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Reproductive Biology of Blood Cockle *Anadara granosa* (Bivalvia: Arcidae) in the Northern Region of the Strait of Malacca

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Abstract – A study on the reproductive cycle of the blood cockle *Anadara granosa* (Bivalvia: Arcidae) was conducted at three different areas in the northern region of the Strait of Malacca. A total of 1,920 samples of adult *A. granosa* (38–71 mm length) were collected from June 2009 until September 2010. Qualitative techniques (gonadal microscopic fresh smear test and histology analysis) as well as quantitative techniques (analysis of condition index and gonadal index) were used to predict monthly gonadal development stages of *A. granosa*. The gonadal index of *A. granosa* from Banda Aceh (Indonesia) ($r = 0.469$, $P > 0.05$) and Pulau Pinang (Malaysia) ($r = 0.123$, $P > 0.05$) did not show any correlation to their condition index, whereas the gonadal index of *A. granosa* from Lhokseumawe (Indonesia) ($r = 0.609$, $P < 0.05$) showed moderate positive correlation to the condition index. During the 16 month sampling period, four reproductive cycles were observed: each from three to six months. The process of releasing gametes is termed dribble spawning, and is the same in all populations. The principle component analysis (PCA) indicated that *A. granosa* reproduction was affected by interaction between internal physiological factors and indigenous environmental factors. In all sampling areas, phytoplankton density played a key role in the reproductive cycle in *A. granosa*. Information on the reproductive biology of this species is essential for species management and to improve the sustainability practices of the fisheries industry. These findings will provide basic information on the biology of the blood cockle *A. granosa* for stock management in the region.

Key words – blood cockle, reproductive cycle, gametogenesis, gonadal index, condition index

1. Introduction

Anadara granosa is one of 7500 bivalve species in the family Arcidae, often called "blood arks" or "blood cockles" (Gosling 2003; Arapov et al. 2010). Their common name refers to the hemoglobin and hemocyanin pigments in their blood and tissue cells, giving their blood a dark red color (Ruppert and Barnes 1994) which has allowed this species to live in oxygen-critical habitats (Broom 1985; Terwilliger and Terwilliger 1985; Cilenti et al. 2010). The species is indigenous to the intertidal mudflats of many Southeast Asian countries, particularly Indonesia, Malaysia and Thailand. *Anadara granosa* are mainly distributed in mangrove forests, muddy vegetation and mixed areas. The intertidal species *A. granosa* is known as a keystone species in mangrove habitats in several areas in the northern region of the Strait of Malacca. This species has also been one of the most important fisheries commodities in Southeast Asia for many years (Borrero 1986; Broom 1985; Suwanjarat et al. 1990, 2009).

The northern Strait of Malacca is an important nursery area for many intertidal organisms and a feeding area for migrating species. Being the most important species in terms of fisheries production, this cockle has become the target of an extensive culturing operation in West Malaysia (Broom 1983). At the same time, harvesting of wild stock of cockles in Sumatra and Java (Indonesia) is at an all-time high to meet the demand for shellfish. In Malaysia, the annual production of blood cockles in 2009 exceeded metric 65,000 tonnes, which is valued at US $36.60 million (DOF 2010). The main blood cockle production areas in Malaysia are concentrated in Kedah (Merbok), Pulau Pinang (Juru), Perak (Kuala Gula, Kula Sangga-Matang, Kuala Trong, Sungai Jarum), Selangor (Kuala Selangor) and Johor (Muar). In Indonesia, this species can be found in abundance on the

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coast of West Sumatra, Central and South Java, East and West Kalimantan and other muddy bottoms in Sulawesi, Maluku and Papua (Khalil et al. 2009). The most recent data available on annual cockle production in Indonesia is from 2009 when it reached 47,437 metric tonnes, or equal to US$ 23.72 million (DKP 2010).

The Northern Straits of Malacca is an important area for the harvesting and culture of the blood cockle *A. granosa* due to the suitability of the habitats for spawning and growth (Mirzaei and Hwai 2016). However, annual production statistics indicate a decrease in stocks in the last decade. This situation may be due to inadequate management of wild cockle populations. Fisheries management is needed to improve policies for the sustainability of the fisheries industry. In depth information on reproductive cycles is necessary for predicting annual recruitment, as well as interpreting growth, mortality, and survival data in the marine culture of species (Shaw 1965; Manzi et al. 1985; Sbrenna and Campioni 1994). This data is lacking for the blood cockle *Anadara granosa* but is essential to optimize aquaculture of this species. This bivalve species can be managed more effectively after evaluating the regeneration capabilities of natural stocks and interpreting growth patterns. Detailed and comprehensive information on gonadal development is also important for economic management of this species (Gribben et al. 2004; Peharda et al. 2006). This study aimed to investigate the seasonal gonadal cycle of the cockle *A. granosa* by using quantitative techniques (gonadal index and condition index) through gonadal fresh smear test and gonad histology (a qualitative technique) from specimens collected from the northern region of the Strait of Malacca.

