [21] and Guest et al. [22]. Egg-sperm bundles collected in June 2012 were targeted for fertilization ecology study. Therefore, after bundle collection, eggs and sperms were separated for subsequent experiments using methods adapted from Oliver and Babcock [6].

2.2.2. Embryonic Development. Spawning of *Platygyra acuta* was observed between 2100 and 2200 h on June 6, 2010. Egg bundles from 10 coral colonies were collected and mixed. Fertilization started at 2325 h, with sperm concentration of around 10^7 sperms mL^{-1}. Early stages of development were examined by sampling the developing embryos in the culture tank at hourly intervals during the first 12 h after fertilization ($T = 0$ to $T = 12$), every 2 h from $T = 12$–24, every 4 h from $T = 24$–72, and every 12 h thereafter. Approximately 50 eggs or embryos of *Platygyra acuta* were collected during each sampling and fixed in 2.5% glutaraldehyde (GA) for 4 h at 4°C. Fixed samples were washed by 0.2 M phosphate buffer (PBS) and postfixed in 1% buffered osmium tetroxide at 4°C. Postfixed samples were washed by 0.2 M phosphate buffer (PBS) and kept in Eppendorf tubes until being ready to be processed. To prepare the samples for scanning electron microscopy (SEM), the samples were dehydrated in increasing concentration of acetone-ethanol. Drying was carried out by using anhydrous acetone as intermediate liquid and tertramethylsilane (TMS) as the transition liquid. After the drying stage, samples were observed under the SEM (S-3400N, Hitachi, Japan) with a working voltage of 20 kV.

2.2.3. Optimal Sperm Concentration on Fertilization Success. The experiment was conducted in June 2012 and was repeated on two successive nights of spawning event. Concentration of undiluted sperm stock was adjusted to about 10^6 to 10^7 sperms mL^{-1}. These suspensions were then serially diluted 10 times with 40 μm filtered sperm-free seawater to provide a standard range of sperm concentrations that ranged from 10^2 to 10^7 sperms mL^{-1}. For each concentration, 50 mL of sperm suspension was added separately to three replicate glass bottles (100 mL). Approximately 100 eggs were added to each of the bottles, placed on a bench at ambient temperature (26°C) and left undisturbed. The number of fertilized eggs, that is, eggs that were at or beyond the two-cell stage, was examined under the stereomicroscope and recorded after 4 h.

2.2.4. Gamete Ageing Effects on Fertilization Success. The experiment was conducted in June 2012 and was repeated on two successive nights of spawning event. In each case, 50 mL sperm suspension (10^6 sperms mL^{-1}) was added separately to three replicate glass bottles (250 mL). Approximately 100 eggs were then added to each of the bottles. In the first day of experiment, each set of mixing was carried out at 30 min intervals for 3.5 h to assess the potential longevity of gametes. In Day 2, mixing was carried out at 1 h intervals for 6 h. This change was necessary after reviewing the results from Day 1. The sperms were kept at a concentration of 10^6 sperms mL^{-1} until the time the gametes were mixed. All experiments were conducted on a bench at ambient temperature and left undisturbed after mixing. The number of fertilized eggs was examined under the stereomicroscope and recorded after 4 h.

2.2.5. Self-Fertilization in Platygyra acuta. To assess the ability for self-fertilization in *P. acuta*, egg bundles collected from the same colony ($n = 5$) were stirred gently to break up the bundles and left undisturbed for at least 4 h. The number of fertilized eggs, as indicated by the presence of cleavage, was counted under the stereomicroscope. This experiment was conducted in 2012 and repeated in 2013 spawning event.

2.3. Data Analysis. All data were arcsine-transformed and tested for normality using one-sample Kolmogorov-Smirnov test and for homogeneity of variances using Levene's test. One-way analysis of variance (ANOVA) was then used to determine if there was any significant difference ($P < 0.05$) in the effect of sperm concentrations on the percent fertilization success in Experiment 1, as well as the effect of gamete age on the percent fertilization success in Experiment 2. Post hoc comparisons of means to find significant groupings were done using Tukey's HSD tests. SPSS version 16.0 for Windows (SPSS Inc., USA) was employed in all statistical analyses.

