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TEMPORAL VARIATION IN SHELL GROWTH RATE OF COCKLE *ANADARA GRANOSA* IN RELATION WITH ITS REPRODUCTIVE CYCLE

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ABSTRACT A study was conducted on the relationship between the reproductive cycle and shell growth rate of *Anadara granosa* (Linnaeus, 1758). Gonadal maturity stages, increment width, and environmental factors were examined by means of a field enclosure experiment in Balik Pulau, Penang Island, Malaysia, from December 2011 to November 2012. Histological analysis of gonads showed that gametogenic activity of *A. granosa* occurred throughout the year, with maximum spawning activity observed from April 2012 to late June 2012 (15%–28%) in males and from March 2012 to late June 2012 (19%–44%) in females. Shell cross-section analysis showed that the increment widths of both sexes in the growing group (indeterminate and developing stages) and the spawning group (ripe and spawning stages) ranged from 35–57 to 8–17 μm, respectively. Seawater temperature and salinity recorded on a daily basis throughout the study period ranged from 22–33°C and 29–31, respectively. A comparison of increment width and gonad development stages in different environmental conditions showed that shell growth in the growing group decreased when seawater temperature and salinity decreased slightly. In contrast, increment widths in the spawning group were much narrower and were influenced by energy consumption during ripe and spawning stages. This study shows spawning breaks in shell structure are considered markers for identifying the period of sexual maturity. Therefore, spawning breaks are suitable proxies for interpretation of the temporal changes in shell microgrowth lines in terms of reproductive cycle of cockle and understanding the number of spawning periods throughout the year.

KEY WORDS: cockle, *Andara granosa*, reproductive cycle, shell microgrowth increment, growth pattern, spawning season

INTRODUCTION

Growth of aquatic animals can be observed using the growth lines that appear in the shells of molluscs and in the otolith and scales of fish species. Growth lines in animals can give a good indication of their life cycles, as well as environmental factors that influence their life histories (Mirzaei et al. 2015). The high-resolution variations in response to environmental and physiological changes in cockle shells make them a good representative species for examining how these changes influence growth (Gibson et al. 2001, Schöne et al. 2003, Witbaard et al. 2005, Ambrose et al. 2006, Gosselin et al. 2006, Limpanont et al. 2010, Karney et al. 2011, Liu et al. 2011).

Cockle shells possess a series of lines, termed "microgrowth lines" interspaced by "growth increments" (i.e., the area between two microgrowth lines), which can be clearly viewed in shell sections (Lavaud et al. 2013). Microgrowth lines are darker and narrower than the growth increments, which are thicker and are located between the microgrowth lines. Cockle shells grow according to the production of consecutive microgrowth lines and growth increments, which together can be considered as a growth pattern. Growth patterns are typically influenced by local environmental conditions or physiological changes such as reproduction (Kanazawa & Sato 2008, Mirzaei & Shau Hwai 2015).

Interruptions in growth patterns can appear due to environmental changes including thermal shock, excessively hot or cold seasons, neap and spring tides, shell margin abrasions, and spawning period (Gibson et al. 2001). However, the formation of irregular microgrowth lines is less affected by environmental factors and more closely related to physiological activities, especially reproduction (Cerrato et al. 1991, Schöne et al. 2005). Physiological changes during the reproductive season have a negative effect on the pattern of shell growth. Specifically, spawning breaks are visible in most cockle shells as microgrowth line production ceases during spawning (Thompson et al. 1980, Sato 1995, Schöne et al. 2005, Nishida et al. 2012). An analysis of the impact of reproduction on the various aspects of shell growth is important for both studies of life history and morphological evolution. However, very few studies have assessed the relationship between shell microgrowth lines and reproductive cycle in marine molluscs [but see studies on *Tellin nitidotellina nitidula* (Kawai et al. 1993) and *Phacosoma japonicum* (Sato 1995)].

In Peninsular Malaysia, *Anadara granosa* of the family Arcidae is a commercially important species. Blood cockle production in 2008 reached the highest value of USD 28 million, whereas it reached to USD 23 million in 2011 (Mirzaei et al. 2015). The reproductive cycle of *A. granosa* varies geographically (Suwanjarat et al. 2009, Khalil 2013). Thus, analyses of the physiological and environmental impacts on the growth of *A. granosa* are important for studies of both life history and morphological evolution. Therefore, it is necessary to determine the pattern of maturity and spawning season and their relationships with environmental factors. In this study, the different maturity stages of *A. granosa* and the impact of local environmental changes and spawning breaks on the microgrowth lines in their shell structure were investigated.

MATERIALS AND METHODS

Site Preparation and Sample Collection

A total of 600 similarly sized *Anadara granosa* (~10 mm) were stained with shell dye (Alizarin Red) at a concentration of

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30 ppm before being transferred to the study site. The cockles were placed in a plastic mesh cage (1.5 m × 1.5 m × 2 m) located at an intertidal site (exposed during all low tides) in Balik Pulau (5° 20′05.50′′ N, 100° 11′35.32′′ E), Penang Island, Malaysia (Fig. 1). Forty samples were collected each month between December 2011 and November 2012. In the laboratory, soft tissues were gently removed from inside the shells and were used for histological examination and shell valves were prepared for shell cross-sectioning.

Histology

Histological analysis was carried out according to the protocol described by Matias et al. (2013). The gonadal tissue was cut into small pieces (5 mm) and immediately immersed in Bouin's fixative for 24 h. A serial dehydration process was conducted using increasing concentrations of an alcohol solution to remove excess water from the gonad sample (Khalil 2013). The clearing process was carried out by immersing the gonad specimen in a xylene solution. The gonad sample was then impregnated with paraffin wax using a tissue embedding system (Leica EG1160) to maintain tissue integrity and to facilitate cutting by the microtome (Leica RM 2135). Sections were mounted on glass slides, stained with hematoxylin to aid observations and then sealed with a cover slip using DPX glue.