2. Materials and Methods

Collecting of samples

A total of 120 samples of adult *A. granosa* were collected

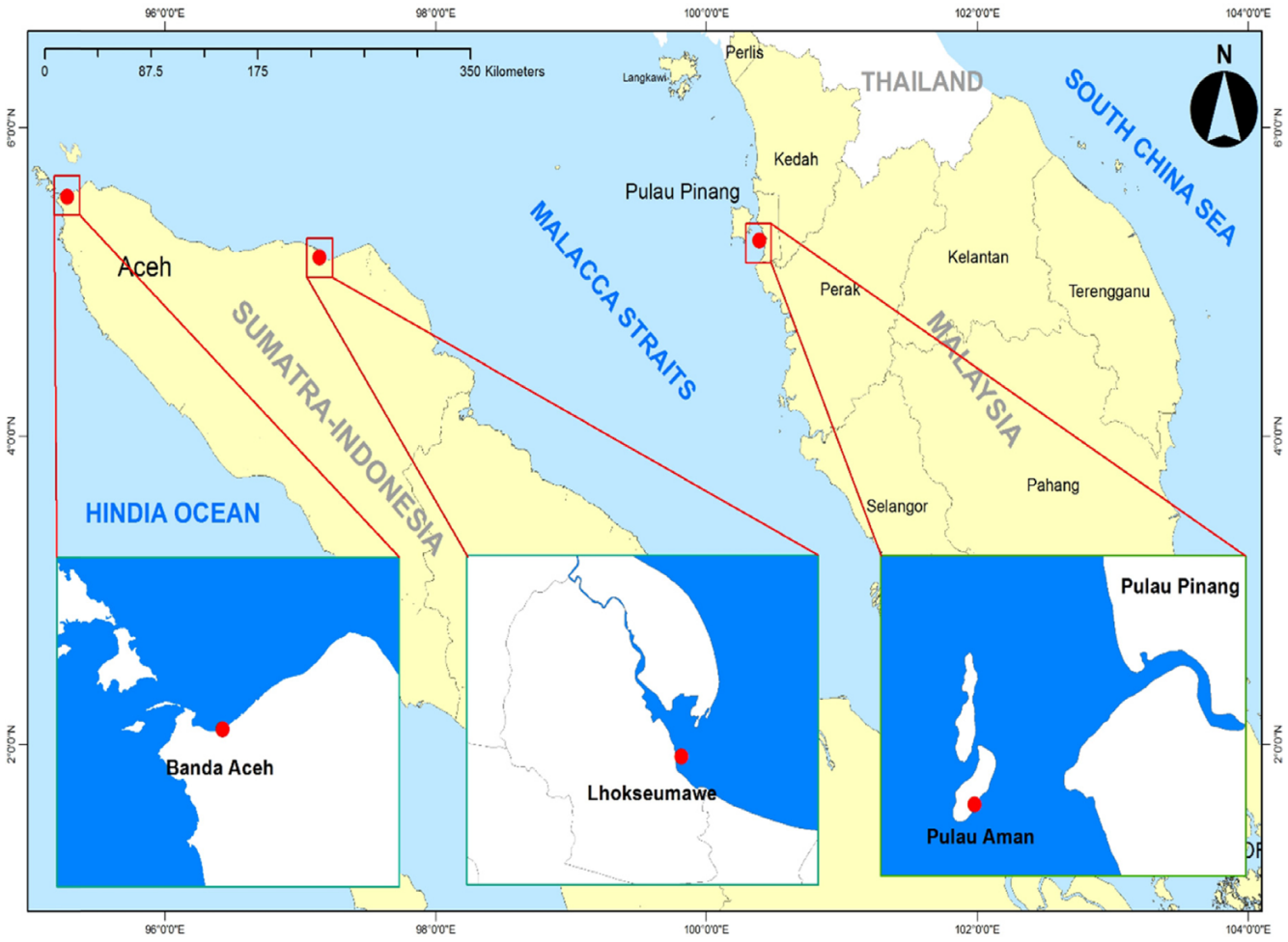

Fig. 1. Sampling location

monthly from June 2009 till September 2010 from the natural habitat in Banda Aceh (532'34.67"N–9517'2.54"E), Lhokseumawe (0509'35.3"N–09708'29.4"E) in Aceh, Indonesia and Pulau Pinang (516'9.66"N–10023'27.37"E) in Malaysia (Fig. 1). The total number of specimens sampled was 1,920 and the adult cockle sizes ranged from 38–71 mm in length. The sampling area was characterized by muddy substrate which was surrounded by mangroves, no wave action and high salinity. The specimens were collected at a depth of 5–30 cm and salinity ranged from 10–33 ppt. Sampling activity in the field was conducted once a month over the specified time frame during low tides. The live specimens were collected manually with the aid of a harrow, which was run through the muddy area to pull bivalves to the surface. After collecting, the specimens were stored in isotherm containers and immediately transported to the laboratory. The samples were cleaned to fully remove all fouling organisms and other adherences.

Qualitative technique

Gonadal microscopic fresh smear test

A total of 40 specimens per sampling site were randomly allocated for the gonadal microscopic fresh smear test each month. All the specimens were dissected with a dissecting needle and pipette. The fresh smear procedure was adopted to observe the gonad content under a compound light microscope (magnification = 100 x) to analyze the stages of the gonadal development. The sex and gametogenesis stages were identified using image analysis, which included 4 stages: (+1) indeterminate, (+2) developing, (+3) developed and (+4) spawned (Rajagopal et al. 2006).

Histology analysis

A total of ten gonad specimens from each of the three sampling sites were allocated for histological analysis each month. Slides were prepared through the process of embedding paraffin wax into the tissue. Haematoxyline and Eosin coloration were used for tissue coloring (Howard et al. 2004). The initial process requires dehydration of the specimen tissue. Dehydration was done through a series of steps of immersing the sample into varying concentrations of alcohol. The sample was embedded into a mold of wax next and kept in a refrigerator overnight before preparing it for HE coloration. The solutions used for histology included bouins, alcohol (50%, 70%, 80%, 90%, 95% and absolute alcohol), xylene, liquid wax, histosolve, HE solution and 1.5% ammonia. A microtome was used to cut 5–7 µm thick tissue sections which were mounted on a glass microscope slide. The light compound microscope was used to analyze the gonad structure to recognize the sex and gametogenesis stages (divided into: (+1) indeterminate, (+2) developing, (+3) developed and (+4) spawned).

Quantitative method

Analysis of condition index (CI)

The water displacement method was used to determine the condition index. A total of 30 specimens (size range: 38–71 mm in length) from each sampling station were examined from June 2009 to September 2010. Each specimen was measured for the following: dry flesh weight, wet weight of shell in grams (g) and internal cavity volume (ml). Fresh cockle tissue including its shell was weighed using digital balances. The flesh was dried at 105°C for 72 hours to a constant weight. The volume of the shell internal cavity was calculated by means of subtracting the volume of the shell (ml) from the total wet volume (ml). These data were used to calculate the condition index using the formula described by Lawrence and Scott (1982):

Condition index = dry flesh weight × 100 / shell internal cavity volume (cm^3)

Analysis of gonadal index (GI)

Gonadal index was calculated based on the formula proposed by Gosling (2003) and Kim and Lee (2008):

Gonadal index = Σ n individual from each stage level gonad stage / n total specimen for each sampling batch

The gonadal index (GI) was calculated for each sampling month through the gonadal microscopic fresh smear test and histological analysis to estimate the proportion of the gonadal stages (indeterminate, developing, developed and spawned). The GI value was ranked to: 1 (all individuals' gonads in the samples were in spawned stage), 2 (all individuals' gonads in the samples were in indeterminate stage), 3 (all individuals' gonads in the samples were in developing stage) and 4 (all individuals' gonads in the samples were in developed stage).

Statistical analysis

Raw data was compiled and entered into Microsoft Office Excel 2011 (Macintosh version) for processing and analyzing of minimum and maximum values, averages, and the standard

deviation as well as to generate graphs. One-Way ANOVA statistical analysis and post hoc test were used to determine significance level ($P < 0.05$ and $P < 0.01$) in the values of each data cluster. Pearson correlation test was also utilized to determine and understand the relationship between differing variables (CI and GI). The principle component analysis (PCA) was used to analyze the correlation between parameters which were affected by reproductive activities in each sampling area. These statistical analyses were conducted using SPSS (Statistical Package for Social Science) release 20.0 for Macintosh.

3. Results

Gonadal structure of *Anadara granosa*

Gonadal microscopic fresh smear analysis

The description of gonad structure of *A. granosa* based on microscopic fresh smear analysis was categorized as shown below:

Stage 1 (indeterminate).

Male and female: Determination of sex cannot possibly be determined. Gonadal compound appeared to be empty and filled up only by network of connecting tissues. Unused residual of gametes can be found.

Stage 2 (developing).

Male: The gonadal compound turned cream in color. Gametes have been very active and the testis was filled with spermatogonia and spermatid. Spermatozoa were also found in limited numbers and sometimes found in tailed form and actively swim.

Female: The gonadal compound turned orange in color. Gametes in ovary have begun to appear, which are previtellogenic oogonia, oocytes and a limited number of oocytes vitellogenic. Oocytes were scattered and filled inside the follicle. Nuclei in oocytes vitellogenic have started forming and are clearly visible. Oocytes have uneven sizes.

Stage 3 (developed).