3. Results

3.1. Gametogenesis of Platygyra acuta. Sampled *Platygyra acuta* colonies covered three spawning events in 1998, 1999, and 2011; all had a single annual gametogenic cycle and similar annual patterns of gametogenesis with oogenesis occurring for 6-7 months from November 1998 to May 1999 and from November 2010 to June 2011 (Figures 2 and 3). Spermatogenesis was monitored only in 2011 and was found to occur two months before spawning from May to June 2011. Oocytes first appeared in *P. acuta* tissues in both November 1998 (104.89 ± 17.10 μm) and November 2011 (95.9 ± 21.7 μm) (Figure 3). Their sizes increased through the gametogenic cycle to reach the largest mean (±SD) size of 264.44 ± 44.65 μm, 329.1 ± 25.7 μm, and 257.1 ± 11.8 μm, respectively, in June 1998, May 1999, and June 2011. Spermaries first appeared in May 2011 with a mean (±SD) size of 40.3 ± 5.2 μm (Figure 3). They developed rapidly within

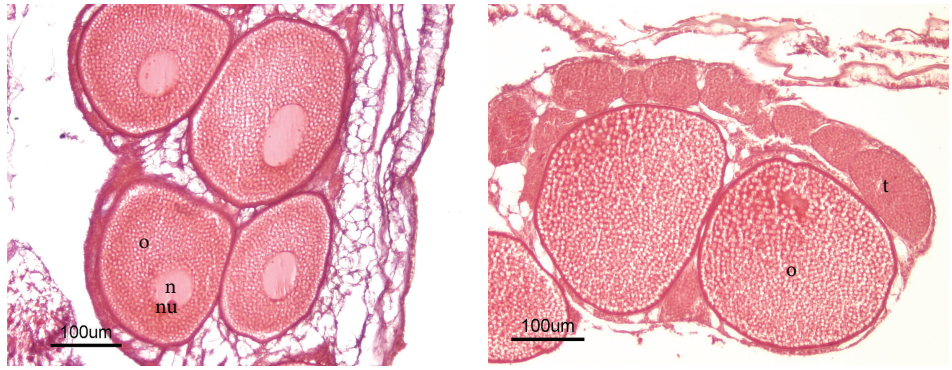

Figure 2: Developing oocytes and spermaries in *Platygyra acuta*. o: oocytes, n: nucleus, nu: nucleolus, and t: spermaries.