Classification of Gonadal Development Stages

The development stages of gonads were identified using the criteria described by Suwanjarat et al. (2009). Based on histological examination and microscopic observation, developmental stages were categorized as indeterminate, developing, ripe, or spawning (see Table 1).

Preparation of Shell Cross-Sections

Shell cross-sections were prepared from a single valve of each animal using the standard method described by Richardson (1987). The valve of each animal was marked in pencil from the umbo to the ventral margin along the maximum growth line (Fig. 2). Marked valves were embedded in an epoxy resin (a 2:1

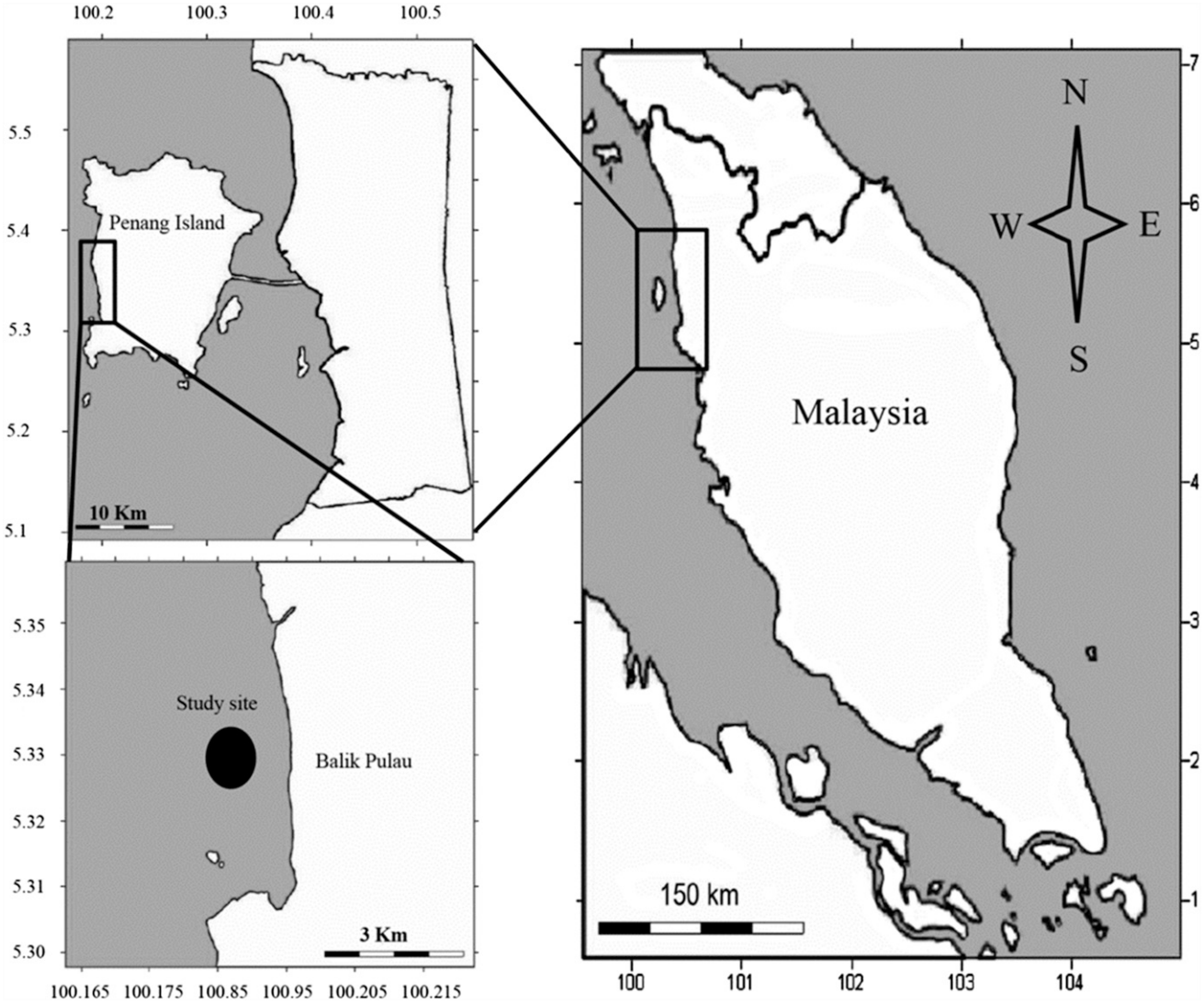

Figure 1. Location of *Anadara granosa* study site in Balik Pulau, Penang Island, West Coast of Peninsular Malaysia.

TABLE 1.

Classification of gonadal development stages in male and female *Anadara granosa* (Suwanjarat et al. 2009).

