Songklanakarin J. Sci. Technol.
31 (5), 471-479, Sep. - Oct. 2009

Songklanakarin Journal
of Science and Technology
http://www.sjst.psu.ac.th

Original Article

Reproductive cycle of *Anadara granosa* at Pattani Bay and its relationship with metal concentrations in the sediments

Jintamas Suwanjarat[1]*, Chinnawat Pituksalee[2] and Suppattana Thongchai[1]

[1] *Department of Biology, Faculty of Science,*
Prince of Songkla University, Hat Yai, Songkla, 90110 Thailand.

[2] *Division of Fisheries Technology, Faculty of Science and Technology,*
Prince of Songkla University, Muaeng, Pattani, 90400 Thailand.

Received 9 June 2008; Accepted 20 July 2009

Abstract

The reproductive cycle of the blood clam *Anadara granosa,* obtained from two different areas at Pattani Bay (Rusamilae and Laem-nok), on the eastern coast of southern Thailand was examined histologically between July 2005 and June 2006. The specimens were analyzed to determine the variability in their cycles under different environmental conditions. Variations of Cd, Cu, Zn, Mn, and Pb concentration in the sediments and clam tissues were measured. Results showed that gametogenesis of *A. granosa* occurred throughout the year with slightly different spawning periods at the two sites. The gonad index indicated that the sexual maturity of *A. granosa* at the Rusamilae site was greater than that at the Laem-Nok site, suggesting that the Rusamilae site is a more suitable location for *A. granosa* breeding and culture than the Laem-Nok site. The concentration of heavy metals (Cd, Cu, Zn, Mn, and Pb) in the sediments from both sites were not significantly different, except that the sediments from Laem-Nok had a significantly higher Pb level than at Rusamilae. The data showed correlation between the metal levels in tissue and the reproductive cycle of *A. granosa* at Laem-Nok. The breeding season of *A. granosa* in Pattani Bay is mainly from July to August. Thus, for the conservation of *A. granosa* in this area harvesting should be avoided during this time, and the best time for collecting this clam at Pattani Bay is suggested to be between February and June which is not the breeding season.

Keywords: *Anadara granosa*, gonadal stage, metal concentration, reproductive cycle

1. Introduction

The blood clam *Anarara granosa* of the family Arcidae is a commercially important bivalve exploited for human consumption through harvesting by fishing and farming. In Thailand, the national production of this edible species is remarkably high with an average yield of 80 million tons per year. In Pattani Province the annual production of the clam *A. granosa* is only about 26,000 tons a year, one of the lowest yielding areas (Department of Fisheries. 2004). Pattani Bay is located in the lower part of the eastern coast of southern Thailand. The coastal zone of the bay is characterized by muddy and sandy sediments with abundant natural invertebrates and widespread clam farming and local fisheries (Swennen *et al.*, 2001). The industries and houses of local people are situated along the shore of Pattani Bay where their wastes, some of which contain heavy metal contaminants, drain directly into the sea. Generally, the re¢productive activities of marine invertebrates, particularly bivalves are mostly influenced by environmental conditions and their gonads may have considerable variations from place to place and from year to year (Broom, 1983; Suwanjarat and Thongboon, 1997; Brown *et al.*, 2002; Tirado *et al.*, 2003; Drummond *et al.*, 2005). Inter-site variations in

*Corresponding author.
Email address: jintamas.s@psu.ac.th

breeding patterns have been observed in bivalve species, and the variations appear to be linked with differences in temperature and food supply (Laruelle *et al.*, 1994; Rodriguez-Rua *et al.*, 2003; Delgado and Camacho, 2005). However, another important environmental parameter that could affect the reproduction is the metal level in sediments (Gagne *et al.*, 2002; Meneghtti *et al.*, 2004; Smaoui-Damak *et al.*, 2006). The presence of toxic metals such as Cd can affect the reproduction and recruitment of clams (Siah *et al.*, 2003; Cheggour *et al.*, 2005; Yu, 2005). Previous studies on marine bivalves have shown an increase in gamete alterations and degeneration of the reproductive tissue when the animals were exposed to various contaminants (Lowe, 1988; Clark *et al.*, 2000). In estuarine ecosystems, sediments are an important sink of heavy metals that may result in the accumulation of metals in marine organisms, especially in bivalves due to their feeding behavior (Gosling, 2003). The blood clam *A. granosa* primarily gains nutrition from deposit-feeding that can cause the accumulation of heavy metals and chemical pollutants in their tissues. At present, the basic knowledge about fecundity, reproduction periods, growth, and circumstances of death of most species in the Gulf of Thailand is relatively poor even for commercially important food species (Swennen *et al.*, 2001). In addition, the knowledge of the reproductive cycle of the blood clam is necessary to interpret the effect of metal bioconcentration in the field. Therefore, it is necessary to observe the relationship between metal burdens in the tissue of *A. granosa* in this area and their gonadal cycle.

The aim of this study was to investigate the annual reproductive cycle of *A. granosa* at two sites, Rusamilae and Laem-Nok in Pattani Bay. The gonadal stages at each month over one year period were examined histologically using a light microscope. Variations in the concentrations of five metals (Cd, Cu, Zn, Mn, and Pb) present in the sediments and the clam *A. granosa* tissue, collected monthly at the same time, were determined using the optical emission spectrometer. Comparisons of the metal levels in the sediments and *A. granosa* soft tissue in the two different areas as well as any observed associations of metal concentrations and the clam reproductive cycles are discussed. Results obtained from this study will be useful in developing strategies for culture and management plans related to the reproductive periods of the clams, and to aid in the protection and conservation of *A. granosa* in Pattani Bay.