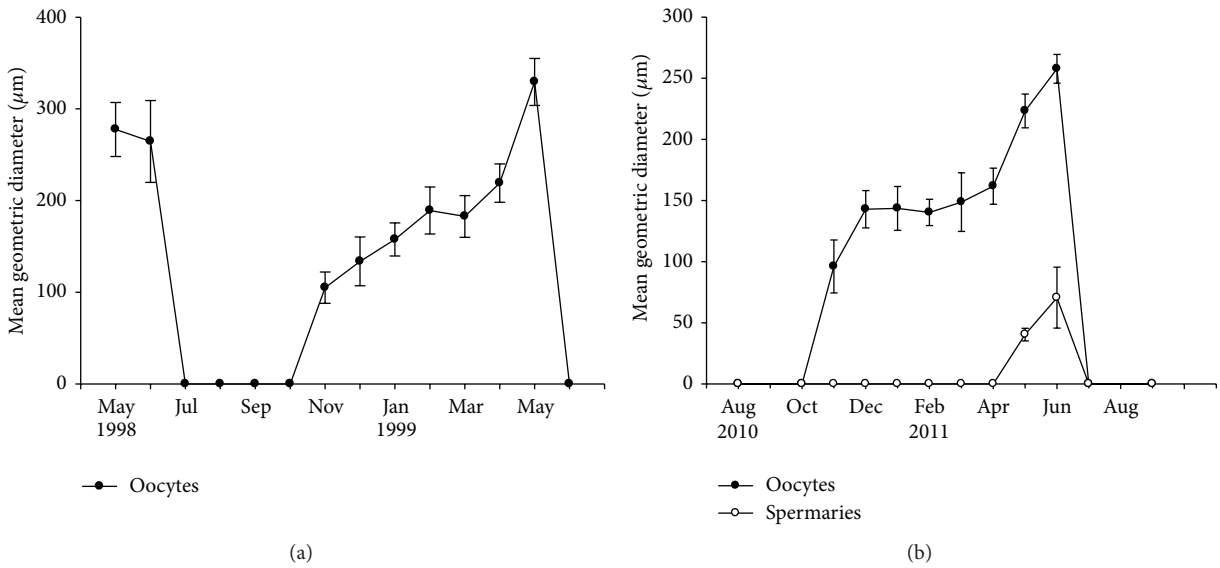

Figure 3: Mean ± SD (n = 5) geometric diameter of oocytes and spermaries (μm) in *Platygyra acuta* colonies during the gametogenic cycle in (a) 1998-1999 and (b) 2010-2011.

a month and achieved their maximum mean size of 70.5 ± 24.9 μm in June 2011. Oocytes and spermaries coexisted in the polyps and all gametes disappeared in the samples of July 2011. Likewise, oocytes were also not detected in the samples of July 1998 and June 1999 (Figure 3). This indicates that *P. acuta* in Hong Kong started gametogenesis in November and mass spawning occurred in May or June in the following year. Two samples collected, one in November 2010 and another in March 2011, within the gametogenetic period were found to contain no gamete. The number of this type of samples is, however, small (5% of sampled colonies).

3.2. Embryogenesis of Platygyra acuta. Average (± SD) temperature of 26.12 ± 1.8°C was maintained throughout the culturing period. Immediately after bundle collection, subsamples of the eggs (Figure 4(a)) released from the bundles were measured under dissecting microscope with the mean (± SD) diameter of the eggs recorded as 375 ± 15.5 μm (n = 20).

The first cleavage was initiated at 2 h after fertilization. Cleavage furrow appeared on one side of the blastomere, creating a heart-shaped zygote (Figure 4(b)). Four equally sized blastomeres were observed at 3 h after the second cleavage (Figure 4(c)). The third cleavage was perpendicular to the first two cleavage planes, creating eight blastomeres that further divided into 16 and 32 blastomeres in 5 h after fertilization (Figures 4(d)-4(f)). After the 32-cell stage, the blastomeres continued to divide into highly irregular shapes (Figures 4(g)-4(h)). Embryos began to flatten and became bowl shaped (cushion stage) at 7 h (Figures 4(i)-4(j)). Smoothening of the embryo surface was observed at 9 h (Figure 4(k)). After 10 h, the irregular shaped embryos expanded and gradually became spherical in shape (Figures 4(l)-4(m)). Invagination of the blastopore began at 16 h (Figure 4(n)), followed by shrinking in diameter of the blastopore (Figure 4(p)). Larvae began to develop cilia and started to rotate at 18 h. Planulae were observed to elongate in the oral-aboral axis and began to be highly mobile at 36 h (Figures 4(q)-4(r)). Formation of two blastopores that resulted in

