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Original Article

Sexual maturation of the blood cockle, *Anadara granosa*, in Matang mangrove estuary, Peninsular Malaysia

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Abstract: The sexual maturation and spawning period of blood cockle in Matang mangrove estuary was studied by naked eye and histological observations of the gonads from July 2010 to April 2011. The high spawning period was from November to February. However, at one station where bottom sediment exhibited a severe reduction in potential of −100 mV lower, immature individuals were common. These results suggest the redacting environment inhibits the sexual maturation of blood cockle.

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Introduction

The blood cockle, *Anadara granosa*, is an ark shell species bearing hemoglobin. The valve of the adult blood cockle forms a thick oval shape, and the surface has about 18–21 ribs and many small nodules (Narashimham, 1988b). This species lives on the surface of muddy bottoms from the intertidal zone to the subtidal zone at depths less than about 5 m distributing mainly in the coastal waters of India, China, West Japan and North Australia (Narashimham et al., 1984; Nakao et al., 1989; Faulkner, 2010). In Southeast Asia, this cockle species is an important species for aquaculture, and natural blood cockle spats are used for seeds to sow cultures (Watanabe, 2009).

Sowing cultures of blood cockle is very popular in the west coast of the Malay Peninsula, particularly the coastal areas of Penang, Perak, and Selangor districts (Pathansali and Song, 1958). However, sites with natural high density have been limited to the coastal waters of Johor and Selangor in recent years. Therefore, it is important to clarify the current status of blood cockle reproduction in the Penang and Perak districts to ensure the sustainable production of blood cockle seed in those areas. However, little is known about the sexual maturation or main spawning period of the blood cockle in these districts. Therefore, unstable factors of the occurrence of the natural spat fall area are unclear. Thus, the natural spat area depends on factors such as defective maturation of adult blood cockles, depletion of the planktonic larvae, and high mortality of the spat in the early life stage. In this study, 3 survey stations were set up in the cockle culture grounds of Matang mangrove estuary on the west coast of Peninsular Malaysia. The sexual maturation and spawning period of blood cockle were studied by naked eye observations at the visceral site as well as histological observations of the gonads.

Materials and Methods

Survey and environmental measurement: Three sampling stations (St. 1-3) were set in Matang mangrove estuary in Peninsular Malaysia from July

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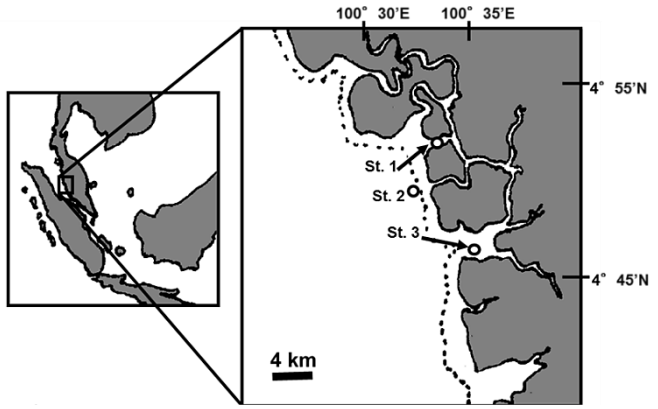

Figure 1. Locations of the 3 sampling stations in Matang mangrove estuary, Peninsular Malaysia.

2010 to April 2011 (Fig. 1). Temperature, salinity, and dissolved oxygen in bottom-layer water (i.e., 20 cm above the seabed) at each sampling station were monitored by a water quality sensor (AAQ-RINKO, JFE Advantech Inc., Japan). In addition, adult blood cockles were sampled to study their sexual maturation. Also, surface sediments (i.e., 0–1 cm) of the 3 stations were monitored for the redox potential by an oxidation reduction potential (ORP) sensor (RM-20, TOA-DDK Co. Ltd., Japan) in November 2011. The blood cockles collected from each station were transported to the laboratory of the Fisheries Research Institute in Penang. The cockles were dissected, their visceral sites were observed, and their gonads were fixed in 10% seawater–formalin solution.