2. Methods

2.1 Specimen collection

The clams *A. granosa* (30-40 clams/site/month) and surface sediments (3 areas/site) of their habitat were sampled monthly from two different sites at Pattani Bay over a period of 12 months during July 2005 to June 2006 (samples were not available in November due to the monsoon). The first site "Rusamilae" is a free fishing area and less affected by industrial activities, whereas the second site "Laem-Nok" is located close to the industrial area and considered to be more influenced by human activities. These two study sites are approximately three kilometers apart (Figure 1). The temperature and salinity of the seawater were recorded each month at both sites during the sampling. Clams were transported alive to the laboratory at the Department of Biology, Prince of Songkla University, for further processing. The whole soft tissue were removed from the shell and weighed (fresh weight, FW) to the nearest of 0.001 g.

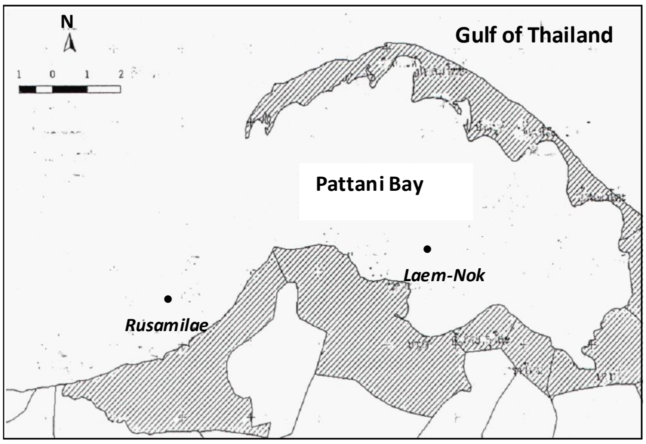

Figure 1. Location of sampling sites in Pattani Bay.

2.2 Sample preparation for metals analysis

The monthly collected sediments were wet digested by high purity nitric acid and the solution was processed according to the method described by Loon (1985) and Gunzler and Williams (2001). Soft tissues of the clams were heat dried at 550°C for 12 hrs. The ash was also digested by high purity nitric acid and further processed according to the method described by Boss and Fredeen (1997). The concentrations of Cd, Cu, Zn, Mn, and Pb in the sediments and tissues of *A. granosa* were analyzed using an inductive coupled plasma optical emission spectrometer (ICP-OES, P. Elmer Opt 4300DV) at the Central Equipment Division, Faculty of Science, Prince of Songkla University.

2.3 Histological analysis

Determination of the gonadal stages was carried out through histological sections of the gonads. The flesh containing most parts of the gonads were removed and immediately fixed in Bouin's fluid for 24 hrs, then passed to 70% alcohol, dehydrated in an ethanol series and infiltrated with paraffin. Sections were cut into 5 μm thicknesses, stained with Harris's haematoxylin and acid eosin (Bancroft and Gamble, 2002) and mounted on a microscope glass slide. The sex of the clam and any histological changes in the gonads of individuals were examined using a light microscope.

2.4 Gonadal index

The gonadal index (GI) was calculated for each sampling month through histological analysis of the gonadal stage to estimate the proportion of resting, developing, mature, spawning, and spent individuals as described by Gosling (2003). In this study, GI was ranked from 1 (all individuals in the sample were in the resting or spent stage) to 3 (all individuals were in the mature stage). An increase in the value of the gonad index implies gonad development, while a decrease implies spawning, spent, and resting stage.

2.5 Statistical analysis

Differences in metal concentrations in the sediments and soft tissues according to the month and site were tested by an analysis of variance (two ways ANOVA, P<0.05). A linear regression and correlation coefficients were determined using Excel 2003 to test for the relationship between two parameters.

3. Results

3.1 Environmental parameters

The mean value of the temperatures was approximate 30°C with the range of 28-31°C at the Rusamilae site and 28-32°C at the Laem-Nok site. There was no significant difference in the temperatures between the two sites (P>0.05). The average salinity of 30.33 ppt at Rusamilae was significantly higher than that of 20 ppt at Laem-Nok (P<0.05).

The average values of Cd, Cu, Zn, Mn, and Pb concentrations and ranges in sediments and *A. granosa* soft tissues from the two study sites for the 12 month period show that the concentrations of heavy metals in sediments from both sites were not significantly different, except that the mean Pb level in the sediments from Laem-Nok was significantly higher than that of Rusamilae (P<0.05). Metal concentrations in all tissue samples of *A. granosa* were lower than those in the sediments for all analyzed metals except Cd and Cu. There was no correlation between the metal levels in the sediments and in the *A. granosa* tissues (r = 0.001). Figures 2 and 3 showed seasonal variations of metal concentrations in sediments and tissues, respectively. Monthly metal variations in the sediment were detected for Cd, Zn, Mn, and Pb concentration at Rusamilae (P<0.05) and Cu, Zn, and Mn at Laem-Nok (P<0.05). All sediment metal concentrations at Rusamilae were highest in December and significantly higher than those at Laem-Nok (P<0.05). Comparing the metal levels in soft tissue, there were no significant differences between the two locations although monthly metal variations in the soft tissues were significantly different at both study