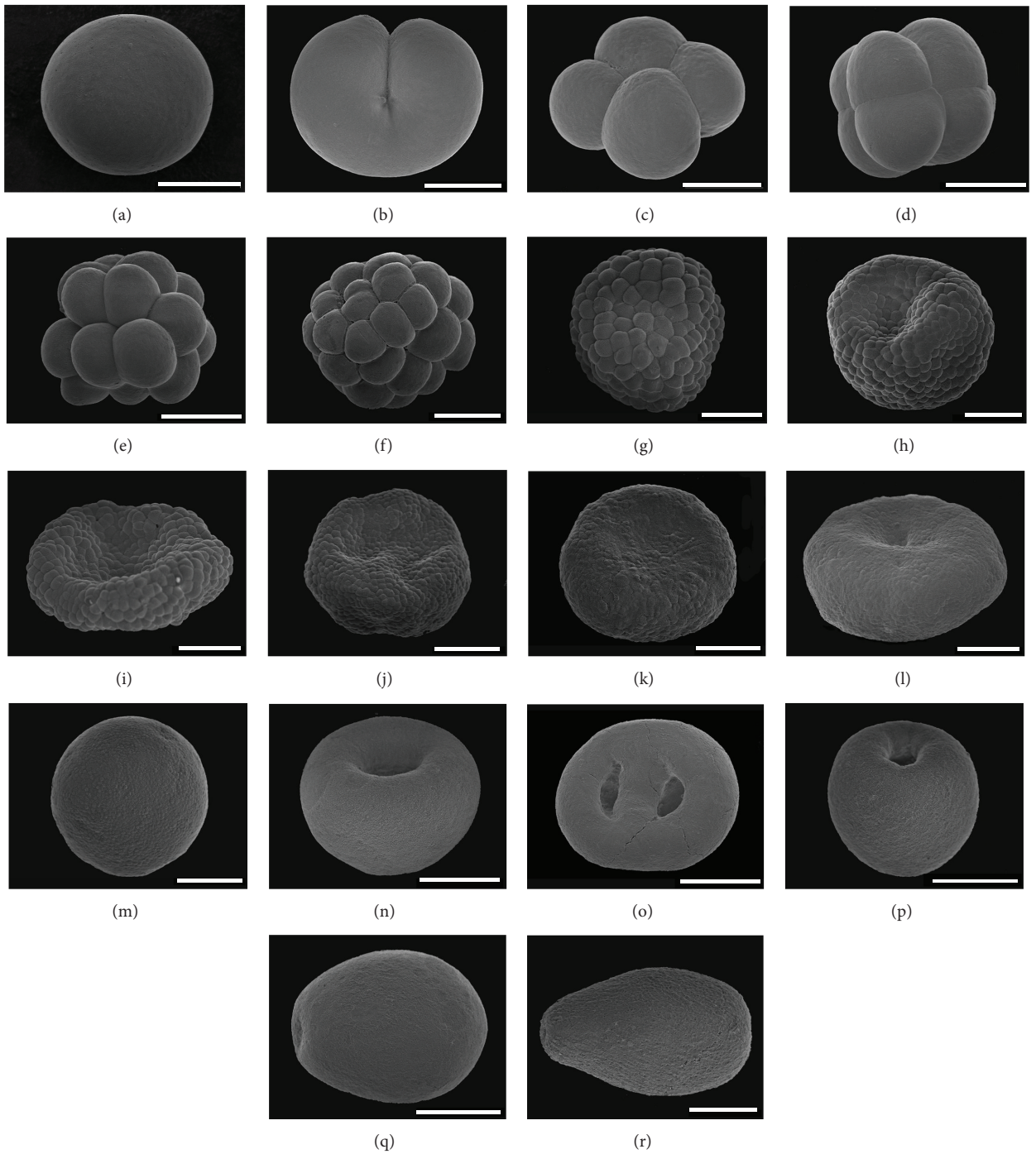

Figure 4: SEM micrograph of embryonic developmental stages of the broadcast-spawning scleractinian coral, *Platygyra acuta*. (a) Egg, where fertilization may or may not have occurred. (b) First cleavage. (c) 4-cell stage. (d) 8-cell stage. (e) 16-cell stage. (f) 32-cell stage. (g)-(h) Blastomeres continued to divide into highly irregular shapes. (i)-(j) Cushion stage. (k) Smoothening of embryo surface. (l)-(m) Embryos expanded and became spherical shaped. (o) Abnormal embryo. (n)-(p) Blastula stage. (q)-(r) Elongated stage. Scale bar = 200 μm.

separate invagination was observed but only in two embryos during sampling at 20 and 22 h. This phenomenon is likely to be an abnormal growth pattern (Figure 4(o)).