Gonadal stages	Histological characteristics	
	Male	Female
Indeterminate	This stage is also called the inactive stage, during which the sexes cannot be distinguished. In the undeveloped stage, connective tissue can be observed. There is no trace of gonad development and specimens cannot be sexed visually. In this phase, some residual gametes, which are derived from the gamete release stage, are occasionally found. These gametes will be reabsorbed and there is no progress in development	
Developing	Follicles start to grow, and connective tissue is reduced. Starting from the outer layers to the center, all developmental stages are present, that is, spermatogonia, abundant spermatocytes, smaller spermatids, and ripe spermatozoa with their pink tails. The average diameter of the follicles at this stage is 117.77 ± 19.58 μm	Follicles begin to grow and fill with oogonia and developing oocytes (increased size and irregular shapes) associated with the follicular wall. Some ripe oocytes with a large nucleus may be observed free in the lumen. The average diameter of the follicles at this stage is 136.2 ± 22.12 μm and the average diameter of oocytes is 24.81 ± 6.19 μm
Ripe	Follicles are enlarged and stratified. Spermatozoa dominate and are tightly packed in the follicle, whereas spermatogonia are restricted to a thin layer at the periphery of the follicle. Only a small amount of connective tissue is present. The average diameter of the follicles at this stages is 186.16 ± 14.47 μm	Follicles are large, distended, and filled with free, polyhedral-shaped, ripe oocytes with a large nucleus and a small nucleolus. The interfollicular space decreases. Few oogonia and immature oocytes remain attached to the follicle wall. The average diameter of the follicles at this stage is 215.13 ± 38.40 μm and the average diameter of oocytes is 30.01 ± 6.80 μm
Spawning	Gamete release starts. Follicles present small, empty spaces with many free residual spermatozoa. Some follicles have irregular shapes with contracted membranes and a small diameter. Spermatogonia are not found in this stage	Oocytes are released from the follicle. Follicle walls look broken and empty, but still distended. Much residual material is seen. Some types of phagocytes appear in the space between the residual oocytes, which now look rounded

ratio of epoxy resin to epoxy hardener) for 24 h to protect the valves during cutting and grinding procedures.

The valve/resin moldings were then fixed on a low-speed saw with a diamond-impregnated blade (Buehler Ltd., Lake Bluff, IL). The low speed made it possible to cut the fragile material without fracturing. The valves were cut through the pencil mark from the umbo to the ventral margin of the shell. Shell sections were polished using sequential grit sandpapers (240, 400, 600, 800 and 1200 Buehler carborundum grits) to remove epoxy resin from the cut surfaces of the valves. The cut valve surfaces were then polished with an aluminum oxide powder on a semi-automatic polishing machine (FORCIMAT—FORCIPOL 300–1V). The cut valve was then removed, rinsed in tap water, and dried. Etching was conducted in a 0.1% solution of hydrochloric acid (HCl) for 1 min so as to leave the aragonite granules that can distort the cross-section image at the surface of the valve section.

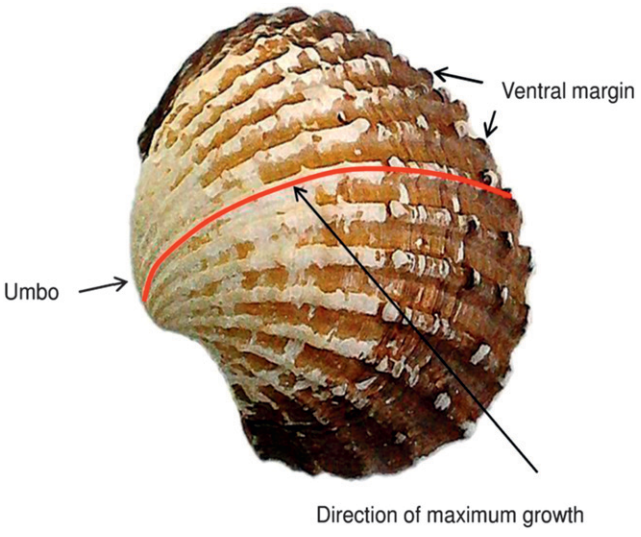

Figure 2. Cutting direction from umbo to ventral margin along the maximum growth line.

Growth Pattern Periodicity

The experiment was designed to examine whether tidal change, emersion, and daily rhythms had any effect on shell banding formation in *Anadara granosa*. The total number of growth bands was counted in each shell between the Alizarin red staining point and the shell margin. A *t*-test was used to determine whether band number was associated with number of days, tidal patterns, or emersion events. The mean difference between pairs of expected and observed values was used to determine whether the values differed significantly from zero.

Increment Width Measurement

The etched shell cross-section was examined under a light stereomicroscope (Olympus SZ61; Olympus Optical Co. Ltd., Tokyo, Japan) at ×100 magnification and photographed (Xcam Alpha; The Imaging Source GmbH). For each sample, microgrowth increments were measured from the shell margin to the Alizarin red staining point using microscopic image

analysis software (Analysis Image Processing Version 5.1; Olympus Soft Imaging Solutions 1989–2008).

Measurement of Environmental Factors

Daily seawater temperature and seawater salinity were measured using a HOBO Pendant Temp/Light logger and a hand-held refractometer (RHSN-10ATC BUILT), respectively. The logger was calibrated to record hourly seawater temperatures for the 1-y study period and seawater salinity was measured daily at the study site for the period between December 2011 and November 2012.

Statistical Analysis

To determine the relationship between shell increment width and the reproductive cycle, monthly samples were divided into two groups, namely, a growing group (indeterminate and developing stages) and a spawning group (ripe and spawning stages). Two-sample independent *t*-tests were used to compare the means of the increment widths between the growing groups and the spawning groups as well as the means of the increment widths between males and females. Correlation analysis was used to determine the linear relationship between increment widths in spawning and growing groups with each environmental variable. A P value <0.05 was considered statistically significant and all data are presented as means ± SE.

RESULTS

Microscopic Description of the Male Gonad

In the indeterminate stage, when the gonad was empty and only connective tissue was present, the sexes were indistinguishable and connective tissue can be observed. Some residual gametes following release could be found in the gonads (Fig. 3A). The developing stage was characterized by the presence of spermatogonia on the follicular wall. Spermatocytes and spermatids were most abundant, and a number of spermatozoa were found toward the end of the developing stage (Fig. 3B). In the ripe stage, follicles were full of spermatozoa, the tails of which were pointed toward the center of the lumen. Only a small amount of connective tissue is present. The average diameter of the follicles at this stages is 186.16 ± 14.47 µm (Fig. 3C). Spermatozoa are released during the spawning stage, leaving an empty space in the follicular lumen. Some follicles have irregular shapes with contracted membranes and a small diameter (Fig. 3D).