Male: The gonadal compound turned a more concentrated cream color as a result of highly condensed developed spermatozoa. The spermatozoa have already developed their tail and are swimming actively. Sometimes, spermatids can still be found in small numbers.

Female: Gonadal compound turned an intense, concentrated orange due to formation of highly condensed oocytes. Gametes were generally mature oocytes. Oocytes are in polyhedral form. The nuclei within the oocytes have matured and grown larger in size. The yolks were found in most of the mature oocytes. Previtellogenic oocytes can still be found in small amounts.

Stage 4 (spawned).

Male: Gonadal compound reduced drastically. Spermatozoa have diminished. Unused residual spermatozoa can be found inside the lumen.

Female: Gonadal compound turned bright orange due to the lowest concentration of oocytes. Mature oocytes were found in small amounts, but these are expected to be residue or absorbed as phagocytes. Most of the oocytes had no shape and the nuclei appeared to have shrunk and disappeared.

Gonadal histology analysis

Stage 1 (indeterminate).

Male and female: The stage is also called the dormant stage; the sexes cannot be distinguished. Undeveloped gonads' content during this stage only consisted of connecting tissues and a handful of residual gametes leftover from the previous spawned stage (stage 4) (Fig. 2).

Stage 2 (developing).

Male: Gonad was gradually filled up with spermatogonia, spermatocyte, and a small quantity of spermatozoa. The average diameter of the follicles at this stage was 117.77 ± 19.58 μm in size (Fig. 3a).

Female: Oocytes occur in a range of sizes and were generally not the same shape (irregular). Gonad was gradually filled up with oogonia as well as vitellogonia oocyte and vitellogenic oocytes, the nuclei have uneven shapes. The average diameter of the follicles at this stage was 136.21 ± 22.12 μm, whereas the average diameter of oocytes was 24.81 ± 6.19 μm in size (Fig. 4a).

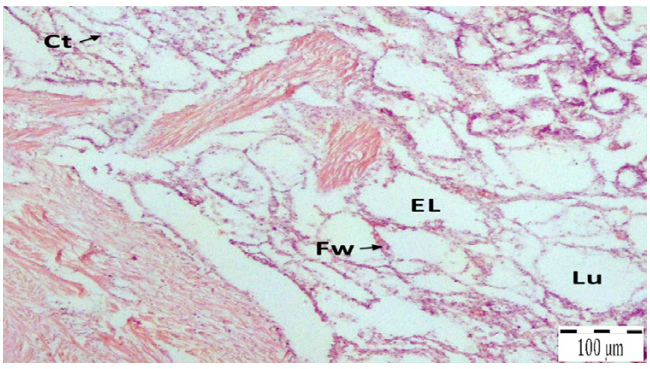

Fig. 2. Gonadal structure of *Anadara granosa* based on histology analysis at indeterminate stage. FW: follicle wall; Lu: Lumen; EL: empty lumen; Ct: connective tissue

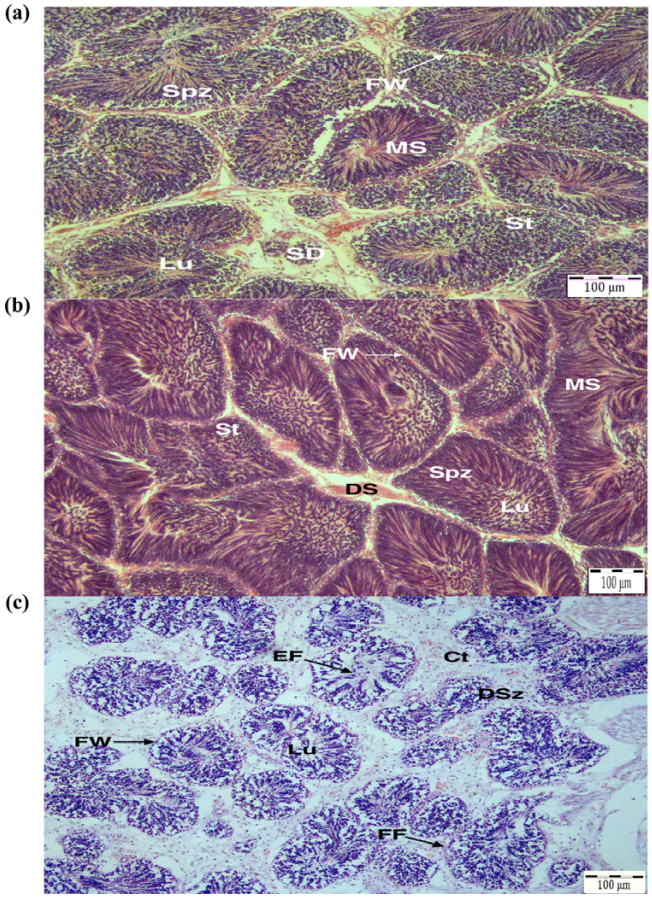

Fig. 3. Gonadal structure of male *Anadara granosa* based on histology analysis: (a) Stage 2 (Developing); (b) Stage 3 (Developed); (c) Stage 4 (Spawned). FW: follicle wall; Lu: lumen; Spz: spermatozoa; MS: mature spermatozoa; SD: sperm ductus; St: spermatid; DS: degenerative space; DSz: degenerative spermatozoa; FF: follicle fragment; EF: empty follicle; Ct: connective tissue

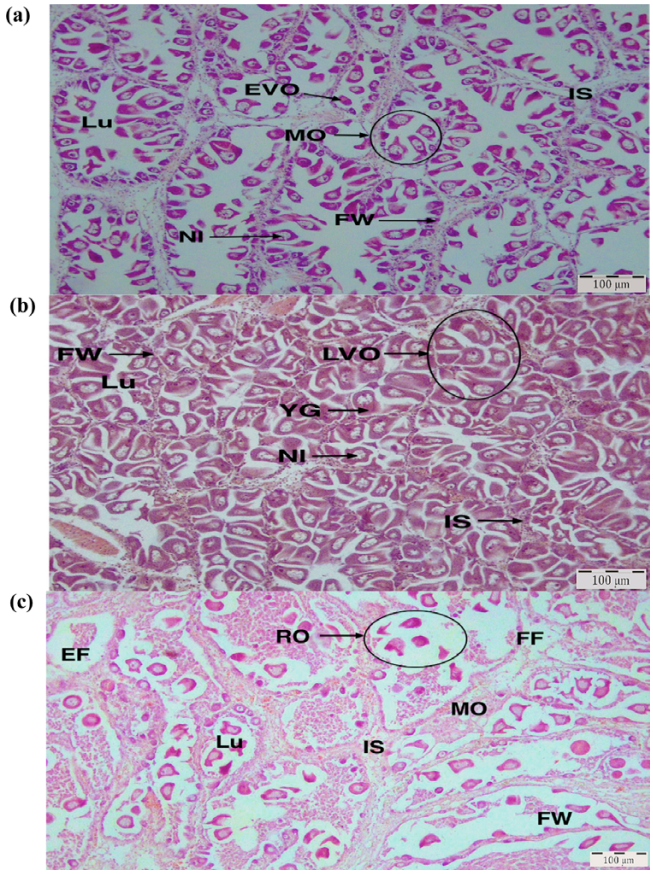

Fig. 4. Gonadal structure of female *Anadara granosa* based on histology analysis: (a) Stage 2 (Developing); (b) Stage 3 (Developed); (c) Stage 4 (Spawned). FW: follicle wall; Lu: Lumen; EVO: early stage of vitellogenic oocyte; LVO: late stage of vitellogenic oocyte; MO: mature oocyte Nl: nucleus; FF: follicle fragment; EF: empty follicle; RO: residual oocyte; IS: interfolicular space; YG: yolk granule

Stage 3 (developed).