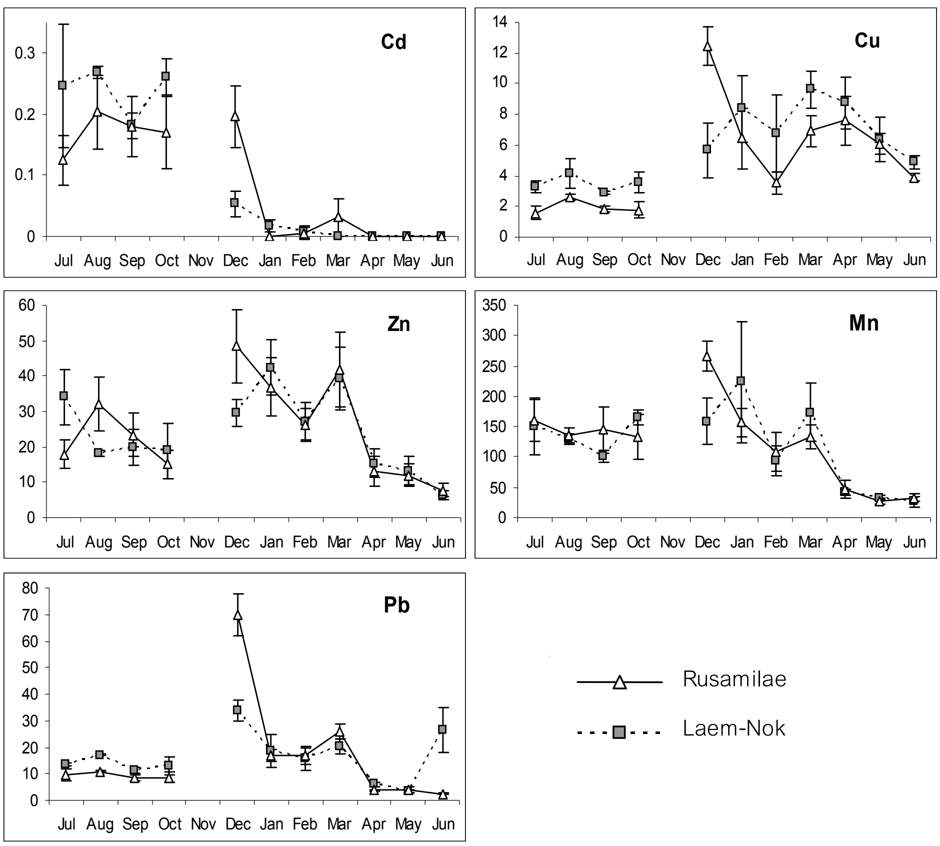

Figure 2. Annual variation of five metal concentrations (mg/kg) in sediments at Rusamilae and Laem-Nok.
* Data not available in November.

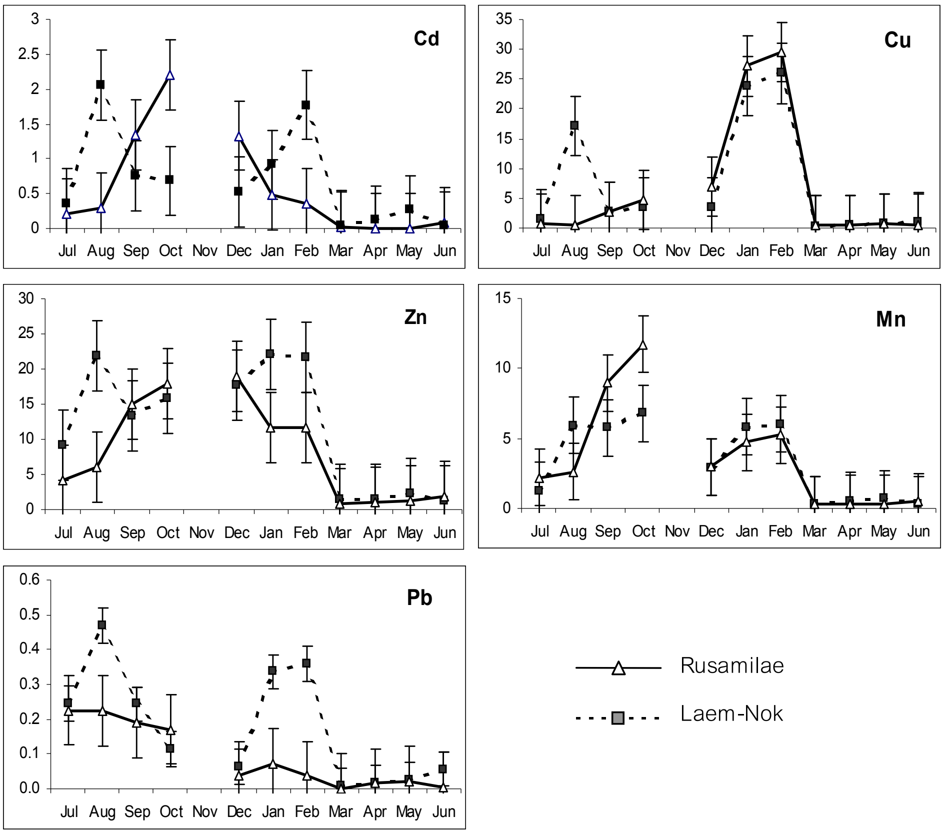

Figure 3. Annual variation of five metal concentrations (mg/kg) in *A. granosa* soft tissue at Rusamilae and Laem-Nok. *Data not available in November.

sites. Cd, Cu, and Mn levels in Rusamilae clams were significantly high in October, February, and October, respectively, while Cd, Cu, Zn, and Pb levels in Laem-Nok clams were synchronously high in August and February ($P<0.05$).