Naked eye observations: A total of 246 blood cockles with shell lengths ranging from 16.8–39.1 mm were used for naked eye observations of the visceral site. The shells were observed, and the thickness of the visceral site was assessed; specimens were classified into 3 groups according to thickness. Class I (immature stage): thickening is not observed at the visceral site; the midgut gland tissue is visible through the membrane of the organ; however, the status is not visible on gonadal tissue (Fig. 2I). Class II (growth or regression stages): thickening on the visceral site is slightly observed, and gonadal tissue is visible through the membrane tissue of the visceral site (Fig. 2II). Class III (mature stage): marked thickening is observed at the visceral site, and gonadal tissue is filled at the site. Sex was

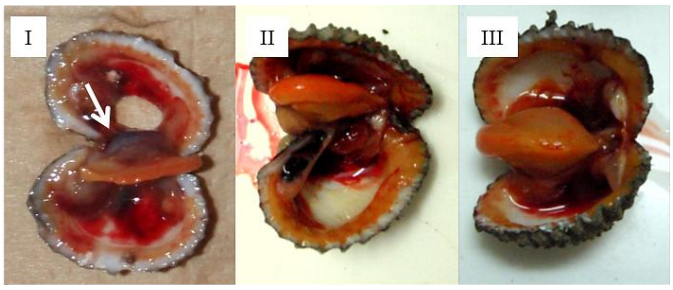

Figure 2. Naked eye observations of the visceral site (arrow in photo I) were used to classify specimens into 3 categories: (I) immature, (II) developing, and (III) mature.

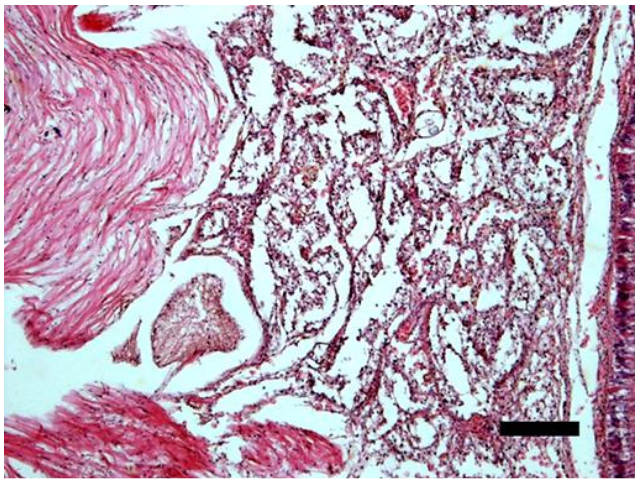

Figure 3. Photomicrograph of immature stage. No traces of gametes are observed, so the sex is indistinguishable. H&E staining. Scale bar = 100 μm.

determined according to appearance (Fig. 2III). As a measure of thickness, individuals in the classes I, II, and III were scored 0, 1, and 2, respectively. The points were subsequently averaged for each survey, and plotted with standard errors on a graph.

Histological observations: The same samples were reused for histological observations. After dehydration in 70–100% alcohol, fixed tissue was embedded in paraffin by replacing the lemosol. Tissues were cut from paraffin blocks into 7–10 μm sections, which were subsequently double-stained with Meyer's hematoxylin and eosin and observed under an optical microscope. In addition, the gonads were classified into 5 stages: the immature, developing, mature, spawning, and spent stages. The stages are described in detail below.

Immature stage: Sex is indistinguishable in the gonads. It is difficult to observe germ cells in the gonads, and the site is mainly occupied by connective tissue or an empty genital tube (Fig. 3).