Male: Gonad was mainly dominated by spermatozoa content. Interfollicular space at this stage was seen to be experiencing constriction due to the growing follicle size. Spematogonia were still found in limited number and typically found on the side wall of the follicle. The average diameter of the follicles was 186.16 ± 14.47 µm in size (Fig. 3b).

Female: Gonad was characterized by the dominance of vitellogenic oocytes with a visibly large nucleus. Lumen space was dominated by the polyhedral oocyte vitellogenic shape which was untouched or free from the follicle wall. The cytoplasm of mature oocytes had been filled by a number of yolk granules. The average diameter of the follicles was 215.13 ± 38.40 µm and oocytes were 30.01 6.80 µm in size (Fig. 4b).

Stage 4 (spawned).

Male: Spermatozoa seemed to be reduced, as the follicle appeared almost empty. Spermatogonia were not found (Fig. 3c).

Female: Residual oocytes were present. The follicles' wall seemed to be damaged and unfilled. Phagocytes were found around the residue oocytes (Fig. 4c).

Gonadal development cycle

The gonad percentage (for each stage) was compared between the three sampling locations: Banda Aceh (Indonesia), Lhokseumawe (Indonesia) and Pulau Pinang (Malaysia). Figures 5a, 6a, 7a, as well as 5b, 6b and 7b depict the computation of gonad percentages per month for all the 4 phases discussed covering a span of 16 months, from June 2009 until September 2010, through gonadal microscopic fresh smear analysis

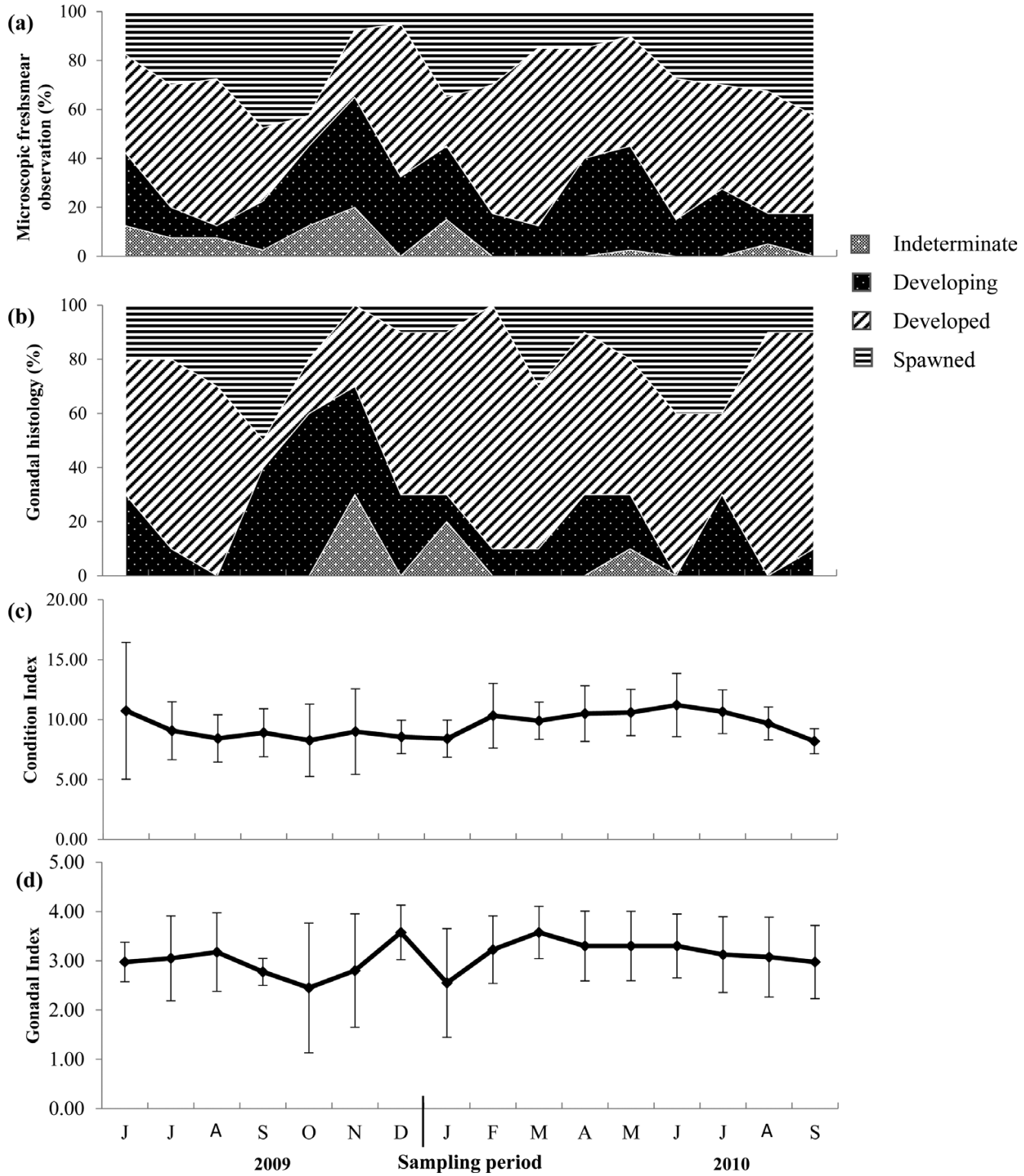

Fig. 5. *Anadara granosa* gonadal development pattern from Banda Aceh, Indonesia (June 2009–September 2010)

and gonadal histology analysis, respectively. Figures 5c, 6c and 7c, as well as 5d, 6d and 7d depict the monthly condition index (CI), and monthly gonadal index (GI), respectively, covering the same 16 months.