3.3. Optimal Sperm Concentration on Fertilization Success. In both experimental days (10th and 11th of June 2012), spawning of *Platygyra acuta* was observed between 2050 and 2210 h. Fertilization success varied significantly with different sperm concentrations (Day 1: $F_{(5,12)} = 182.57$, $P < 0.001$; Day 2: $F_{(6,14)} = 132.48$, $P < 0.001$). High percent fertilization successes (>95% in Day 1 and >73% in Day 2) were obtained at a broad sperm density range between 10^4 and

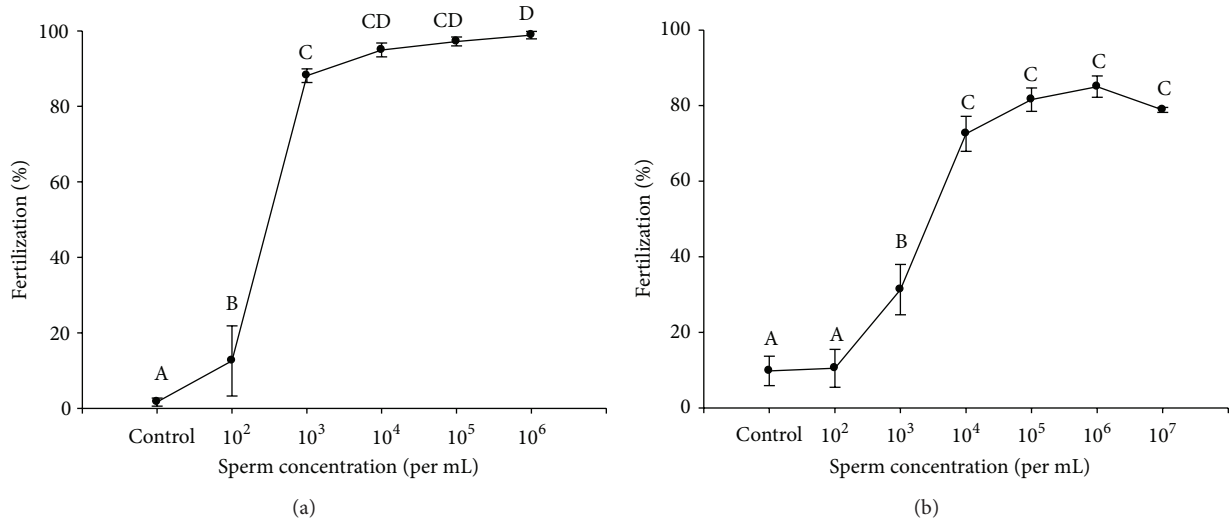

Figure 5: Mean ± SD (n = 3) percent (%) fertilization success of the coral *Platygyra acuta* under different sperm concentration treatments. Experiment was conducted on (a) Day 1, June 10, 2012, and (b) Day 2, June 11, 2012. Data indicated with the same letter showed no significant difference in % fertilization as determined by Tukey's HSD post hoc test. Percent fertilization of eggs in sperm-free filtered sea water (FSW) served as the control to evaluate the effectiveness of sperm wash in separating eggs from the sperms before mixing.

10^6 sperms mL^{-1} (Figures 5(a) and 5(b)). Mean (±SD) percent fertilization success was lowest at sperm concentration of 10^2 sperms mL^{-1} on Day 1 (12.6 ± 9.3%) and Day 2 (10.5 ± 5.0%). Very high sperm concentration of 10^7 sperms mL^{-1} could only be obtained on Day 2 of the experiment and showed no sign of significant inhibition of fertilization (Tukey's HSD test: $P = 0.066$) (Figure 5(b)). However, percent fertilization success varied at sperm concentration of 10^3 sperms mL^{-1} in the two experimental days with 88.1 ± 1.8% observed in Day 1 but only 31.3 ± 6.7% in Day 2. Highest fertilization success was achieved at 10^6 sperms mL^{-1} in both days with 98.9 ± 1% in Day 1 and 85.0 ± 2.8% in Day 2.