Microscopic Description of the Ovary

The sexes could not be distinguished in the indeterminate stage. At this stage, connective tissue and some residual gametes following gametic release could be found in the gonads (Fig. 4A). During the developing stage, immature oocytes were observed attached to the basal membrane. Various sizes of developing oocytes were found, which possessed basophilic cytoplasm containing irregularly shaped nuclei. The average diameter of the follicles at this stage is 136.2 ± 22.12 µm and the average diameter of oocytes is 24.81 ± 6.19 µm (Fig. 4B). The size of oocytes increased in the ripe stage. Acidophilic cytoplasm and yolk granules were clearly visible and ripe oocytes were mostly free in the lumen. The average diameter of the follicles at this stage is 215.13 ± 38.40 µm and the average diameter of oocytes is 30.01 ± 6.80 µm (Fig. 4C). During the spawning stage, the number of ripe oocytes decreased and empty spaces were observed in the follicular lumen. Some types of phagocytes appear in the space between the residual oocytes, which now look rounded (Fig. 4D).

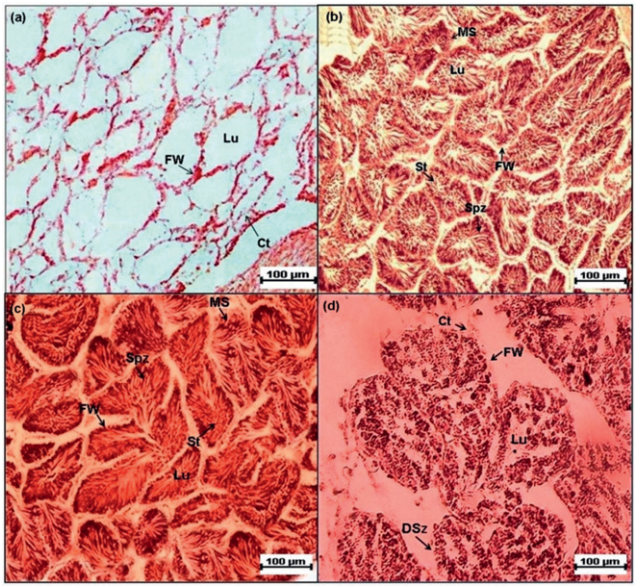

Figure 3. Photomicrograph of a histological section of (A) indeterminate, (B) developing, (C) ripe, and (D) spawning stages of a male gonad in *Anadara granosa*. FW, follicular wall; Lu, lumen; Ct, connective tissue; MS, mature spermatozoa; St, spermatid; Spz, spermatozoa; DSz, degenerative spermatozoon.

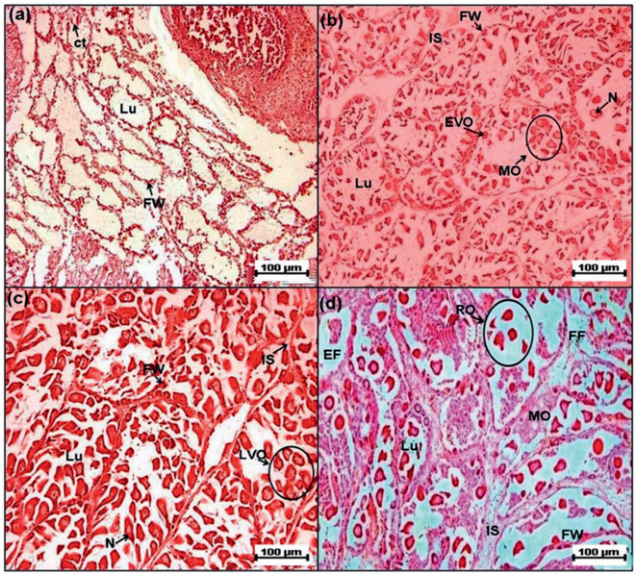

Figure 4. Photomicrograph of a histological section of (A) indeterminate, (B) developing, (C) ripe, and (D) spawning stages of a female gonad in *Anadara granosa*. FW, follicular wall; Lu, lumen; Ct, connective tissue.; MO, mature oocyte; LVO, late vitellogenic oocyte; N, nucleus; IS, interfollicular space; RO, residual oocyte; FF, fragment follicles; EF, empty follicles.

***Reproductive Cycle of* Anadara granosa**

The indeterminate stage predominated for both sexes throughout the study period. The percentage of samples in the indeterminate stage was highest in December 2011 (57%), followed by August 2012 (54%), November 2012 (54%), June 2012 (40%), and July 2012 (30%), whereas the lowest percentage was in April 2012 (8%).

The developing stage was observed for both sexes throughout the year, with the highest percentages of females at this stage during July 2012 (46%), March 2012 (42%), February 2012 (35%), and January 2012 (27%). The frequency of females undergoing this stage was lowest in December 2011 (21%) and November 2012 (21%). A similar trend for the developing stage was observed for male *Anadara granosa*, with the highest frequencies in this stage during January 2012, February 2012, and March 2012 (46%, 37%, and 28% respectively), but with an additional peak in August 2012 (48%).

The maximum frequency of the ripe stage in female *Anadara granosa* occurred during April (57%), followed by January (50%), February (40%), and March (38%). For males, the ripe stage was observed with the highest frequency in December 2011 (40%) and February 2012 (45%). The spawning stage was observed throughout the study period (December 2011 to November 2012) in both male and female *A. granosa*; however, in female, peaks in the spawning period were observed in April 2012 (15%) and late June (28%). For males, the peak spawning period (19%–44%) was observed from March 2012 to late June 2012, with a secondary peak observed in October 2012 (21%) (Fig. 5).