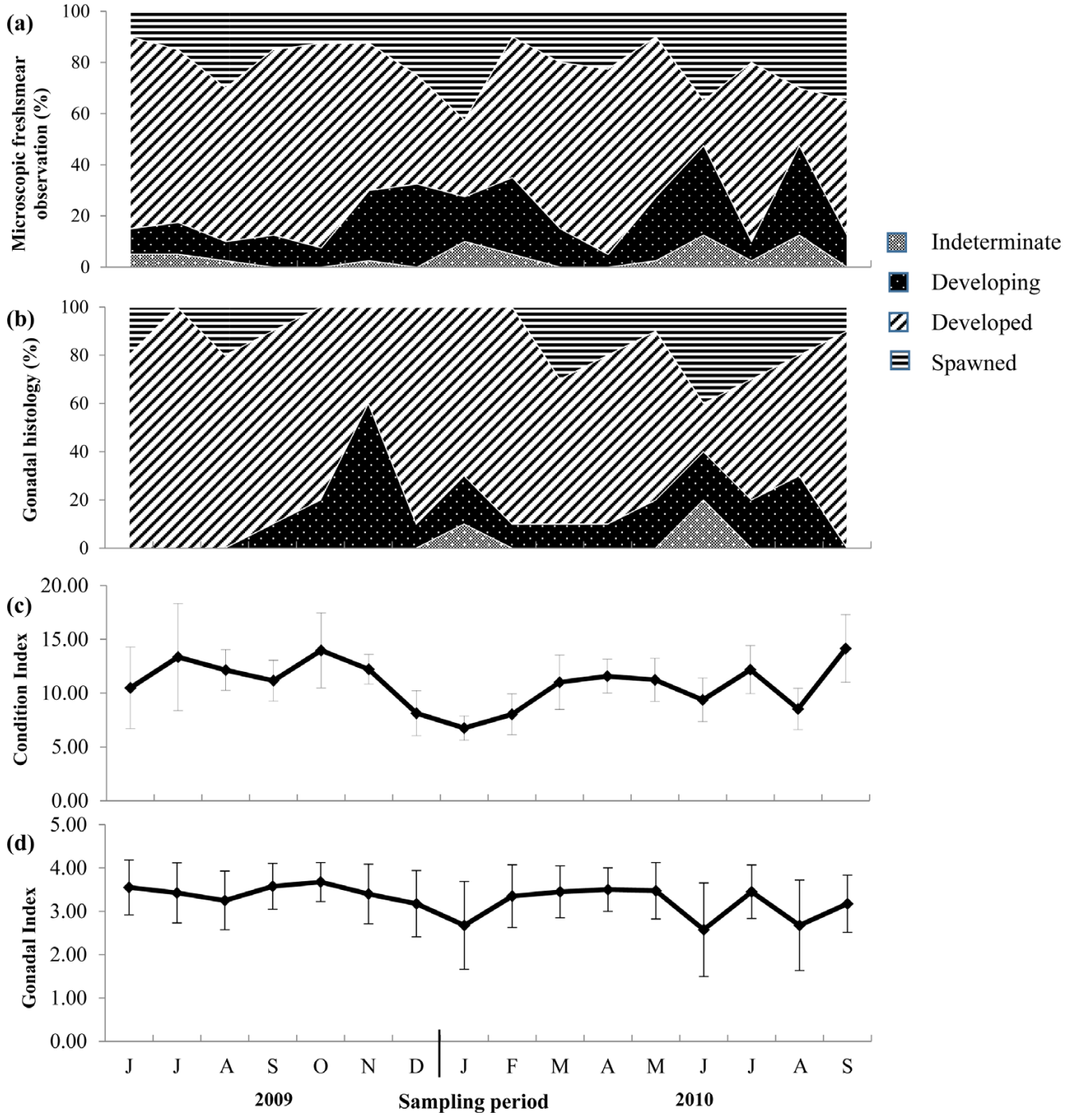

Fig. 6. *Anadara granosa* gonadal development pattern from Lhokseumawe, Indonesia (June 2009–September 2010)

Environmental variable

Monthly seasonal variations of environmental parameters in the three different sampling areas are reported in Table 1. During the study period, water temperature, salinity and phytoplankton density fluctuated significantly compared to other environmental parameters. The principle component analysis (PCA) was conducted to evaluate the comprehensive relationship between environmental factors and reproduction variables in the *A. granosa* populations in Banda Aceh, Lhokseumawe, and Pulau Pinang (Fig. 8).

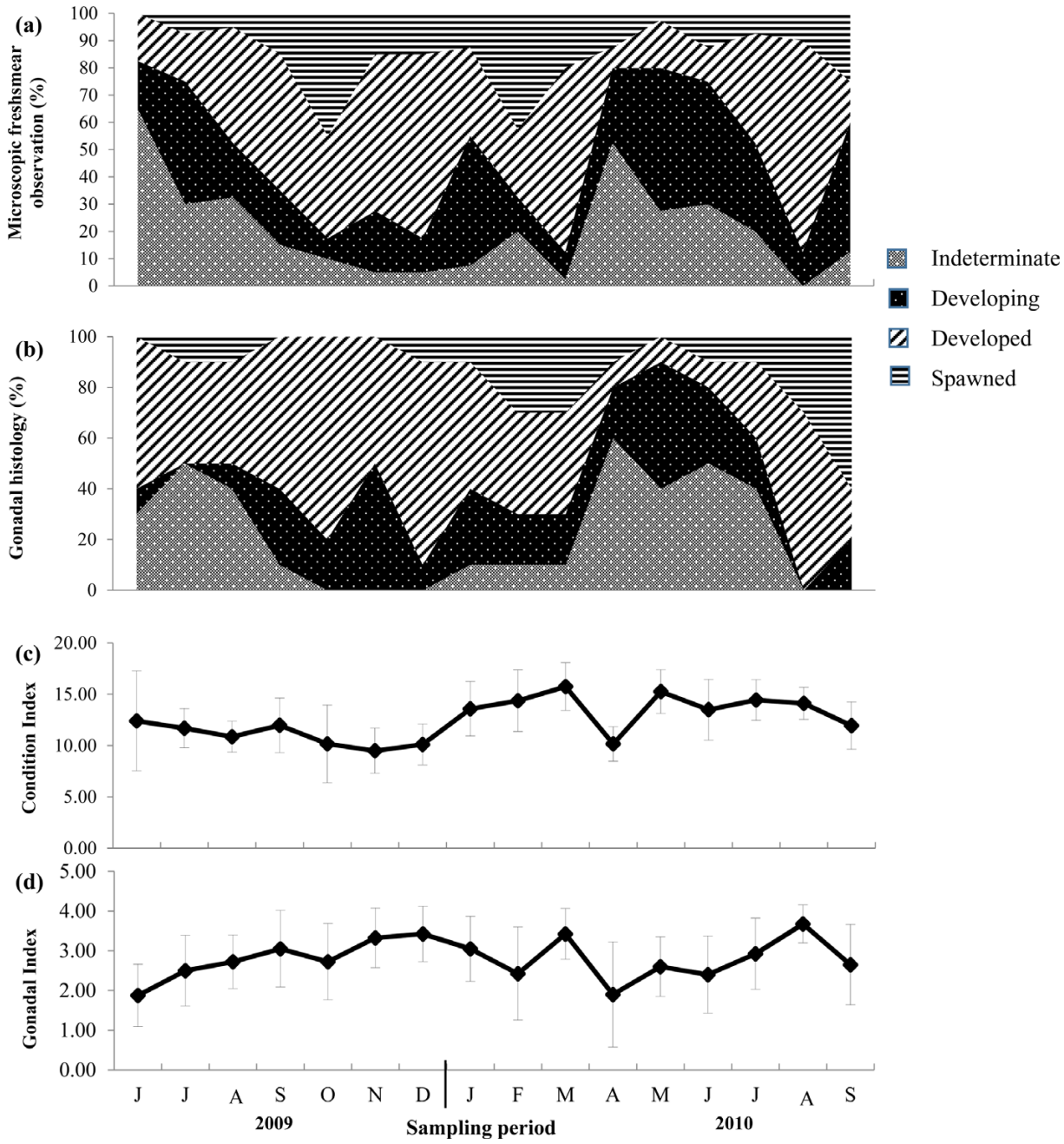

Fig. 7. *Anadara granosa* gonadal development pattern from Pulau Pinang, Indonesia (June 2009–September 2010)