3.4. Gamete Ageing Effect on Fertilization Success. In both experimental days, egg bundles were collected from tagged colonies that spawned between 2050 and 2210 h. The fertilization experiment started a bit later after sperm wash to separate sperms from the eggs. Therefore, time = 0 indicated here referred to 2330 h, around 2.5 h after spawning. Fertilization success varied with different ages of gametes (Day 1: $F_{(8,18)} = 112.39$, $P < 0.001$; Day 2: $F_{(7,16)} = 187.87$, $P < 0.001$). Mean percent fertilization success was relatively high for the first 3 h ($T = 0$ to $T = 3$ h) of the experiments, ranging from 96% to 94% in Day 1 and from 75% to 73% in Day 2 (Figures 6(a) and 6(b)). Significant reduction in fertilization success was observed after 3 h (Tukey's HSD test: $P < 0.05$) with only 38.0 ± 3.1% fertilization success at 6 h after the commencement of the experiment (Figure 6(b)).

3.5. Self-Fertilization in Platygyra acuta. No fertilized eggs or very low fertilization <0.6% was observed in this experiment either in 2012 or in 2013, indicating that eggs and sperms from the same colony of *P. acuta* cannot self-fertilize.

4. Discussion

In the present study, oocytes appeared from November to May or June, suggesting that the duration of the oogenic cycle is between six and seven months. The spermatogenic cycle was much shorter. Spermaries started to appear only approximately two months before spawning. Harrison and Wallace [3] reported that broadcast spawning coral species release their gametes subsequent to the full moon after gamete maturation. The disappearance of oocytes and spermaries after May (1999) or June (1998, 2011) therefore indicated that spawning of *P. acuta* in Hong Kong occurs after the full moon in May or June each year. Spawning of *P. acuta* has also been confirmed by field observations. Our results also supported those from previous studies from other localities suggesting that most *Platygyra* species are hermaphroditic broadcast spawners, with oocytes and spermaries present within the same polyp [7, 8, 23, 24].

Broadcast spawning of *Platygyra* species has been documented in different regions [4]. However, there are only two studies describing in detail the length and pattern of gametogenic cycles of *Platygyra* species, including *P. daedalea* in Kenya [25] and *P. pini* in Singapore [24]. Single annual gametogenic cycles were found in majority of *P. daedalea* colonies in Kenya, whereas a low proportion of the population spawned biannually. Duration of oogenic cycle lasted between six and seven months and spermatogenic cycle lasted five months [25]. Two spawning seasons were observed in *P. pini* colonies in Singapore, but whether the two spawning seasons were caused by multiple gametogenic cycles or population split spawning is not known. Duration of oogenic cycle of *P. pini* lasted between five and eight months and spermatogenic cycle approximately two to three months [24]. In the present study, *P. acuta* has a single annual gametogenic cycle, with comparable length of oogenic cycle as that of both

