REPRODUCTIVE CYCLE OF THE RAZOR CLAM Solen regularis DUNKER, 1862 IN THE WESTERN PART OF SARAWAK, MALAYSIA, BASED ON GONADAL CONDITION INDEX

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Abstract: Razor clam or 'ambal' is a highly-priced bivalve collected as food source from several intertidal areas located in western Sarawak, which currently is an unregulated fishery. This study monitored the reproductive cycle of razor clam, *Solen regularis*, collected from the intertidal beaches of Asajaya Laut and Buntal using gonadal condition index (GCI). Sampling was performed at a two-weeks interval or monthly for two years from March 2007 to February 2009. A total of 30 specimens were dissected per sampling. The male gonads appeared beige in colour while female gonads were whitish. Throughout the study, the mean GCI ranged from 0.000 (\pm 0.000) to 0.247 (\pm 0.077) at Asajaya Laut and 0.000 (\pm 0.000) to 0.253 (\pm 0.079) at Buntal. Based on the mean GCI pattern, it is concluded that this razor clams has five stages of reproductive cycle as follows; i) gonadal development, ii) maturation, iii) spawning, iv) spent, and v) resting period. Spawning period for both sites was similar, from end of March-April to September and followed by a short spent stage from end of September-October to November and a resting period from end of October-November to January in the following year. The results obtained in this study could provide important knowledge in determining the spawning season which will benefit razor clam fishery for sustainable management and potential species for aquaculture in future.

KEYWORDS: Reproductive cycle, Gonadal Condition Index, Solen regularis, Asajaya Laut, Buntal

Introduction

Razor clams are soft bottom infaunal marine bivalves with more or less narrow and long shells, gaping at both ends (Cosel, 1990). In Sarawak of East Malaysia, the razor clams under Family Solenidae are commonly known as 'ambal' by the local people. It is one of the famous seafood and commercially sold, not only in the western part of the state but also the northern part. There are three different species of Solenidae razor clams being harvested: i) Solen regularis Dunker, 1862 (ambal biasa), ii) S. lamarckii Deshayes, 1839 (ambal jernang) and iii) S. sarawakensis Cosel, 2002 (ambal riong) (Hung and Ruhana, 2007). Period of razor-clamming season is about five months, starting from October to February coinciding with the occurrence of lowest low tides during the day (Rahim and Tan, 2008) that is linked to the degree of mudflat exposure. Among the three species, *S. regularis* is the most common species collected in the western part of Sarawak because it is widely distributed from the high-tide to the low-tide area, thus making an easy assess for collection by the local people throughout the year (Rahim and Tan, 2008; Rinyod and Rahim, 2009). Razor clams have a great commercial value in the market with a selling price ranging from USD3.50 to USD7.36 per kg (exchange rate USD1.00 = RM3.40), depending on the demand, species and seasons (Ruhana *et al.*, 2007). As a delicacy at seafood restaurants, one kilogram of razor clams sells for USD14.70 to USD17.65 per dish.

Distributions of razor clams in Sarawak are abundant in the intertidal sandy beaches and mudflats of Kuching and Samarahan Division (Pang, 1992). The popular razor-clamming areas

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in Sarawak are Asajaya Laut, Bako, Buntal, Muara Tebas, Moyan Laut, Sambir, Sebandi and Serpan (Rahim and Tan, 2008). Razor clams can also be found in numerous intertidal localities along the west coast of Labuan Territory Federation and sandy beaches of Teluk Mengakong, Pulau Banggi and Kudat, Sabah (Ridzwan, 1993). In Peninsular Malaysia, razor clams clamming-areas are along the mudflats of Kuala Langat, Selangor and Tanjung Lumpur beach, Pahang (Rahim and Tan, 2008).