4. Discussion

Gonad development for *Anadara granosa*

The recorded CI values for the samples indicated significant varying values every month for samples from the same sampling location as well as those from different sampling locations. The difference in the trend of CI value indicated the status of the population of blood cockles throughout the year. A high CI value implies the gonad has already reached maturity. However, CI is not always linearly correlated to its breeding pattern. This can be shown from the comparison of the monthly CI vs GI values. The GI value is an assumed indication of the breeding status. A sudden drop in GI value signifies the occurrence of spawning activities. From this analysis, there was no linear correlation between CI and GI values for samples from Banda Aceh and Penang. However,

Table 1. Mean monthly seasonal environmental parameter at the sampling areas from June 2009 to September 2010

Environmental parameter	Jun. 2009	Jul. 2009	Aug. 2009	Sep. 2009	Oct. 2009	Nov. 2009	Dec. 2009	Jan. 2010	Feb. 2010	Mar. 2010	Apr. 2010	May 2010	Jun. 2010	Jul. 2010	Aug. 2010	Sep. 2010	Average
Temperature (°C)																	
Banda Aceh																	
Minimum	26.32	24.27	24.29	25.43	26.90	25.00	25.39	25.79	22.96	22.84	25.47	21.87	25.90	22.82	25.27	26.00	24.78
Maximum	30.97	30.44	30.45	29.88	32.61	31.93	31.95	32.48	32.93	32.10	31.40	31.03	30.93	32.11	30.98	30.38	31.41
Lhokseumawe																	
Minimum	28.82	28.82	26.81	28.07	27.71	27.03	28.45	28.06	28.96	28.48	28.60	28.23	27.95	28.23	27.23	27.40	28.05
Maximum	31.08	31.71	30.06	31.17	30.87	30.33	30.81	30.68	30.75	31.10	30.90	31.65	31.27	31.39	30.84	31.17	30.99
Pulau Pinang																	
Minimum	27.23	27.52	26.65	27.10	25.87	27.93	26.05	23.68	25.61	26.74	26.37	26.29	26.67	26.74	26.65	27.37	26.53
Maximum	31.63	31.10	31.45	30.60	30.90	31.43	30.58	28.35	30.46	31.71	31.13	30.68	31.53	31.32	31.45	31.30	30.98
Salinity (ppt)																	
Banda Aceh	32.27	31.35	29.98	27.47	30.06	27.20	26.45	29.68	31.50	31.16	29.30	30.71	30.85	31.29	30.45	28.27	29.87
Lhokseumawe	31.00	30.97	31.16	31.20	31.03	29.07	30.94	31.16	31.46	31.84	30.57	31.26	30.90	30.81	30.65	31.27	30.95
Pulau Pinang	29.33	28.52	26.39	26.87	27.35	26.13	25.23	28.06	29.00	28.94	28.67	26.48	29.70	29.32	31.06	30.40	28.22
pH																	
Banda Aceh	7.65	8.02	8.03	8.17	7.80	8.02	7.91	8.17	8.08	8.06	8.02	7.97	7.74	8.27	7.94	8.23	8.01
Lhokseumawe	8.13	7.88	8.04	8.06	8.17	8.13	7.98	8.21	7.89	8.18	7.91	7.84	8.08	7.93	7.99	8.13	8.03
Pulau Pinang	8.02	7.49	7.85	8.14	8.04	8.07	7.79	8.02	7.95	7.86	8.08	8.21	7.91	7.86	7.33	7.54	7.89
Dissolved oxygen (mg/L)																	
Banda Aceh	6.53	6.81	6.96	6.05	5.97	6.12	6.05	5.95	6.10	5.84	6.23	5.86	6.68	5.84	6.32	5.47	6.17
Lhokseumawe	6.01	6.38	6.47	6.14	6.28	6.04	6.07	6.10	6.97	6.02	6.17	5.98	6.02	5.89	6.28	6.13	6.18
Pulau Pinang	7.20	5.20	5.20	4.90	5.13	5.21	5.09	5.29	5.20	5.64	5.87	5.39	7.67	5.64	6.29	5.87	5.67
Turbidity (NTU)																	
Banda Aceh	17.40	29.30	8.61	9.02	10.86	9.12	19.16	14.09	16.03	10.27	18.98	13.83	10.27	34.29	9.74	10.48	15.09
Lhokseumawe	43.20	30.50	36.50	66.90	31.60	15.18	103.00	93.67	29.13	37.30	64.92	38.95	35.30	49.98	46.90	98.30	51.33
Pulau Pinang	29.30	17.36	15.11	13.09	17.27	74.30	57.80	77.10	109.67	107.00	98.00	76.00	103.40	107.00	76.65	93.12	67.01
Orthophospate (mg/L)																	
Banda Aceh	0.05	0.03	0.04	0.13	0.03	0.02	0.00	0.40	0.00	0.07	0.13	0.07	0.03	0.09	0.08	0.53	0.11
Lhokseumawe	0.05	0.01	0.02	0.07	0.01	0.01	0.01	0.70	0.00	0.04	0.06	0.04	0.04	0.08	0.07	0.01	0.08
Pulau Pinang	0.10	0.05	0.08	0.06	0.03	0.01	1.00	0.01	0.16	0.13	0.09	0.08	0.52	0.13	0.15	0.81	0.21

Table 1. Continued

Environmental parameter	Jun. 2009	Jul. 2009	Aug. 2009	Sep. 2009	Oct. 2009	Nov. 2009	Dec. 2009	Jan. 2010	Feb. 2010	Mar. 2010	Apr. 2010	May 2010	Jun. 2010	Jul. 2010	Aug. 2010	Sep. 2010	Average
Nitrate (mg/L)																	
Banda Aceh	0.71	0.03	0.11	0.73	0.75	0.01	0.04	0.05	0.05	0.09	0.23	0.63	0.18	0.05	0.53	0.65	0.30
Lhokseumawe	0.68	0.14	0.03	0.03	0.20	0.01	0.00	0.03	0.01	0.10	0.77	0.58	0.10	0.17	0.07	0.98	0.24
Pulau Pinang	0.80	0.02	1.30	0.73	0.03	2.02	0.03	1.02	0.11	0.14	0.61	0.42	0.64	0.14	0.65	1.76	0.65
Nitrite (mg/L)																	
Banda Aceh	0.05	0.02	0.02	0.03	0.75	0.01	0.03	0.03	0.03	0.03	0.05	0.05	0.05	0.03	0.04	0.05	0.08
Lhokseumawe	0.03	0.03	0.04	0.04	0.05	0.03	0.44	0.03	0.43	0.02	0.08	0.07	0.02	0.08	0.03	0.04	0.09
Pulau Pinang	0.03	0.03	0.04	0.06	1.09	1.68	0.18	1.00	0.13	0.12	0.09	0.03	0.14	0.12	0.39	0.87	0.37
Ammonia (mg/L)																	
Banda Aceh	0.87	0.20	0.16	0.15	0.11	0.68	0.06	0.13	0.08	0.09	0.19	0.79	0.18	0.06	0.59	0.13	0.28
Lhokseumawe	0.19	0.17	0.25	0.25	0.14	0.35	0.23	0.30	0.19	0.27	0.39	0.49	0.27	0.21	0.43	0.24	0.27
Pulau Pinang	0.24	0.18	0.14	0.25	0.42	0.11	0.61	0.15	0.68	0.54	0.65	0.98	0.65	0.54	0.82	0.65	0.48
Phytoplankton density (cell/L)																	
Banda Aceh	1831.67	1446.67	851.67	1178.33	630.00	991.67	1201.67	385.00	1773.33	1388.33	1516.67	1785.00	1738.33	1283.33	1341.67	1108.33	1278.23
Lhokseumawe	1656.67	1365.00	711.67	2601.67	3010.00	1435.00	4001.67	1365.00	2986.67	2415.00	2333.33	2298.33	2415.00	1050.00	1003.33	2310.00	2059.90
Pulau Pinang	4340.00	4001.67	1470.00	11713.33	4340.00	4281.67	2636.67	4561.67	4235.00	5751.67	5693.33	6090.00	5728.33	5751.65	2905.00	7910.00	5088.12