Microgrowth Pattern Periodicity

The mean numbers of microgrowth bands deposited in the shell layer were almost the same as the total number of tidal emersions in fortnightly periods from December 2011 to January 2012. There was no significant difference between the number of observed microgrowth lines and number of tidal emersions for shells in the intertidal area ($P > 0.05$) (Fig. 6). However, the number of microgrowth lines during the study period was significantly different from the number of days and tidal changes for samples obtained from the intertidal area ($P < 0.05$).

Shell Increment Width Measurements

The shell increment widths of individuals in the indeterminate and developing stages (growing group) were relatively stable from December 2011 to early June 2012, ranging from 47 to 57 μm.

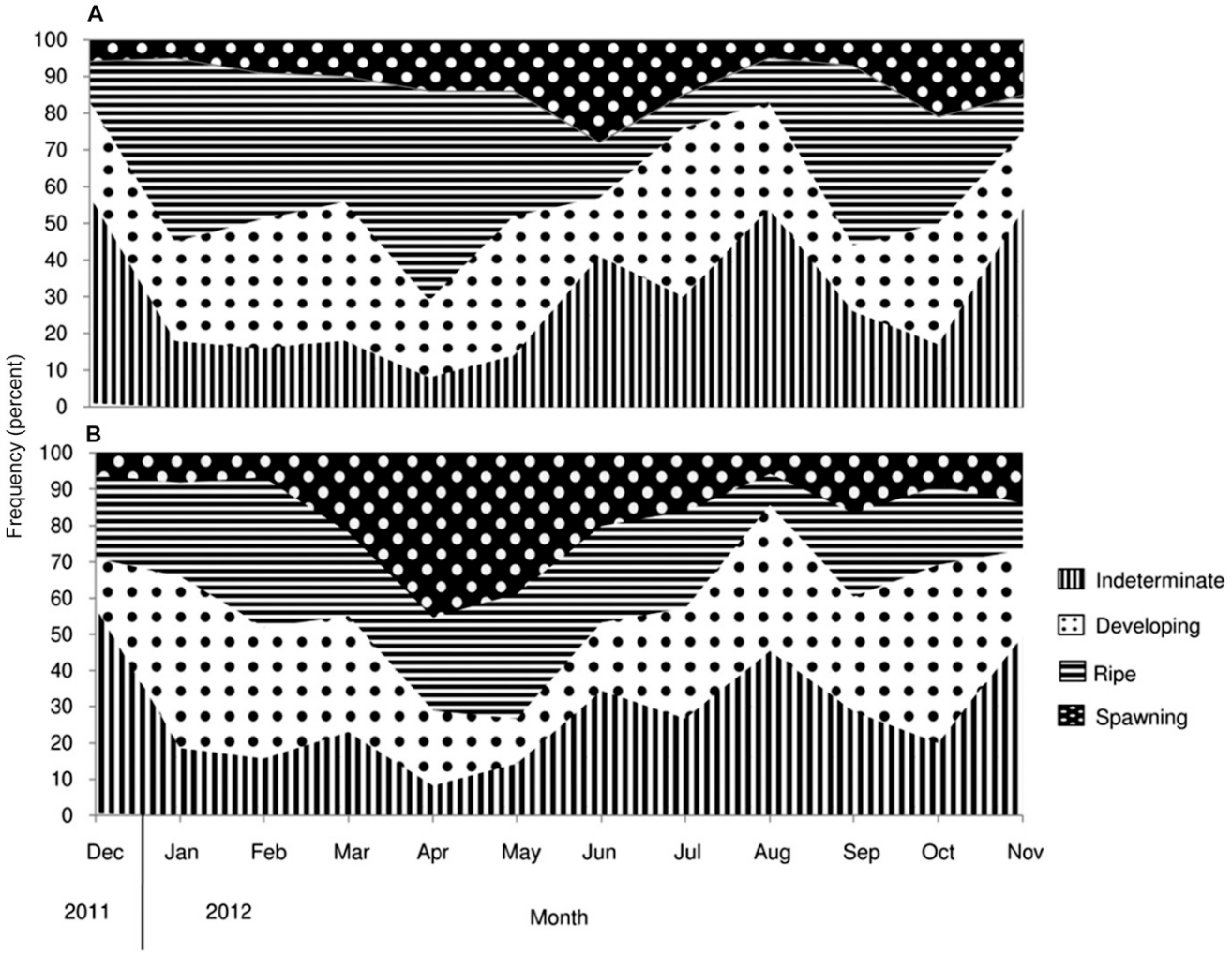

Figure 5. Distribution of gonadal stages in (A) female and (B) male *Anadara granosa* in Penang Island, Malaysia (December 2011 to November 2012).