Currently, collection of razor clams is an unregulated fishery which causes depletion of stocks in its natural habitat (Rahim and Tan, 2008). During the low tide of spring tide in the clamming season, approximately 300 people could be seen harvesting the clams for about three to five hours per day (Rinyod and Rahim, 2009). It was recorded that the density of razor clam S. regularis in Asajaya Laut had decreased by 63.3% from 2005 to 2006 (Rahim and Ruhana, 2009). Several short-period studies on razor-clam reproduction had been conducted (Afzalina, pers. comm.; Diomira, pers. comm.) at Asajaya Laut and Buntal with the usage of gonadal condition index (GCI) as an initial approach to determine the sexual development and to monitor the reproductive cycle. However, data obtained was inadequate because of the short study duration to produce a complete annual reproductive cycle, which is important for conservation and management purposes.

Gonadal condition index (GCI) study was first demonstrated by Darriba *et al.*, (2004) in order to create an appropriate GCI for the study of razor clam *Ensis arcuatus* reproductive cycle in Spain. Later, another study on GCI was also reported on other razor clam species *E. siliqua* (Darriba *et al.*, 2005). Both studies agreed that GCI (based on gonadal weight) is an efficient tool that may prove a valuable technique in fishery management to recognise the reproductive stage of the razor clam. Thus, the present study is uses the same technique in order to study the reproductive cycle of *S. regularis*.

Information on the reproductive cycle will provide important knowledge for restocking

of razor clams in their natural beds. Besides, razor clams provide subsistence income for the local people and could be a potential species for aquaculture. Therefore, further knowledge and information about reproduction of razor clams are needed. This will benefit the razorclam fishery management and aquaculture in the future towards a sustainable natural resource of razor clams in Sarawak. The objectives of this research are to study the reproductive cycle of *S. regularis* at Asajaya Laut and Buntal and to determine the spawning period of the razor clam which will be used as initial information for conservation and management purposes.

Materials and methods

Sampling

Razor clams (Solen regularis) were taken from two selected intertidal sandy beaches and mudflats of Asajaya Laut (N 01° 36' E 110° 36') and Buntal (N 01°42' E110°22'), which are located in the western part of Sarawak (Figure 1). Specimens were collected randomly from the mid-tide area towards the low-tide area of the study sites through traditional method by hiring local people. A maximum number of 30 specimens were collected at the twoweek interval or monthly, starting from early March 2007 to end of February 2009 with 40 and 39 samplings at Asajaya Laut and Buntal, respectively. However, specimens were bought from the local people in early March 2007, middle of November 2007 and early February 2008 at Asajaya Laut. Sampling in June 2007 for both sites, May 2007 for Asajaya Laut and February, August and September 2008 for Buntal were conducted only once due to poor weather conditions. From end of March to Jun 2008, 20 specimens were examined monthly due to the limited availability of specimens.

Gonadal condition index (GCI) study

Live specimens of razor clam were transported back to the laboratory for GCI study. Number of specimens dissected depended on the availability of specimens collected. Only specimens with shell length of about 4 cm or bigger were

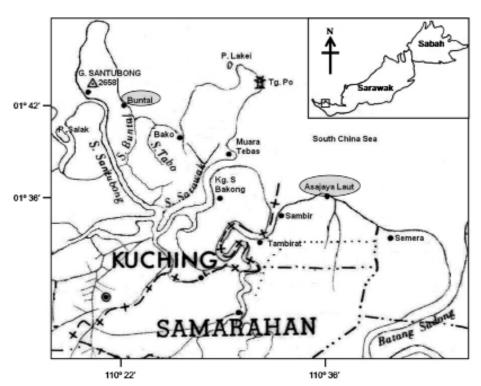


Figure 1. Location of the study sites (highlight in grey colour) Asajaya Laut (N 01° 36' E 110° 36') and Buntal (N 01° 42' E110° 22').