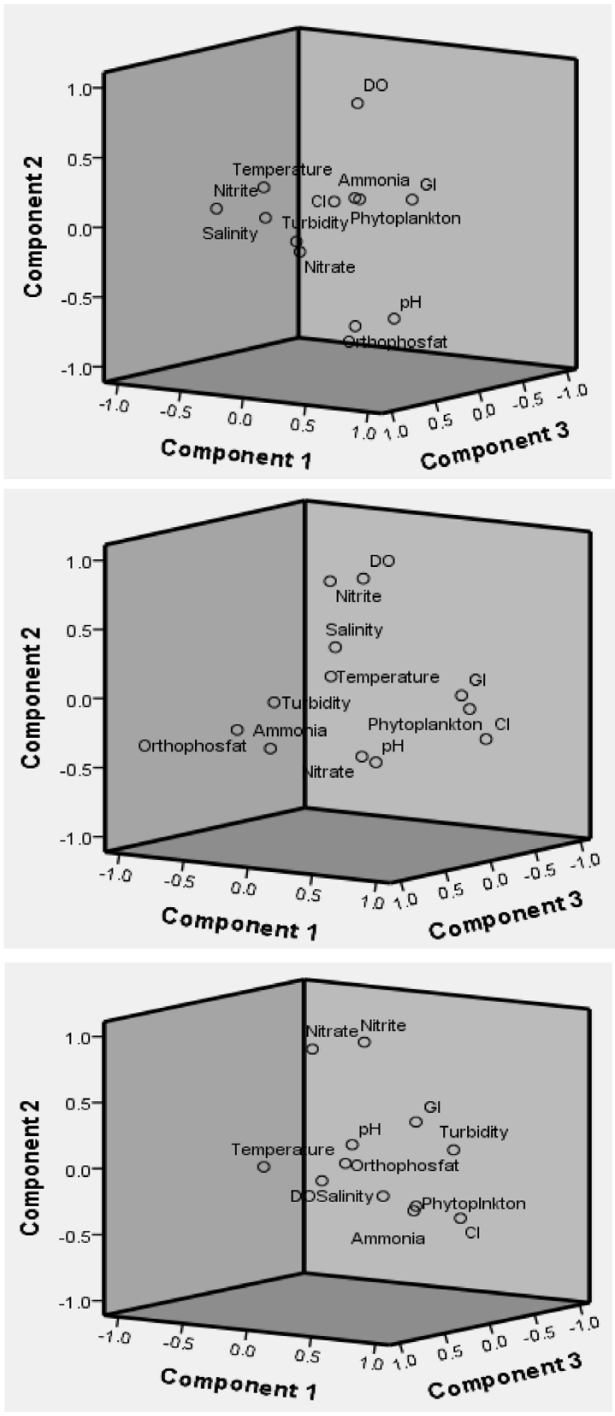

Fig. 8. Principle component analysis (PCA) plot for the reproductive factor component on *Anadara granosa* population. (a) Banda Aceh, Indonesia; (b) Lhokseumawe, Indonesia; (c) Pulau Pinang, Malaysia

a linear correlation between these values was noted for samples from Lhokseumawe. These were tested with the Pearson correlation test, which indicated that CI values for samples from Banda Aceh ($r = 0.469$ at $P > 0.05$) and Penang ($r = 0.123$ at $P > 0.05$) has no significant correlation to their respective GI, but there is a mild correlation for samples from Lhokseumawe ($r = 0.609$ at $P < 0.05$). A negative correlation has also been reported in studies of other bivalve species. Herrmann et al. (2009) reported a negative correlation between CI and gametogenesis cycle for *Amarilladesma mactroides* (Reeve, 1854). Mladineo et al. (2007) also reported zero correlation between CI and GI for the bivalve *Modiolus barbatus* (Linnaeus, 1758). The same applies to *Mercenaria mercenaria* (Linnaeus, 1758) from the Gulf of Narragensett in the United States, as reported by Marroquin-Mora and Rice (2008).

The GI values obtained throughout the year indicate high diversity in reproductive patterns among the three sampling locations. This is expected due to the differences in habitat conditions as well as breeding seasons. Blood cockles from all three sampling locations showed a rapid transition from gonad development to maturation phase. GI analysis shows that spawning activity happened every month throughout the year with varying intensity. The GI value increases during gametogenesis and decreases after spawning. The fast-paced transition could be a strategy for the blood cockles to increase the amount of gamete released whilst favorable environmental conditions are present. This behavior is characteristic of the reproduction of invertebrates in tropical regions. Species have been shown to adopt opportunistic strategies to develop the gonadal matter from energy available from food rather than from energy stored inside somatic parts (Cárdenas and Aranda 2000). Freites et al. (2010) found that *Anadara notabilis* exhibits a continuous reproductive cycle throughout the year and that particulate organic matter, temperature and food availability were regulating factors of the reproduction of *A. notabilis*.

This study of blood cockles' GI shows that it has a breeding cycle lasting an average of 3~6 months across the three sampling locations (Banda Aceh, Lhokseumawe and Pulau Pinang). During the 16 month sampling period, four reproductive cycles were observed. For the *A. granosa* population from Banda Aceh (Indonesia), cycle I occurred from June to October 2009, cycle II from November 2009 to January 2010, cycle III from February to April 2010, and cycle IV from April to September 2010. In Lhokseumawe (Indonesia), cycle I started from June to August 2009, cycle II from September 2009 to January 2010, cycle III from February to June 2010 and cycle IV from July to September 2010. For the *A. granosa* population in Pulau Pinang (Malaysia), cycle I started from June to October 2009, cycle II from November 2009 to February

2010, cycle III from February 2010 to April 2010 and cycle IV from April 2010 to September 2010. All three populations started the first cycle around June 2009 and ended the fourth cycle also around the same time, September 2010. The population from Lhokseumawe (Indonesia) showed a tendency to spawn faster compared to the other two populations. However, during the third cycle, populations from Banda Aceh (Indonesia) and Penang (Malaysia) exhibited a more rapid and shorter cycle lasting approximately 2~3 months, compared to Lhokseumawe (Indonesia) which took about 5 months.