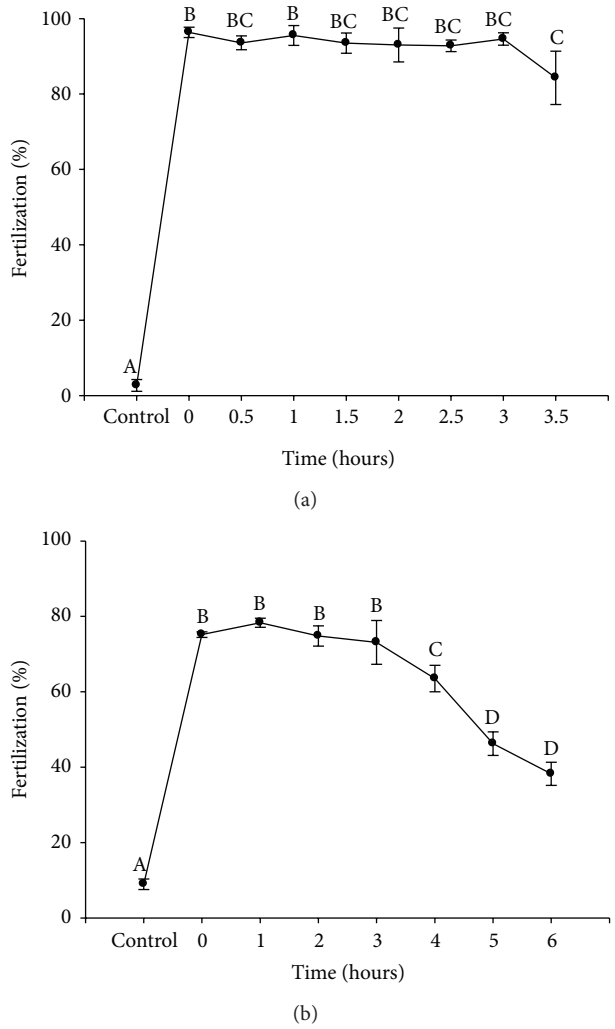

Figure 6: Mean ± SD ($n = 3$) percent (%) fertilization success of the coral *Platygyra acuta* gametes mixed at different times after spawning to examine the effect of ageing. Eggs and sperm were of the same age at the time of mixing. Experiment was conducted on (a) Day 1, June 10, 2012, and (b) Day 2, June 11, 2012. Data indicated with the same letter showed no significant difference in % fertilization as determined by Tukey's HSD post hoc test. The background controls in Day 1 and Day 2 were 1.7.0 ± 1.1% and 9.8 ± 3.9%, respectively.

species of *Platygyra* described above. Its spermatogenic cycle is, however, more comparable to that of *P. pini*. Biannual spawning is not restricted to equatorial reefs [24]. Future research is needed to investigate whether single annual gametogenic cycle is the more common reproductive mode at marginal reef communities. Having annual gametogenic cycle may be one of the adaptive reproductive strategies to ensure high sperm density for fertilization success [6].

Our study is the first detailed examination of the embryonic development of *P. acuta*. In general, the developmental stages are similar to those described for *P. sinensis* [7] and *P. contorta* [11]. The embryogenetic pattern of *P. acuta* also fits that of robust corals described by Okubo et al. [11]. However, Okubo et al. [11] documented the formation of blastopores with separate invagination in *P. contorta*, with the two pores that subsequently merged. In our current study, this stage with two pores was only observed in two but not in the rest of >100 embryos. We therefore suspect this to be an abnormal growth stage for *P. acuta*. It is still possible that this stage was very short and hence could have been missed between our sampling intervals of one h, or such characteristic of double invagination is not shared among *Platygyra* species.

While the general embryogenetic patterns and stages of *P. acuta* are similar to those of many of the other coral species examined thus far, they differ from those of the branching *Acropora* spp. [11, 26]. Instead of forming a very thin single layer prawn-chip stage which is typical for *Acropora* species, *P. acuta* develops a cushion stage (Figures 4(i) and 4(j)) which subsequently expands and develops a depression on its side referred to as "pseudoblastopore" by Okubo et al. [11]. This is then followed by a return to spherical shape before commencing a second invagination (Figure 4(m)). It is reported that the timing required for coral embryo to complete embryonic development through to larval motility stage varies among families and species. *Acropora hyacinthus* and *Pectinia lactuca* embryos were motile at 48 h and 18 h, respectively [27]. *Favites pentagona* and *F. abdita* embryos started swimming approximately 15 and 22 h after first cleavage [11]. In this study, *P. acuta* developed cilia and started to rotate 18 h after fertilization. This is comparable to *P. contorta* whose embryos were reported to start swimming 19 h after fertilization [11]. In both the present and *P. contorta* studies, the culturing temperature for embryonic development was maintained around 26°C.

Fertilization successes of *P. acuta* were strongly influenced by sperm concentrations. The pronounced decrease in percent fertilization success at low sperm concentrations is believed to be due to the decreased probability of successful egg-sperm encounters [6]. Our results were comparable to those from Oliver and Babcock [6] for *P. sinensis*, but with even broader optimal sperm concentrations recorded from 10^4 to 10^7 sperms mL^{-1}. Inhibition of fertilization was reported when sperm concentration was greater than 10^6 sperms mL^{-1} [6], but such inhibition was not observed in the current study. However, it should be noted that the level of statistical significance to evaluate the difference in fertilization success between the highest sperm concentration of 10^7 sperms mL^{-1} and the lower sperm concentrations was marginal (i.e., $P = 0.066$). Significant inhibitory effect might be detected with more replicates or in repeated experiments. Nonetheless, we were unable to test sperm concentration higher than 10^7 sperms mL^{-1}. The overall fertilization success in the second day of spawning was also lower, with the highest only around 80%. This suggests that the quality of the eggs and sperms may differ between spawning days and may ultimately affect the optimal concentration of sperm for successful fertilization. In the present study, the most striking difference on optimal sperm concentration for fertilization success between the two days of spawning was observed at 10^3 sperms mL^{-1} concentration. The reason for a much lower percent fertilization success at this sperm concentration in the second day of spawning is not known but may be related

to the lower quality of the sperms or their activity from subsequent days of spawning. Additional experiments should be carried out to evaluate this hypothesis.