analysed. Total shell length and total wet weight were measured using analogue vernier caliper and digital balance (Setra EL-410S) to the nearest 0.001 cm and 0.001 g, respectively. Gonad was noted for colouration and appearance and removed carefully from the flesh, starting from the digestive gland until the interior foot and weighed. Sexually-undifferentiated individual terms were used to refer to individual where the sexes could not be distinguished due to lack of gonad content. Microscopic observation was done to clarify the sexes using compound microscope (Leica) at 100× and 200× magnification in order to observe the sperm and eggs. The weight of shells dried at 60°C for 3 hours were recorded. All measurements were done in triplicate on the same specimen. GCI was obtained by the formula = Fresh Weight of Gonad Tissue / Shell Dry Weight (Darriba et al., 2004).

Presentation of data and statistical analysis

GCI data were shown using mean and standard deviation (S.D.) as estimators of central trend of the sample as describe by Darriba et al. (2004). Statistical analysis was done using SPSS software version 16 to test normality of the dataset and homogeneity of variance. Since variances were not homogenous, a nonparametric Kruskal Wallis H was used to test significant changes in the mean GCI over the sampling period at each site.

Results and discussion

Sex determination and biometric analysis

The sexes of *S. regularis* cannot be distinguished externally. Therefore, gonad examination through dissection was required. Through macroscopic observation, the gonad appeared in two different colourations where female was whitish with milky texture, while male was beige with granular texture. This observation of *S. regularis* has similar results with other species

of razor clams i.e. Ensis arcuatus (Jeffreys 1865) (Darriba et al., 2004) and E. siliqua (Linnaeus 1758) (Darriba et al., 2005) but differ from the descriptions given for S. marginatus (Pulteney 1799) (Remacha-Triviño and Anadón, 2006). However, these criteria cannot be used in certain months due to the different stages of gonadal development, thus sex clarification needs to be done through microscopic observation where there are presence of sperm and eggs in different individuals.

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A total of 1,134 individuals were sampled from Asajaya Laut and 1,078 individuals from Buntal throughout the two years study period. In Asajaya Laut, individuals analysed were 330 females, 364 males and 440 sexually undifferentiated with percentages of 29.10%, 32.10%, and 38.80%, respectively (Figure 2a). In Buntal, there were 336 females, 344 males and 398 sexually undifferentiated with percentages of 31.17%, 31.91%, and 36.92%, respectively (Figure 2b). In some cases, individuals with invasion of endoparasites in their gonad were placed together in the sexually-undifferentiated state since it was impossible to determine the sex (Figure 2a and 2b). The mean total shell length of the individuals analysed was 6.072 ± 0.977 cm and mean total wet weight was 5.810 ± 1.274 g in Asajaya Laut, while in Buntal it was $5.844 \pm$ 0.565 cm and $4.836 \pm 1.577 \text{ g}$, respectively.

Throughout the study period, the numbers of individuals with the presence of gonad content were more than sexually-undifferentiated individuals. For both study sites, more than 60% of individuals with the presence of gonad content were observed from March until end September 2007, from end January to end October 2008 and end January to end February 2009 with exceptions in June and early September 2007 and early August 2008 at Asajaya Laut while at Buntal it was in early March and early September 2007. For both locations, majority of the individuals were in sexually-undifferentiated state from early October 2007 until early January 2008. Similar trend was observed in the next cycle, although majority of them entered the sexually-undifferentiated stage a month later (November 2008).