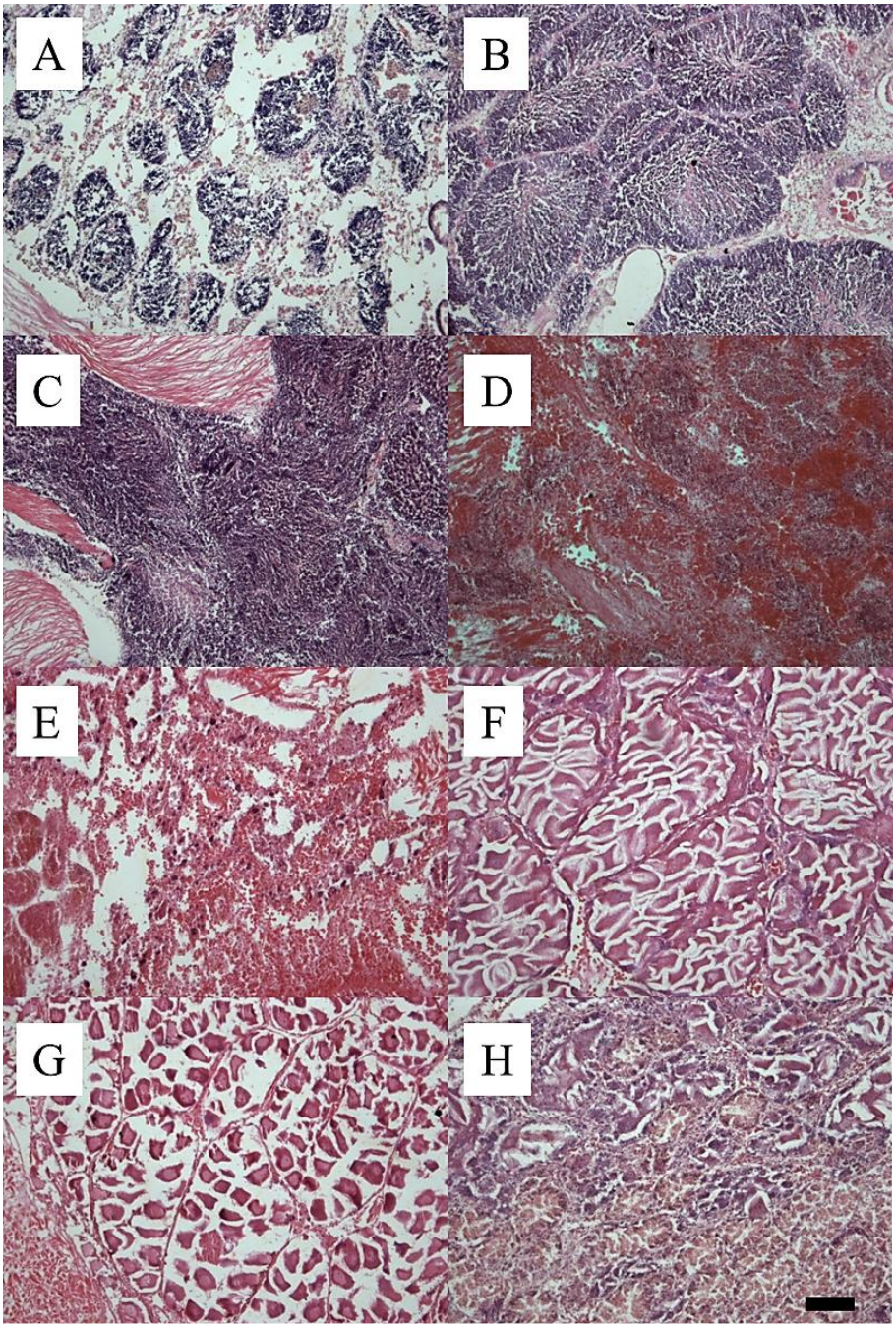

Figure 4. Photomicrographs of testes (A–D) and ovaries (E–H) at each reproductive stage. (A, E) developing, (B, F) mature, (C, G) spawning, and (D, H) spent stages. H&E staining. Scale bar = 100 μm.

Developing stage: Sex discrimination is possible starting from this stage. In males, the genital tube is enlarged and the spermatogonia appear along the wall (Fig. 4A). In the late stage, spermatocytes move toward the center of the genital tube and a small number of sperm cells appear. In females, the genital tube is enlarged and oogonia appear along the wall (Fig. 4E). In addition, the ovary becomes a capsule-like tube structure, and oocytes and oogonia are attached to the tube wall.

Mature stage: In males, sperm and sperm cells occupy a large part of the genital tube; the sperm are arranged radially in center of the tube (Fig. 4B). In females, there are many mature or late developing oocytes in the ovary; the oocytes transform into irregular or polygonal shapes (Fig. 4F).