3.2 Reproductive cycle

Based on the histological structure, the ovarian and testicular developmental stages were classified into 5 stages: resting; developing; mature; spawning, and spent stages as described in Table 1. The five-stage gametogenic cycles of male and female *A. granosa* from Rusamilae and Laem-Nok were established as a percentage distribution of gonadal stages in each month (Figures 4 and 5). The histological changes of ovary and testis during the reproductive cycle are illustrated as in Figures 6 and 7. Gametogenesis in both male and female *A.granosa* occurred continuously throughout the year. At Rusamilae, the developing stage took place from July-October in male and in October in female, and declined in the following months. The mature stage was regularly found in most months with the highest peak in August (56.25%) and July (55.25%) for male and female, respectively. In December, 100% of male gonads were in spawning stage, while the high values of this stage in females appeared in December 2005 (50%) and January 2006 (62.5%). However, this study showed that the spawning period of both sexes was synchronized at Rusamilae with three peaks in September, December, and July. In contrast, at Laem-Nok, the spawning period in the male and female *A. granosa* had only one synchronous peak in June. From July to October, male and female *A. granosa* from this location were in the spent stage. The mature stage in the males from Laem-Nok showed the highest peak in August, the same period as that at Rusamilae. The resting stage in the Rusamilae male was scanty and found only in April and June with low percentages while that in the Laem-Nok male appeared all year around except for January.

3.3 Gonadal index

The monthly GI of male and female *A. granosa* from both sites is presented in Figure 8. Generally, the GI values increased during gametogenesis and decreased during spawning and resting. The data show that the values of the GI of the Rusamilae clams were close to 2.0 through most of the sampling period (mean > 2.0), while those of the Laem-Nok clams were mostly below 2.0 (mean < 2.0). This indicated that sexual maturity of the population at the former site was greater than that at the latter. A comparison of GI (Figure 8) to the distribution of the reproductive cycle (Figures 4 and 5)

Table 1. Description and criteria for the gonadal stages of *Anadara granosa*.

Gonadal stages	Histological characteristics	
	Male	Female
Resting:	Luminal follicles are narrow and empty. Connective tissue is present among the follicles (Figure 6A).	Follicles are small with the appearance of few oogonia along the follicle wall . (Figure 7A)
Developing:	Presence of spermatogonia which lined the follicle wall. Spermatocytes and spermatids are abundant. A number of spermatozoa are found in late development (Figure 6B-C).	Immature oocytes attached to the basal membrane. Various sizes of developing oocytes with basophilic cytoplasm are found (Figure 7B).
Mature:	Follicles are full of spermatozoa with their tails pointing towards the center of lumen (Figure 6D).	Oocytes increase in size. Acidophilic cytoplasm and yolk granules are remarkable formed. Ripe oocytes are mostly free in the lumen (Figure 7C).
Spawning:	Spermatozoa released causing the empty space in the follicular lumen (Figure 6E).	Ripe oocytes decrease in number and empty spaces in the follicular lumen are observed (Figure 7D).
Spent:	The alveoli are empty. Some spermatozoa remained in the lumen (Figure 6F).	Follicle degenerates, few residual germ cells remained in the lumen (Figure 7E).

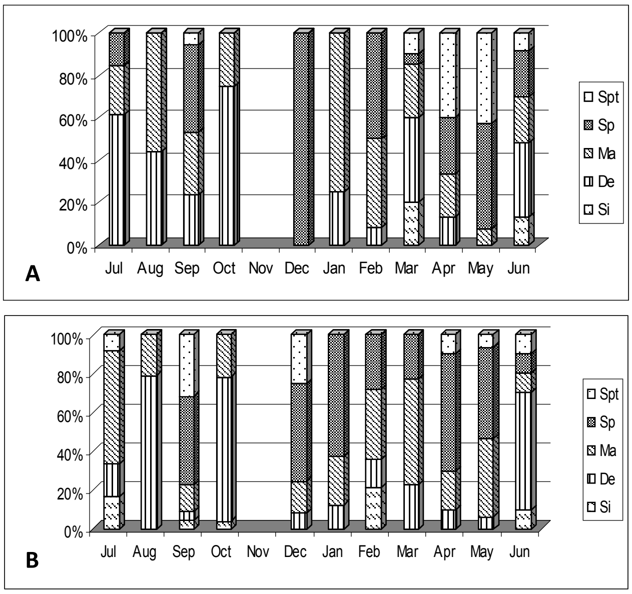

Figure 4. Percentage distribution of gonadal stages in male (A) and female (B) *A. granosa* from Rusamilae area during June 2005 and July 2006. (Spt-spent; Sp-spawn; Ma-mature; De-developing; Si-resting).
*Data not available in November.