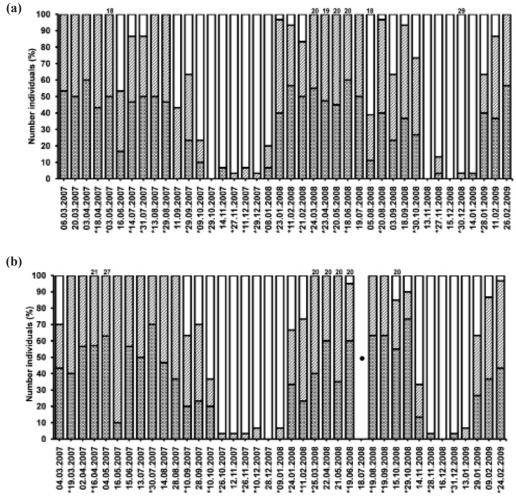
The numbers of individuals with presence of gonad content and sexually undifferentiated were observed to be slightly different at both locations which might be due to the random collection of individuals during samplings. In addition, sexually-undifferentiated presence of individuals (in June 2007 and early August 2008 at Asajaya Laut, while in early March and September 2007 and middle of October 2008 at Buntal) before the resting period would probably result from emptying out of gonad tissues due to spawning event. After spawning, the gonad was restored as the number of individuals with presence of gonad content increased in the next sampling. On the other hand, increment in the number of sexually-undifferentiated individuals from October until early January indicates the end of spawning season where all gametes were

completely released and those individuals had entered their resting stage. Thus, all these can also be indicative of the annual reproductive behaviour of the razor clams over the study period that will be further elaborated in the next section.

Gonadal condition index (GCI) study

Generally, the mean GCI values at both study sites gradually increased from January to March and decreased from September to December. For Asajaya Laut specimens, the mean GCI ranged from 0.000 ± 0.000 to 0.247 ± 0.077 (Figure 3a) while for Buntal specimens it was from 0.000 ± 0.000 to 0.253 ± 0.079 (Figure 3b). The minimum GCI value at Asajaya Laut was recorded at the end of October 2007, November and December 2008 and Buntal was at the end of December 2007 and 2008. On the other hand, the maximum value throughout the study period was recorded at early April 2007 and June 2008 at Asajava Laut and Buntal, respectively.

Based on the observation of mean GCI pattern, it is suggested that razor clams at both locations have five stages of reproductive cycle: i) gonadal development (during the increment of GCI value); ii) maturation (during the maximum GCI values); iii) spawning (indicated by the decreasing in GCI value after the maximum);



Date of sampling

Figure 2. Number of females (\square), males (\square) and sexually undifferentiated (\square) of Solen regularis from (a) Asajaya Laut and (b) Buntal from March 2007 to February 2009. Notes: * indicate the presence of endoparasites in gonad of some individuals; • indicate no sample is available; samples with less than 30 individuals were stated by number.

iv) spent (occurred after last spawning which indicated the end of active phase), and v) resting period (during the minimum GCI values). Over the sampling period, changes in mean GCI values at both study sites were observed to be statistically significant (Asajaya Laut: Kruskal Wallis H = 858.99, df 39, p < 0.001 and Buntal: Kruskal Wallis H = 817.10, df 37, p < 0.001). Therefore, GCI can indicate the different stages of reproductive cycle in *S. regularis*.

Based on the above stages and their descriptions, the gonadal development (stage i) of *S. regularis* at Asajaya Laut and Buntal occurred from end of January until end of March early April which was about three months when the GCI values showed monthly increments. Gonad maturation followed by spawning (stage ii and iii) at Asajaya Laut occurred from early April to September 2007 with maximum GCI in early April 2007 while in the next cycle it was slightly earlier from end March until September 2008 with