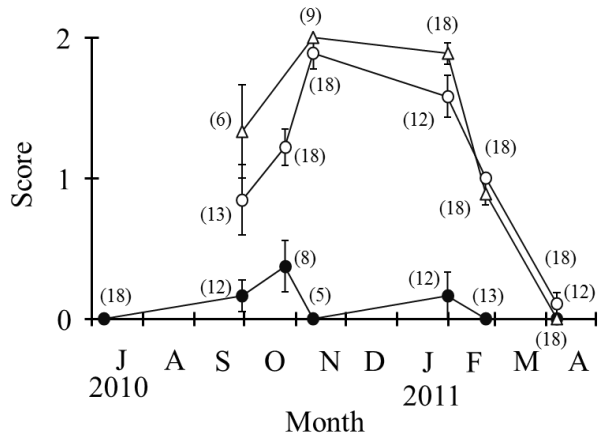

Figure 5. Changes in the thickness of the visceral site at the 3 sampling stations. Values represent mean ± SE. Numbers in parentheses are the numbers of samples. Station 1: ●, Station 2: △, and Station 3: ○.

Spawning stage: In males, some sperm have been released, and many sperm and sperm cells remain in the genital tube (Fig. 4C). In addition, some of the genital tube is contracted and partially collapsed after spawning. In females, a tube in the ovary is observed, because mature eggs have partially spawned (Fig. 4G). Oocytes in the late developing or mature stages remain in much of the genital tube. In addition, mature eggs usually exhibit nuclear disappearance because of germinal vesicle breakdown.

Spent stage: In males, the genital tube is almost devoid of sperm, which have completely spawned, although a few sperm remain (Fig. 4D). In females, the genital tube is deformed and folded, and much of the tube is completely empty (Fig. 4H).

Results

Naked eye observations: The thicknesses of the viscera of blood cockle specimens collected from St. 2 in September were observed. The thickness increased in November (Fig. 5), continuing until January. The average score decreased in February, and the thickness had completely contracted by April. Changes in the thickness of blood cockles at St. 3 exhibited similar changes as those of St. 2. The thickness increased from September to November, peaking from November to January, regressing in February, and completely regressed by April. On the other hand, at St. 1, thickening was almost 0 points in July, which continued until the last survey in April.

Table 1. Occurrence of each developmental stage of *Anadara granosa* in Matang mangrove estuary from July 2010 to April 2011.

	Station	n	I	Male				Female			
				II	III	IV	V	II	III	IV	V
Jul.	1	18	18								
	2	ns									
	3	ns									
Sep.	1	12	11	1							
	2	6	1	1	1			2	1		
	3	13		6	4			2	1		
Oct.	1	8	6					2			
	2	ns									
	3	18		3	6	3			6		
Nov.	1	5	5								
	2	9			7				2		
	3	18			5	5			6	2	
Jan.	1	12	10	2							
	2	18			4	3			4	5	2
	3	12	3		1	2				3	3
Feb.	1	13	12					1			
	2	18			2	4	5		1	1	5
	3	18	1			8	1			4	4
Apr.	1	18	18								
	2	12	6			1	4				1
	3	18	5			1	5				7

I: Immature, II: Developing, III: Mature, IV: Spawning, V:Spent
ns: no sample

Histological observations: The histological changes in the gonads of both sexes from St. 2 and St. 3 were almost the same. Specimens were mainly classified as being in the developing and mature stages in September, the mature and spawning stages in November and January, the spawning and spent stages in February, and the spent and immature stages in April (Table 1). Individuals in the spawning stage were first observed in males from St. 3 in October; individuals in this stage at St. 3 were observed until April. In addition, males in the spawning stage at St. 2 were observed from January to April; thus, individuals in the spawning stage were observed later than at St. 3. Females in the spawning stage were first observed at St. 3 in November until February. In addition, individuals in the spawning stage at St. 2 were observed from January to