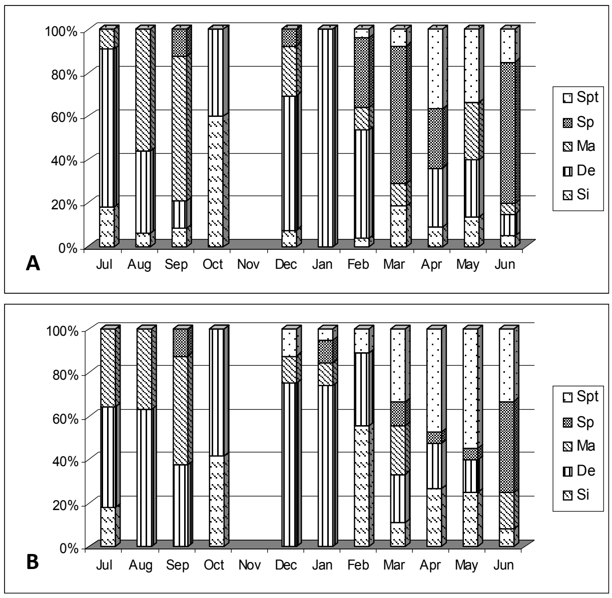

Figure 5. Percentage distribution of gonadal stages in male (A) and female (B) *A. granosa* from Laem-Nok area during June 2005 and July 2006. (Spt-spent; Sp-spawn; Ma-mature; De-developing; Si-resting).
*Data not available in November.

revealed that the maximum GI values were attained during the mature stage. The GI values of the Rusamilae male was above 2.0 from July 2005 to February 2006, then decreased in March to May and began to increase again in June. The GI curves of both sexes at Rusamilae were synchronously high from July - August and December - February implying the beginning of the breeding season of *A. granosa* at this site. Similar situation was found at Laem-Nok with the GI values of male and female clams being high in August-September and December-January, revealing a slight delay of the breeding season compared to Rusamilae.

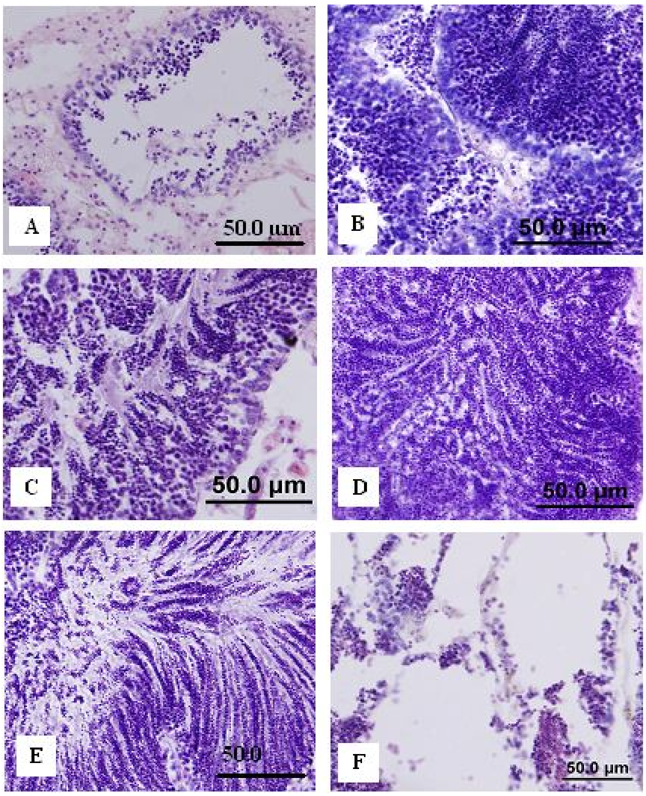

Figure 6. Photomicrographs of *A. granosa* testis (H&E). A = resting stage, B - C = developing stage, D = mature stage, E = spawning stage, F = spent.

4. Discussion

This study revealed that the seasonal changes in the stages of gametogenesis in *A. granosa* were not obviously related to changes in the studied environmental parameters. An influence of environmental conditions on bivalve reproduction has been reported by previous authors, with the most important parameters being the temperature and availability of food (Rodriguez-Rua *et al.*, 2003; Darriba *et al.*, 2004; Delgado and Camacho, 2005), as well as the levels of environmental instability (Power *et al.*, 2004; Trivino and Anadon, 2006). In this study, the monthly and the mean values of the seawater temperature were not significantly different between the Rusamilae and Laem-Nok sites. In general, temperature plays an important role on the reproductive cycle of bivalves particular in the temperate zones, where the temperature fluctuates and is widely different between summer and winter. Spawning took place in the warmer months in response to the increases in temperature and presence of phytoplankton (Rodriguez-Rua *et al.*, 2003), but the razor clam *Ensis arcuatus* was in its sexual resting stage in summer and spawned in winter (Darriba *et al.*, 2004). Gametogenesis of *A. granosa* at Rusamilae and Laem-Nok occurred throughout the year yet with different spawning periods and no relationship was found between the water temperature and the spawning period. The spawning periods of the Rusamilae male and female were synchronized with