Gamete ageing effect experiment indicated that viability of the gametes for successful fertilization is relatively short. The capability for fertilization remained high for up to 3.5 h after the experiment started. Given that the start of the experiment ($T = 0$) was about 2.5 h after spawning, this indicates that highest gamete viability could only last for 6 h. Nonetheless, the duration of this viability is already much longer than that reported for other corals. Oliver and Babcock [6] showed thatfertilization for *P. sinensis* remained high for only up to 2 h after spawning. The mechanisms behind such prolonged viability of *P. acuta* gametes in the present study are not clear. Apparently, given that the supply of gametes is usually limited in a marginal environment, such prolonged viability may be a reproductive strategy to enhance fertilization success by increasing the chances of egg-sperm encounter over time. It is also not clear whether the loss of viability was largely due to the eggs or the sperms or both. It has been suggested that the loss of viability may be related to a drop in sperm motility due to depletion of its limited energy reserves [6]. Prolonged gamete viability of more than 5 h has been demonstrated in other marine invertebrates, such as the abalone *Haliotis laevigata* [28] and mussel *Mytilus edulis* [29]. Even longer sperm longevities of more than 24 h were reported in the ascidians *Ascidia mentula* [30]. Although eggs and sperm of the coral *P. acuta* were still fertilizable for up to 6 h after release, the age of the gametes for optimal fertilization is comparatively short.

Plasticity in coral gametes released over the spawning period of several days has been documented before. Hédouin and Gates [31] reported a significant difference in the fertilization success of *Montipora capitata* between two consecutive spawning nights within a month and between spawning months under copper exposure. It has also been shown that the number, size, and lipid and protein concentrations of gametes released at different spawning days were different, thus contributing to differences in their quality [30, 31]. The generally much lower fertilization success observed in the second day of spawning in *P. acuta* observed in the present study may be part of an adaptive reproductive strategy for this coral. The best and most mature egg bundles could be released immediately upon receiving the first cue of a favorable condition for fertilization to ensure a higher probability of fertilization success. Those released in the second or subsequent days may thus be of a lesser quality. Further investigations should be conducted to assess this hypothesis.

Results of self-fertilization experiment indicated that eggs and sperms from the same colony of *P. acuta* were not able to self-fertilize. Scleractinian corals have been reported to demonstrate a self-recognition response [32]. However, the degree of specificity may vary with species [33]. One thing to be noted was that although self-fertilization experiment in this study was ended at least 4 h after gamete mixing, it has been reported that the effectiveness of the barrier that inhibits self-fertilization may decrease several hours after spawning [34]. There remains a possibility that the level of self-fertilization success might increase beyond 4 h after spawning in *P. acuta*. Nevertheless, the ability to avoid self-fertilization, together with successful fertilization under lower sperm concentration and prolonged gamete longevity, will all provide time for gametes from different colonies to mix, hence enhancing the chance of successful cross-fertilization in *P. acuta* under the natural condition [6].

5. Conclusions

Reproductive ecology and strategy of corals from marginal environment are understudied. Yet, marginal environment could be refuge for future coral expansion under the threat of global climate change. Our study provided the first detailed documentation on gametogenic cycles, embryonic development, and fertilization ecology of *P. acuta*, one of the most dominant massive coral species in subtropical marginal nonreefal coral communities in Hong Kong. Our study also contributed new insights to the understanding of the reproductive strategy of corals. These baseline pieces of information and insights may shed light on further understanding coral responses to and adaptations in a changing ocean environment and could contribute to the development of strategies for their conservation and protection.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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