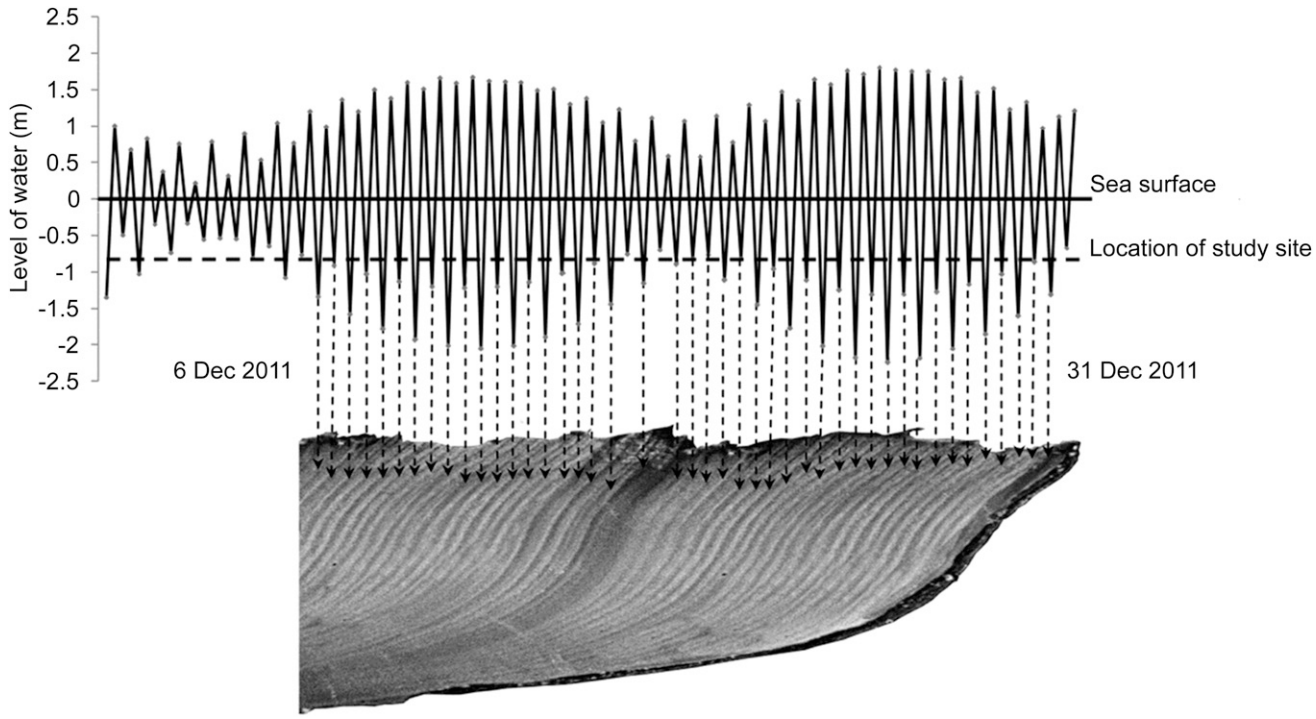

Figure 6. Shell microgrowth pattern formed from 6 to 31 December 2011 for an individual (shell length: 13 mm, age: 5 mo) of *Anadara granosa* collected from Pinang Island, Malaysia.

Shell increment widths then decreased to 35–45 μm from early June 2012 to early September 2012. Increment widths again increased ranging between 47 and 57 μm during the last 2 mo of the study period (October to December 2012) (Fig. 7).

Throughout the study period, shell increment widths of cockles in the spawning group typically ranged from 8 to 17 μm. However, during the spawning stage, increment widths decreased to less than 5 μm for some individuals, resulting in a spawning break within the shell structure (Fig. 8). Based on gonad histology analysis, gonad maturation progressed from the spawning stage to indeterminate (spent) stage following the spawning break, with the increment widths gradually increasing to 47–57 μm during the indeterminate and developing stages. The independent sample (two-tailed) *t*-test showed there were no significant differences between increment widths of males and females in the spawning ($P = 0.827$) and growing ($P = 0.79$) groups; however, there was a significant difference between increment widths in the spawning and growing group ($P < 0.005$) (Tables 2 and 3).

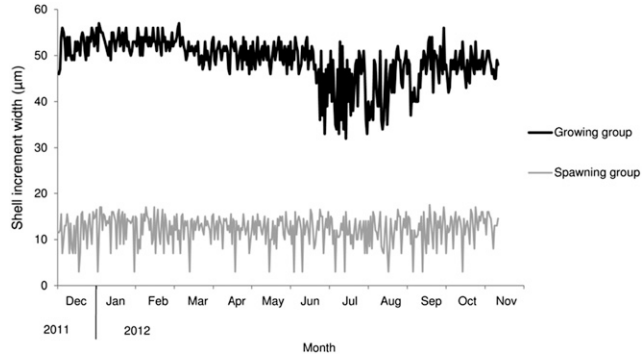

Figure 7. Shell increment width of the two groups (growing and spawning) of *Anadara granosa*, collected from Balik Pulau, Penang Island, Malaysia (December 2011 to November 2012).

Environmental Parameters

Daily variations in seawater temperature ranged from a maximum of 33°C to a minimum of 22°C over the course of the study period. Figure 9A shows the frequent significant fluctuations in seawater temperature observed during December 2011 and January 2012. Between late January 2012 and late June 2012, seawater temperature remained relatively stable (between 28°C and 33°C) for a period of 3 mo, before increasing to 30°C in late September 2012. During the last 2 mo of the study period (October and November 2012), the temperature remained relatively stable (28–33°C).

In general, seawater salinity remained relatively stable, ranging between 29 and 31 throughout the 1-y study period (Fig. 9B). Three periods of fluctuating seawater salinity were observed in December 2011, from April 2012 to late May 2012 and from late September 2012 to late November 2012. Seawater salinity dropped sharply and reached to the lowest point (11) on December 12, 2011. The second lowest seawater salinity was 14 on March 6, 2012 and October 27, 2012.