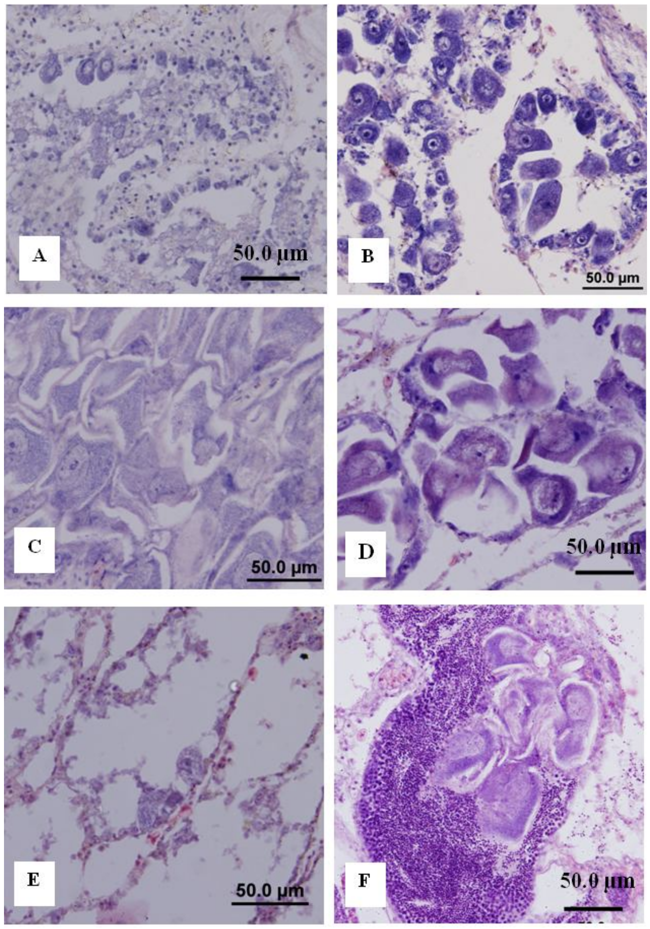

Figure 7. Photomicrographs of *A. granosa* ovary (H&E). A = resting stage, B = developing stage, C = mature stage, D = spawning stage, E = spent, F = hermaphrodite.

three spawning peaks in September, December, and July. At the Laem-Nok site, the spawning periods were less and the male and female clams spawned synchronously only in June. This is a different behavior from *A. granosa* on the west coast of South Thailand where spawning period occurred twice a year in August and November (Suwanjarat and Parnrong, 1990). The resting stage of the male gonad cycle in Rusamilae was found only in April and June and then only at low percentages, while the resting stages of the Laem-Nok male appeared all year round. This indicated that the envi-ronmental conditions at Rusamilae were more suitable for gametogenesis than at Laem-Nok where more industrial activities are located nearby. The environment at Rusamilae seems to be more favorable for the male clam to develop a new set of sex cells for succeeding gametogenic cycles while spawning at the same time. Gametogenesis of the clams occurs rapidly, the gametes are released as soon as they mature and recovery is fast, resulting in a new phase of development and maturation between successive spawns that makes it difficult to distinguish between spawning and mature stages. However, the determination of the gonad index through histological analysis is another useful method

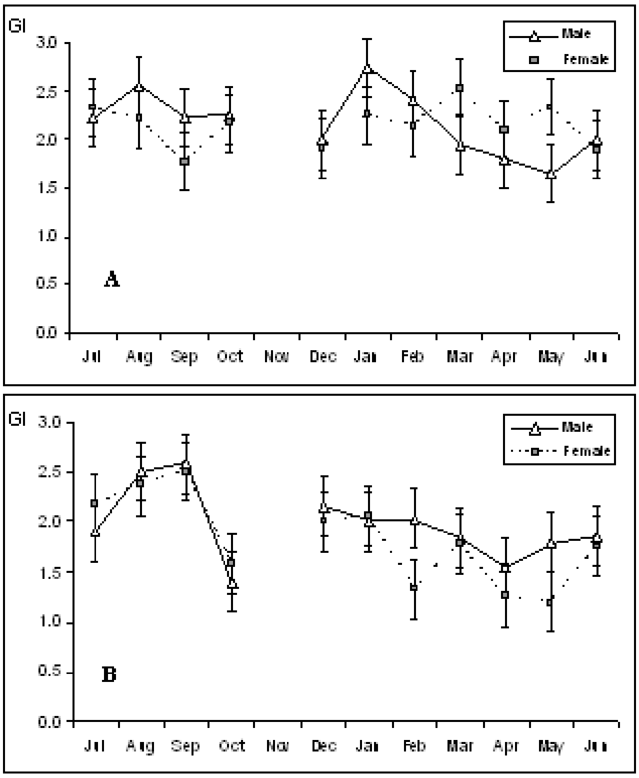

Figure 8. Seasonal variation in gonadal index (GI) of male and female at Rusamilae (A) and Laem-Nok (B).
*Data not available in November.

to assess the sexual maturity and gonad condition. The GI indicated that the number of sexual maturity of *A. granosa* at Rusamilae was greater than that at Laem-Nok. The minimum GI values of males in May at Rusamilae and in October at Laem-Nok coincided with the lowest percentage of individuals in the resting or spent stages indicating inactive gonads. In our study, GI values below 2.0 were observed more often in females than in males due to the female clams having a greater tendency to spawn completely and undergo an inactive phase. Males, on the other hand, have a GI value higher than 2 because they had a faster rate of gametogenic activity (Gosling, 2003).