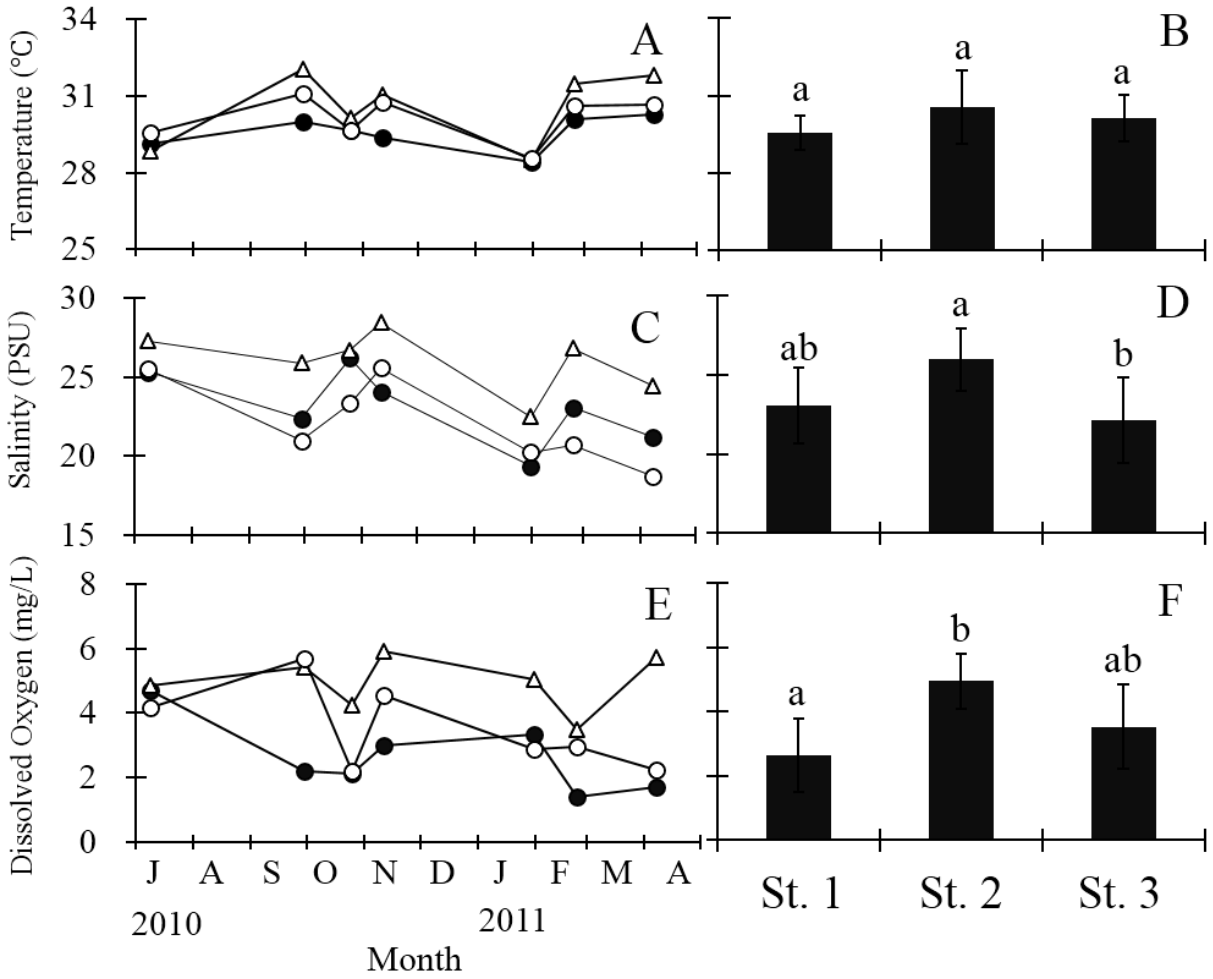

Figure 6. Changes in and averages of water temperature, salinity, and dissolved oxygen on the bottom layer at the 3 stations in Matang mangrove estuary during the survey period. (A) Changes in water temperature. (B) Average water temperature. (C) Changes in salinity. (D) Average salinity. (E) Changes in dissolved oxygen. (F) Average dissolved oxygen. Large bars (means) and small bars (SD) without common letters indicate significant differences in each graph in B, D, and F (P<0.05; Tukey test). Station 1: ●, Station 2: △, and Station 3: ○.

February. On the other hand, all 18 individuals at St. 1 in July were immature; the percentage of immature individuals exceeded 70% in each survey throughout the study period.

Environment: The water temperature at the bottom of 3 stations ranged from 28.4–32.1°C (Fig. 6A). The maximum temperature at St. 1–3 was recorded 30.3°C in April, 32.1°C in September, and 31.1°C in September, respectively. Meanwhile, the minimum temperatures ranged from 28.4–28.5°C in late January (Fig.6), the average temperature was not significant difference during these stations (Fig. 6B). The minimum salinity at St. 1–3 was 19.3 PSU in late January, 22.3 PSU in late January, and 18.7 PSU in April, respectively (Fig. 6C). Regarding the average salinity at each station during the study period, the average salinity at St. 2 was significantly higher than that of St. 3 (P<0.05) (Fig. 6D). The minimum dissolved oxygen concentrations at St. 1 and St. 2 were 1.4 and 3.5 mg/L in February, respectively, and 2.2 mg/L in April and October at St. 3. The average dissolved oxygen concentration at St. 1 was significantly lower than that of St. 2 (P<0.05) but not significantly different from that of St. 3 (Fig. 6F). Finally, the redox potential in the bottom sediment at St. 1 was −100 mV lower in November and was significantly different from those of other stations (P<0.01) (Fig. 7).