Correlation Matrices

Correlations between increment widths and environmental factors (temperature and salinity) are shown in Table 4. The correlation matrices showed a strong direct relationship between increment widths in the growing group and temperature

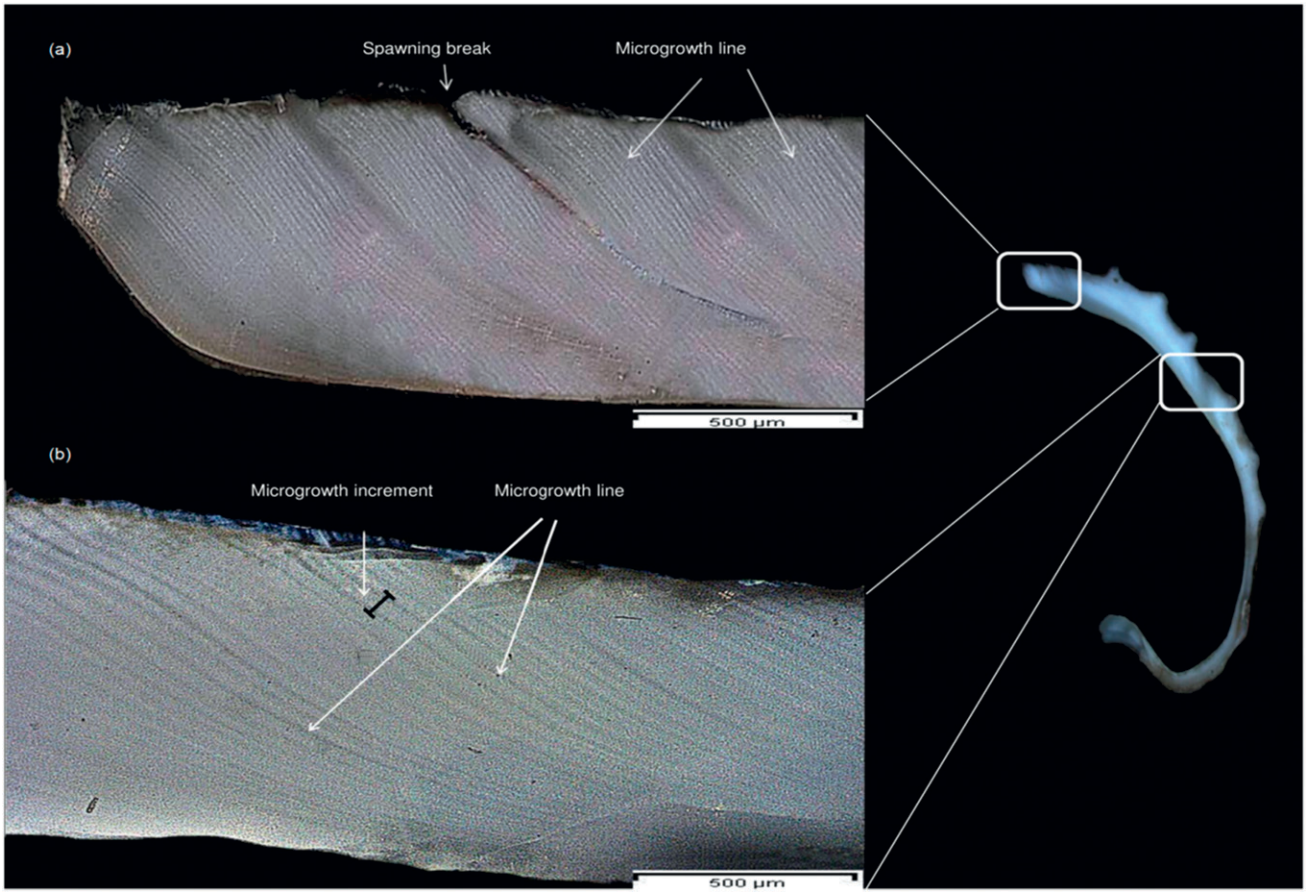

Figure 8. Interior shell section of *Anadara granosa* showing (A) cessation of growth (spawning break) during the spawning stage and (B) wide microgrowth increment during the developing stage.

($r = 0.642$, $P < 0.05$). There was a weak positive correlation between salinity and increment width in the growing group ($r = 0.46$, $P < 0.05$), but there was no correlation between increment width in the spawning group with either temperature or salinity.

DISCUSSION

Histological examination of the gonads showed gametogenic activity and indicated that *Anadara granosa* spawn throughout the year, with a maximum spawning period observed from April to late June. A second peak in spawning occurred in October. Based on environmental data, the main spawning season coincided with high seawater temperatures (30–33°C) and considerable fluctuations in seawater salinity (14) during the 1-y study period. Under the optimal conditions of the growing period, the shell increment width ranged from 47 to 57 µm. Shell increment width patterns decreased with decreasing seawater temperature in the study area. In the spawning group, changes in shell increment widths were masked by the impact of spawning activity.

A possible explanation for the differences between increment widths in spawning and growing individuals might be changes in feeding activity during the spawning season.

TABLE 2.

Two-sample independent *t*-tests between increment widths in males and females of *Anadara granosa* in growing and spawning groups from December 2011 to November 2012.

		Levene's test for equality of variances		*t*-test for equality of means		
		F	**Sig.**	***t***	**df**	**Sig. (two tailed)**
Growing group	Equal variances assumed	0.080	0.77	–0.21	182	0.82
	Equal variances not assumed			–0.21	181.9	0.82
Spawning group	Equal variances assumed	0.43	0.51	0.25	182	0.79
	Equal variances not assumed			0.25	181.57	0.79

TABLE 3.

Two-sample independent *t*-tests between increment widths in growing and spawning groups of *Anadara granosa* from December 2011 to November 2012.