The relationship of the average values of metals concentrations in the sediment between Rusamilae and Laem-Nok was not significant except Pb. The Pb level in Laem-Nok is significantly higher than that in Rusamilae ($P<0.05$). The waste products drained from the industries located along the shore as well as the waste drains from human activities around Laem-Nok possibly caused high Pb concentrations in the sediments. Although there was no correlation between the metal levels in the sediment and in the *A. granosa* tissues ($r= 0.001$), but the average values of metal concentrations in *A. granosa* tissues from both study sites were lower than those in the surrounding sediments except for Cu (not significant, $P>0.05$) and Cd. The Cd concentration in soft tissue was significantly high in August, when the reproductive activity of *A. granosa* was mostly in developing as well as maturing stage. During these stages, clams required considerable energy consumption and this led to metal intake and accumulation in tissue particularly the gonads that become an additional temporary storage tissue for metals (Mathieu and Lubet, 1993). The Cd burden in *A. granosa* indicated an accumulation or a low excretion of this metal from sediment particles, somehow as the efficiency of assimilation after ingestion of sediments is dependent on the sediment type, the animal species and its physiological condition (Griscom *et al.*, 2002). Moreover, spawning can mobilize some assimilated metals in bivalves and this can contribute to the seasonal variations noted in bivalve metal concentrations (Smaoui-Damak *et al.*, 2006). Besides Cd, the Cu, Zn, Mn, and Pb concentrations in *A. granosa* tissues was high in August and February and low during March-June when they were in spawning and spent stages implied the correlations of tissue metal concentration to the reproductive cycle. The findings in the clam *Scrobicularia plana* revealed the seasonal variation in tissue metal concentrations linked to patterns of reproductive activity for Cu, Ni, Mn, Fe except Cd and Zn whose tissue concentration may be regulated (Cheggour *et al.*, 2005). On the other hand the period of ripening and spawning of sexually products in the clam *Ruditapes decussatus* led to decrease of Cd concentration in their whole soft tissues (Smaoui-Damak *et al.*, 2006). In the present study, the correlation of tissue metal concentrations to the reproductive cycle at Laem-Nok was found. However, it is ambigulous to interpret that the metal concentrations affected reproductive cycle of *A. granosa* at Pattani Bay.

In conclusion, the reproductive cycle of *A. granosa* at Pattani Bay is an annual cycle of gametogenesis occurring throughout the year with a slight different spawning period between Rusamilae and Laem-Nok sites. There was no direct relationship between the spawning period and the studied environmental parameters such as pH, temperature, and heavy metal concentrations. The relationship of metal concentrations in *A. granosa* tissue with those in the sediments was not found. At Laem-Nok, the metal concentration showed slight correlation to the gonadal cycle of this clam. The Rusamilae site was a more suitable location for *A. granosa* breeding and culture than the Laem-Nok site. The breeding season of *A. granosa* in Pattani Bay is between July and August. Thus, for the conservation of *A. granosa* in this area harvesting should be avoided during this time. This study also indicated that the Cd, Cu, Zn and Mn concentrations in surface sediments from both studied areas in Pattani Bay were below the minimum toxic levels known to cause toxic affects in resident benthic animals (Long *et al.*, 1995).

Acknowledgements

This work was financial supported by the research budget of PSU, and the Graduate School, Prince of Songkla University, HatYai campus.

References

Bancroft, J. D. and Gamble, M. 2002. Theory and Practical of Histological Techniques. 5 th Ed. Churchill Livingstone. pp 796.

Brown, D., Campos, B., and Urban, H. J. 2002. Reproduct-ive cycle of the bivalve clams *Semele solida* (Gray, 1828) (Semelidae) and *Gari solida* (Gray 1828) (Psammobiidae) from Chile. Journal of Shellfish Research. 21(2), 627-634.

Broom, M. J.1983. Gonad development and spawning in *Anadara granosa* (L.) (Bivalvia: Arcidae). Aquaculture. 30, 211-219.

Boss, C. B. and Fredeen, K.J.1997. Concepts, Instrumentation and Techniques in Inductively Coupled Plasma Optical Emission Spectrometry. U.S.A.: The Perkin -Elmer corporation. pp 450.

Cheggour, M. J., Chafix, A., Fisher, N. S. and Benbrahim, S. 2005. Metal concentrations in sediments and clams in four Moroccan estuaries. Marine Environmental Research. 59, 119-137.

Clark, S.L., Tech, S. J. and Hinton, D.E. 2000. Tissue and cellular alterations in Asian clam (*Potamocorbula amurensis*) from San Francisco Bay: toxicological indicators of exposure and effect. Marine Environ-mental Research. 50, 301-305.

Darriba, S., Juan, F. S. and Guerra, A. 2004. Reproductive cycle of the razor clam *Ensis arcuatus* (Jeffreys, 1865) in northwest Spain and its relation to environmental conditions. Journal of Experimental Marine Biology and Ecology. 311, 101-115.

Department of Fisheries. 2004. Statistic of Marine Shellfish Culture Survey 2002. Fishery statistic analysis and research group, Fishery Information Technology Center. No 26. pp 31.