Discussion

Environment: Matang mangrove is about 40,000 ha and has a long coastline of about 50 km from north

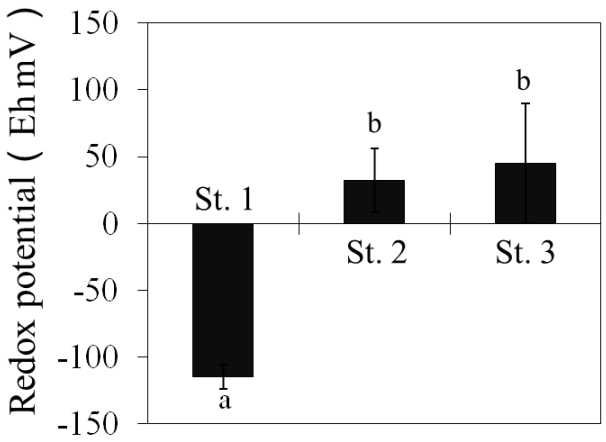

Figure 7. Comparison of redox potential in the surface sediment at the 3 stations in Matang mangrove estuary on November 2010. Large bars (means) and small bars (SE) without common letters indicate significant differences (P<0.01; Tukey test).

to south (Fontalvo-Herazo et al., 2011). In addition, this region has a typical tropical rainforest climate; annual precipitation is 2,000–3,000 mm (Ashton et al., 1999), and average temperature is 22°C at night and 33°C in the daytime with little annual variation (Aldrie Amir, 2012). Therefore, water temperature is stable even in shallow water, which is easily affected by changing temperature. In the present study, the water temperature of the bottom layer was almost constant at the 3 stations throughout the study period. On the other hand, salinity exhibited clear changes among surveys, decreasing at 3 stations in September, February, and April. This is considered to be due to freshwater inflow from rivers as a result of heavy rainfall at the time. In addition, salinity remained low during the study period at St. 1 and St. 3, which are located in estuaries; these results indicate both stations are affected by inland water. In addition, dissolved oxygen concentrations were high at St. 2 throughout the study period, while concentrations were often low at St. 3 and particularly low at St. 1. Okamura et al., (2010) surveyed the distributions of dissolved oxygen in the waters of Matang mangrove estuary and found hypoxic waters (dissolved oxygen < 2 mg/L) were distributed in tributary rivers and the inner part of the mangrove during neap tide; furthermore, the hypoxic water mass extended to estuaries and coastal areas during spring tide. Therefore, estuaries affected by land waters, such as St. 1 and St. 3, were considered susceptible to hypoxic water distributing in the inner part of the mangrove. In addition, Okamura et al., (2010) surveyed redox potential in the surface of bottom mud in the same area; their results indicate bottom mud from the coastal to offshore areas is oxidative. However, in the present study, redox potential measurement of bottom mud in November showed oxidative values at St. 2 and St. 3., and a significant reduction of −100 mV lower at St. 1. It is necessary to consider the effect of specific organic loading on the oxidative reduction of sediment around St. 1., because this area has been recently exposed to increasing numbers of net cages for finfish culture. Therefore, remaining feed from these cages may be one of the causes for the observed severe reduction at this site.

Spawning season: The spawning season of the blood cockle in tropical regions is very long. The spawning seasons of both sexes in the Rusamilae waters of Pattani Bay, Thailand off the east coast of Peninsular Malaysia are observed in September and from December to June (Suwanjarat et al., 2009). The appearance of mature blood cockles in Selangor estuary off the west coast of Peninsular Malaysia are observed from September to April (Broom, 1983). In addition, in Kakinada Bay off the west coast of India, the spawning season of the blood cockle differs each year but spawning-stage individuals appear almost year round (Narashimham, 1988a). On the other hand, from the naked eye observations of the visceral site of blood cockles collected at St. 2 and St. 3 in the present study, the thickness index peaked in November, continuing until January and regressing from January to April. In addition, from gonadal tissue observations of the same specimens, males and females in the spawning stage were observed from November to February. Therefore, the peak of spawning season of the blood cockle in Matang mangrove estuary is likely from November to February. The sexual maturation of bivalves is usually closely associated with water temperature and food availability (Mackie, 1984). The