	Levene's test for equality of variances		*t*-test for equality of means		
	F	**Sig.**	***t***	**df**	**Sig. (two tailed)**
Equal variances assumed	34.47	0.00	–113.80	695	0.000
Equal variances not assumed			–114.15	592.63	0.000

Cockles can suddenly stop feeding in the late ripe and early spawning stages (Kanazawa & Sato 2008). Therefore, their growth rates would significantly decrease during spawning period. Moreover, reproduction is one of the most energy-consuming physiological activities, with gametogenesis acting as a regulator thus exerting considerable influence on shell formation. Consequently, the allocation of energy into gamete release leads to different shell growth rates between spawning and growing groups. This conclusion verifies the findings of Lewis and Cerrato (1997) who studied the formation of increments and rings in the shell of *Mya arenaria* and reported that a decline in metabolic activity before the spawning stage was responsible for growth cessation. Sato (1995) found that a sudden decrease in feeding activity during the ripe and early spawning stages led to a decline in the growth of *Phacosoma japonicum* shells. Moreover, Miyaji et al. (2007) reported that mollusc specimens secrete calcium carbonate in their shells at a high rate during their growth period, which necessitates use of almost all of their energy resources. However, due to utilization of energy resources to cover the spawning period, shell increment widths decrease sharply during the spawning stage.

There was a similarity between the spawning period described in this study and those observed in earlier studies (Table 5). Broom (1983) reported that *Anadara granosa* spawned throughout the year, with a maximum spawning period in October. Similarly, Narasimham (1969) observed year-round spawning in the species, but with a period of high intensity from January to April. Khalil (2013) also reported year-round spawning but with peaks between June and September (Banda Ache, Indonesia), April and October (Lhokseumawe, Indonesia), and June and September (Penang, Malaysia). These peaks are consistent with remarks by Sarkis et al. (2006), who stated that the majority of spawning in tropical species occurred exclusively or most intensely during the warmer months. In addition, the developing stage predominated during periods of lower temperatures [July to September (28.5–27°C)].

The histological analysis of the gonads showed that the spawning stage coincided with some frequent strong fluctuations in seawater salinity. This finding is in agreement with those of Toral-Barza and Gomez (1985) who reported that a major spawning period for *Anadara granosa* in the west coast of Malaysia occurred when low salinity continued over a short period (Fig. 9C).

Growth breaks in shell cross-sections of *Anadara granosa* are formed by an interruption in shell growth. These growth breaks arise from various environmental or physiological stressors such as temperature or salinity shocks, shell margin abrasions,

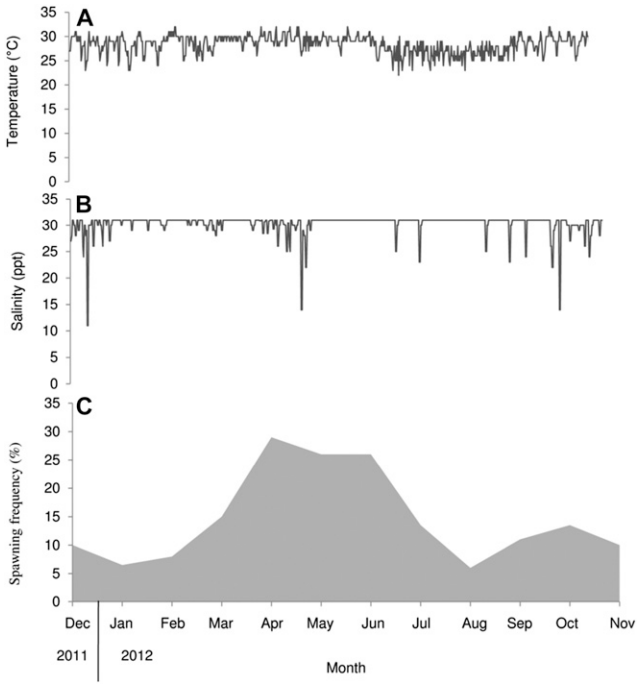

Figure 9. Relationship between spawning period and environmental parameters (temperature and salinity) of *Anadara granosa* from Penang Island, Malaysia (December 2011 to November 2012). (A) Daily seawater temperature; (B) daily salinity; (C) spawning frequency.

TABLE 4.

Correlation matrix between increment widths in growing and spawning groups of *Anadara granosa* and environmental factors.

	Temperature	**Salinity**	**Increment width (mature group)**
Temperature	1	0.29	0.642
Salinity	0.29	1	0.46
Increment width (growing group)	0.642	0.46	1
	Temperature	**Salinity**	**Increment width (immature group)**
Temperature	1	0.29	0.420
Salinity	0.29	1	0.304
Increment width (spawning group)	0.310	0.204	1

TABLE 5.

The maximum spawning period of cockles *Anadara granosa* in different study areas.

Location	Spawning period	Source
Penang Island, Malaysia	April to late June and October	Current study
Banda Ache, Indonesia	June to September	Khalil (2013)
Lhokseumawe, Indonesia	April to October	Khalil (2013)
Pulau Pinang, Malaysia	June to September and January to March	Khalil (2013)
West coast of Malaysia	May, June, and October	Broom (1983)
Kakinada Bay, India	January to April	Narasimham (1969)

spawning, spring tides, and/or strong currents (Thompson et al. 1980, Schöne et al. 2005). A possible means to distinguish spawning breaks from other types of growth breaks is shell increment width characteristics. In the present study, increment widths quickly decreased to less than 5 μm before a spawning break. After the spawning break, when the individuals were in indeterminate and developing stages, the increment widths gradually increased to 47–57 μm. In addition, based on histological analysis, it was obvious that growth breaks in the current study were formed immediately before spawning, when the gonads were still filled with sperm and free oocytes; however, according to Sato (1995), for individuals whose growth break was formed due to environmental stress, increment width rapidly increased after the growth break within the shell cross section. The results are in agreement with Kanazawa and Sato (2008) findings, which showed widely spaced growth increments ceases suddenly before spawning, and after spawning, the growth increments gradually increase in width as growth recovers.

This study has shown that microincrements in shell width of cockles provide a precise indication of the timing of gonad maturity stages. Shell increment width analysis also showed spawning breaks, which are considered markers for identifying the period of sexual maturity, for understanding the number of spawning events that have occurred over the life history of a specimen, and for determining variations in the reproductive cycle from year to year.

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