Delgado, M. and Camacho, A. P. 2005. Histological study of the gonadal development of *Ruditapes decussates* (L.) (Mollusca: Bivalvia) and its relationship with available food. Science Marine. 69(1), 87-97.

Drummond, L., Mulcahy, M. and Culloty, S. 2005. The reproductive biology of the Manila clam, *Ruditapes philippinarum*, from the North-West of Ireland. Aquaculture. 254, 326-340.

Gagne, F., Blaise, C., Pellerin, J., Pelletier, E. and Strand, J. 2002. Health status of *Mya areneria* bivalves collected from contaminated sites in Canada (Saguenay Fjord) and Denmark (Odense Fjord) during their reproductive period. Ecotoxicology and Environmental Safety. 64, 348-361.

Griscom, S. B., Fisher, N. S. and Luoma, S. N. 2002. Kinetic modeling of Ag, Cd and Co bioaccumulation in the clam *Macoma balthica*: quantifying dietary and dissolved sources. Marine Ecology Progressive Series. 240, 127-141.

Gosling, E. 2003. Bivalve Molluscs, Biology, Ecology and culture. Fishing News Books, Blackwell Publishing. pp. 443.

Gunzler, H. and Williams, A. 2001. Handbook of Analytical Technique. Winnies: Wiley-VCH. pp. 320.

Laruelle, F., Guillou, J. and Paulet, Y. M. 1994. Reproductive pattern of the clams, *Ruditapes decussatus* and *R. phillipinarum* on intertidal flats in Brittany. Journal of Marine Biology Association of United Kingdom. 74, 351-366.

Long, E.R., MacDonald, D.D., Smith, S.L., and Calder, F.D. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environmental Management. 19, 81-97.

Loon, V. 1985. Select Methods of Trace Metal Analysis in Biological and Environmental Samples. New York: John Wiley & Sons. pp 375.

Lowe, D. M. 1988. Alterations in cellular structure of *Mytilus edulis* resulting from exposure to environmental contaminants under field and experimental conditions. Marine Ecology Progressive Series. 46, 91-100.

Mathieu, M. and Lubet, P. 1993. Storage tissues metabolism and reproduction in marine bivalves. A brief review. Invertibrate Reproduction and Development. 23, 123-129.

Meneghetti, F., Moschino, V. and Ros, L. D. 2004. Gametogenic cycle and variations in oocyte size of *Tapes phillippinarum* from the Lagoon of Venice. Aquaculture. 240, 473-488.

Otchere, F.A. 2003. Heavy metal concentrations and burden in the bivalves (*Anadara (Senilia) senilis, Crassostrea tulipa* and *Perna perna*) from lagoons in Ghana: Model to describe mechanism of accumulation/excretion. African Journal of Biotechnology. 2(9), 280-287.

Power, A. J., Nunez, J., Mitchell, M., Walker, R.L., and Sturmer, L. 2004. Reproductive pattern of the blood ark, *Anadara ovalis* from the northeastern coast of Florida. Journal of Shellfish Research. 23, 173-178.

Rodriguez-Rua, A., Prado, M. A., Romeo, Z. and Bruzon, M. 2003. The gametogenic cycle of *Scrobicularia plana* (da costa, 1778) (Mollusca: Bivalvia) in Guadalquivir estuary (Cadiz, SW Spain). Aquaculture. 217, 157-166.

Siah, A., Pellerin, J., Amiard, J. C., Pelleteir, E. and Viglino, M. 2003. Delayed gametogenesis and progesterone levels in soft-shell clams (*Mya arenaria*) in relation to in situ contamination to organotins and heavy metal in the St. Lawrence River (Canada). Comparative Biochemistry and Physiolology. 135, 145-156.

Smaoui-Damak, W., Rebai, T., Berthet, B. and Hamza-chaffai, A. 2006. Does cadmium pollution affect reproduction in the clam *Ruditapes decussates* A one-year case study. Comparative Biochemistry and Physiology. 143, 252-261.

Suwanjarat, J. and Parnrong, S. 1990. Reproductive cycle of *Anadara granosa* L., in Jebilung, Satun Province. Songklanakarin Journal of Science and Technology. 12 (4), 341-351.

Suwanjarat, J. and Thongboon, L. 1997. Reproductive cycle of *Amusium pleuronestes* collected from a fish landing in Songkla. Songklanakarin Journal of Science and Technology. 19 (4), 405-415.

Swennen, C., Moolenbeek, R. G., Ruttanadakul, N., Hobbelink, H., Dekker, H. and Hajisamae, S. 2001. The Molluscs of the Southern Gulf of Thailand. BRT. Bangkok, Thailand. Pp 310.

Tirado, C., Salas, C. and Marquez, I. 2003. Reproduction of *Venus verrucosa* L.,1758 (Bivalvia: Veneridae) in the littoral of Malaga (southern Spain). Fishery Research. 63, 437-445.

Trivino, A.R. and Anadon, N. 2006. Reproductive cycle of the razor clam *Solen Marginatus* (Pultency, 1799) in Spain: A comparative study in three different locations. Journal of Shellfish Research. 25(3), 869-876.

Yu, M. 2005. Soil and Water Pollution-Environmental Metal and Metalloids. In Environmental Toxicology. Florida: CRC Press. U.S.A. pp 185-249.