Breeding pattern of *Anadara granosa*

Generally, the bivalve breeding process is characterized by a continual and seasonal pattern (Ceballos-Vazquez et al. 2000), and is iteroparous in nature, continually and repeatedly breeding throughout its entire life span (Dame 1996). Bivalves give birth to their young by means of gametogenesis. This process is then followed by the release of one or several gametes. The process of rearranging the empty gonad with new gametes for the next cycle always is the signal for the beginning of a new breeding cycle (Gosling 2003). Variation in the breeding trend amongst cockle populations of different geographical locations makes it difficult to determine a pattern of gonad development. A well balanced distribution of males to females in blood cockles is supported by the sex ratio analysis done in this study. Gonad development and spawning period was determined to be parallel between the two opposing sexes, a scenario known as synchrony. According to Levitan (1993), synchrony in gonad development of bivalves is crucial to increase the possibility of effective mating. Extended spawning durations from one to two months is a common breeding strategy for bivalve species. Such a strategy is essential to maintain the cockle population over time within its habitat. Generally, sporadic gamete mating will happen concurrently under suitable surrounding conditions. Blood cockles for all three sampling locations, and in general, exhibit a tendency to be characterized as bivalve brachidictics, which means they are capable of undergoing a continual breeding cycle throughout the year, with varying spawning intensity every month. Pathansali (1966), Narasimham (1988) and Broom (1983) reported that *A. granosa* in Peninsular Malaysia and India has a spawning season throughout the year with no apparent seasonal pattern. In comparison, the spawning season of Archidae (genus Anadara) is presented in Table 2.

The information on the reproductive cycle of *A. granosa* provided by this study is crucial for initiating its commercial aquaculture value, as well as for the sustainable management of wild stocks. In the future, data on spawning periodicity might be used to identify trochophore or veliger larvae in wild habitats and for seed collection activities. When bivalve culture production depends on natural seed supply, the timing of seed collection is critical since the potential brood stock is

Table 2. Comparison of spawning period with the highest intensity of releasing gamete in genus Anadara

Species	Location	Spawning period	Sources
A. granosa	Banda Aceh, Indonesia	September and October	Present study
A. granosa	Lhokseumawe, Indonesia	Jun and September	Present study
A. granosa	Pulau Pinang, Malaysia	October, February to March	Present study
A. granosa	Perak	December	Pathansali (1966)
A. granosa	Phuket, Thailand	October to November	Boonruang and Janekarn (1983)
A. granosa	Pulau Pinang, Malaysia	August to September	Broom (1983)
A. granosa	Selangor, Malaysia	September and November	Broom (1983)
A. granosa	West coast, Thailand	August and November	Suwanjawat and Parnrong (1990)
A. granosa	Pattani bay, Thailand	September, December and July	Suwanjarat et al. (2009)
A. trapezia	Sydney, Australia	end of summer	Sullivan (1961)
A. broughtoni	Japan	Jun to August	Kanno and Kikuchi (1962)
A. senilis	Nigeria coast	October and November	Yoloye (1974)
A. broughtoni	Great Bay, Japan	July to September	Dzyuba and Maslennikova (1982)
A. trigonopsis	New Zealand	winter and summer	Booth (1983)
A. trapezia	Sydney water, Australia	winter	Hadfield and Anderson (1988)
A. descripta	Sydney water, Australia	winter and autumn	Hadfield and Anderson (1988)
A. inaequivalvis	Black sea, Turkey	Mei and November	Sahin et al. (2006)
Anadara/Scapharca kagoshimensis	Ariake bay, Japan	autumn to early of summer	Yurimoto et al. (2008)
A. notabilis	Northeastern of Venezuela	Jun and October	Freites et al. (2010)

primed for a short period of time. Information presented here indicates that quantitative methods (condition index and gonadal index) are a precise indicator in *A. granosa* brood stock.

Factors that affected reproduction cycle of *Anadara granosa* in the northern region of the Strait of Malacca

Gametogenesis is affected by the change and interaction of exogenous (temperature, salinity, light, food), and endogenous factors (nervous system, hormones) that could determine the reproductive strategy of bivalve species (Ram et al. 1996; Utting and Millican 1997; Louro et al. 2003; Barber and Blake 2006; Magnesen and Cristophersen 2008). The principle component analysis (PCA) has shown that the principle components which affected the reproductive cycle of the *A. granosa* population in Banda Aceh were gonadal index, condition index, phytoplankton density, orthophosphate, salinity, and water temperature. The principle component analysis for Lhokseumawe showed that there were five variables affecting *A. granosa* reproduction, namely interaction among gonadal index, condition index, phytoplankton density, ammonia, and pH. The reproduction of the *A. granosa* population in Pulau Pinang also revealed the complex interaction of seven principle variables, namely interaction between gonadal index and environmental factors such as salinity, nitrite, ammonia, phytoplankton density, turbidity and dissolved oxygen.

Principle component analysis indicated that the environment parameters modifying the reproduction of *A. granosa* populations were diverse and complex. This analysis also indicated that reproduction of *A. granosa* populations is significantly affected by the interaction of local environment parameters. For example, water temperature was found to be modestly interacting with and affecting components of reproduction in *A. granosa* in Banda Aceh. Dissolved oxygen, nitrite, ammonia, and turbidity variables were only found to be specifically interacting and affecting the components of reproduction in the *A. granosa* population in Pulau Pinang, however these variables were not the factors affecting reproduction in the *A. granosa* population in Banda Aceh and Lhokseumawe. Reproductive physiological factors such as gonadal index and condition index were shown to be affected only by the interaction of several water environment parameters that are dependent on adaptation level (Gillmor 1982; Beninger and Le Pennec 1997).

One of the environmental factors that is known to be strongly correlated with *A. granosa* reproduction is phytoplankton density. This variable is known to interact with and affect gonadal index and condition index as determined by gonadal development stage in the three *A. granosa* populations. Lodeiros and Himmelman (1999) conducted a statistical analysis, namely multiple regression analysis, to see the relationship between environmental factors and reproduction of the bivalve *Lima scabra*. The conclusion of that study found that phytoplankton density was the only primary factor positively correlated to the reproduction of *L. scabra*. Phytoplankton density is the principle factor influencing the reproduction of bivalves (Wacker and von Elert 2003; Villalejo-Fuerte et al. 2005; Kang et al. 2006; Liu et al. 2006; Hernández-Olalde et al. 2007; Calderon-Aguilera et al. 2010). Phytoplankton are also known to be the main source of diet to anadarinid animals (Kasigwa and Mahika 1991).

Gonadal maturation and the fertilization activities of *A. granosa* that correspond with the high level of phytoplankton density is a strategy to increase planktotrophic larval autonomy by increasing the larvae growth rate. The duration of the planktonic phase can be reduced through optimal utilization of the food source (phytoplankton). Himmelman (1975) showed that a high content of phytoplankton in the aquatic environment will stimulate the reproductive period of invertebrate organisms, particularly species that have pelagic larvae. Ram et al. (1992) found that phytoplankton release a type of chemical substance that could stimulate the nervous system of bivalves to make them release gametes.

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