importance of these factors has been confirmed experimentally in Manila clam, *Ruditapes philippinarum* (Mann, 1979; Toba, 1989; Toba and Miyama, 1995). However, in tropical areas such the present survey sites, the water temperature of the bottom layer is stable almost year round. Therefore, it is unlikely water temperature is a primary factor confining the spawning season. On the other hand, according to the average monthly precipitation in Sitiawan, which is about 60 km south of Matang mangrove estuary, referenced from the Malaysian Meteorological Agency website (http://www.met.gov.my/), a rainy season of more than 200 mm/month occurs from October to December. In addition, it is well known phytoplankton in Matang mangrove estuary increases from November to January; in one case, the peak phytoplankton volume was more than 5 times the normal volume (Tarutani et al., 2005). Therefore, the above mentioned studies suggest changing food availability during the rainy season is likely one of the major factors defining the spawning season of the blood cockle in Matang mangrove estuary.

Immaturity: In this study, the visceral sites of blood cockles from St. 2 and St. 3 were remarkably thick; these tissues were confirmed to be developing germ cells by histological observation. Meanwhile, the blood cockles collected in St. 1 did not exhibit the same thickness at the visceral sites; moreover, the gonads were completely immature or some cells in these gonads were slightly developing upon histological observation. Therefore, this behavior observed in blood cockles from St. 1 is considered to be a unique phenomenon, which will be discussed below. Comparing the environments at St. 1 and St. 3, which are located at the river mouth, both stations have almost the same temperature and salinity. However, regarding the average dissolved oxygen concentrations, the average concentration at St. 1 was significantly lower than those at St. 2 and St. 3. In addition, in November, when the blood cockles at St. 2 and St. 3 were in high spawning season, the redox potential at the surface layer of bottom mud was measured at each station; oxidative values were detected at St. 2 and St. 3, whereas a severe reduction below −100 mV was detected at St. 1. Hata (1965) examined air-dried fine seabed mud containing 1% each of minced fish meat and powdered cellulose, which was filled with seawater and incubated at 30°C, and observed the environmental changes. The results show changes in redox potential: the mud was 340 mV on the first day, decreasing to below −100 mV after about 5 days, and to about −250 mV, stabilizing for about 20 days until the end of the experiment. In addition, when the same experiment was set up at different temperatures, the decrease in potential was significant at temperatures exceeding 22°C. Furthermore, the results of that study demonstrate potential is closely associated with bacterial activity. On the other hand, the monitoring of bottom mud in an inner bay of the Danish coast by Jorgensen (1977) revealed seasonal variation in the redox potential; the results show the reduction of surface mud and hypoxic water just above the seabed is likely to occur in high temperatures in summer and disappear in winter.

Considering these findings and the fact that water temperature in tropical areas is stable at about 30°C year round, it is possible, mud with reduced redox potential remains. Furthermore, it is well known that hypoxic water is likely to occur at the boundary layer immediately above the water on sediment (Gundersen and Jorgensen, 1990). Therefore, it is very likely blood cockles, which commonly exist in the boundary layer, are exposed to the hypoxic environment detected in the present study or harsher environments. On the other hand, bivalves in remarkably low-oxygen environments are well known to have anaerobic metabolism including decreased metabolic and filtration rates (Sobral and Widdows, 1997). However, corresponding knowledge in the blood cockle is currently insufficient. Nevertheless, it is quite possible the blood cockle is restricted by food availability when exposed to hypoxic environments. Thus, the specimens collected from St. 1 suggest blood cockles cannot maintain good nutritional conditions for sexual maturation.

Conclusion

The sexual maturation and spawning of blood cockles from 3 stations of Matang mangrove estuary in peninsular Malaysia were estimated by naked eye and histological observations of visceral and gonadal tissues. The results indicate a high period of spawning from November to February during rainy season at 2 stations. However, at one other station, where redox potential was −100 mV lower at the surface bottom in November, maturation was not clearly observed during the study period. Therefore, the results suggest the redacting environment inhibits the sexual maturation of the blood cockle.

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