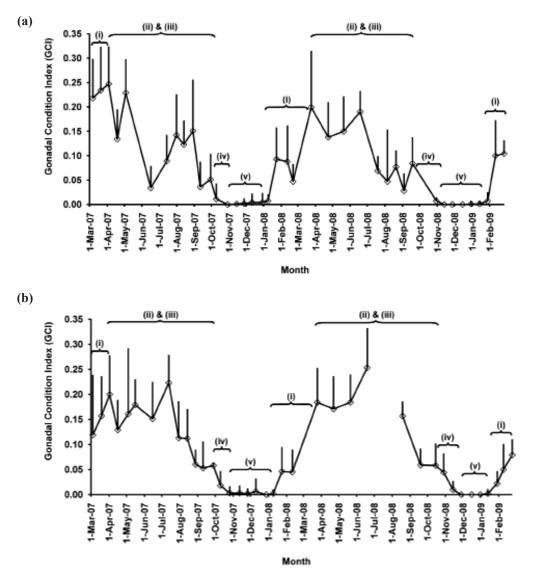


Figure 3. Variation of gonadal condition index (GCI) (mean + S.D.) from March 2007 to February 2009 in males and females of S. regularis at (a) Asajaya Laut and (b) Buntal with five stages of gonadal condition, (i) gonad development, (ii) maturation, (iii) spawning period, (iv) spent and (v) resting period.

maximum GCI in end March. During maturation and spawning stages, large S.D. values in mean GCI were recorded due to spawning event among collected specimens. Similar trend was observed at Buntal but with slight differences in the time of occurrence of GCI maximum (middle of July 2007 and end of June 2008). Maximum values indicated that most individuals were in matured gonadal condition. Sudden drop in the GCI values after the maximum (end of March-April to September) indicated that most individuals had underwent spawning event which occurred in between the six months.

A slight difference was observed in the occurrence of GCI maximum at both study sites, probably due to the high number of individuals with full gonad content during that time. Thus, it is suggested that most of Asajaya Laut individuals mature earlier than Buntal with the evidence of early maximum GCI values. In additions, during the six month spawning period (end of March-April to September) at least three-times the spawning event occurred at both locations after the three GCI maximum, where the GCI values decreased drastically. However, a brief report (Narong, 2007) on four tropical razor clams Solen species, Solen strictus Gould, 1861, S. thailandicus Cosel, 2002, S. corneus Lamarck, 1818 and S. regularis Dunker, 1861 stated that the razor clams were having twice-spawning period during December to April and June to October. Compared to a temperate razor clam E. arcuatus (Darriba et al., 2004), the species has continuous maxima GCI values between December-January to May-June showed a prolonged period of spawning which is similar to the present study for S. regularis (six month) but with different timing. Nevertheless, compared to E. siliqua (Darriba et al., 2005) it has one short spawning period between April to May. The differences in the length of spawning period would probably be due to both genetic and nongenetic adaptations of the species in coordinating their reproductive events with the environment to successfully maximise their reproduction (Newel et al., 1982). In this study, large S.D. values in the mean GCI observed during maturation and spawning stage might be due to the partially-spawned behaviour of the species (Massapina et al., 1999). At both sites, a short period (one month) of spent stage (stage iv) which occurred after last spawning event with continuous decreasing in GCI values indicates the end of spawning period (end September to October). In the next cycle, Asajaya Laut showed similar trend with the previous cycle, but the spent stage for Buntal occurred a half month later than the previous cycle (from middle of October to middle of November). The stage was followed by resting period (stage v) from end of October 2007 which in the next cycle was about one month later until early January in the following year which is about three months.

Although S. regularis is a tropical species, the species does not spawn all year round as in other tropical bivalves like tropical blacklip pearl oyster, Pinctada margaritifera (Pouvreau et al., 2000), fire scallop Lima scabra (Lodeiros and Himmelman, 1999) and cockle Anadara granosa (Pathansali, 1966; Narasimham, 1969; Broom 1983 cited in Broom, 1985). S. regularis did display distinct spawning season or peaks as observed in the GCI values where there were changes occurring throughout the study period with distinct period of minimum GCI values indicative of the resting stage (Figure 3a and 3b). The reproductive stages of S. regularis in this research has similar results with temperate razor clams species E. arcuatus (Darriba et al., 2004) and E. siliqua (Darriba et al., 2005) which stated that minimum GCI value indicated sexual rest while maximum GCI value represented highest percentage of individuals in ripe stage followed by spawning.

GCI can be used to distinguish a phase when the gonad was in a resting period or beginning of gametogenesis, a phase when gonadal development took place, a phase of maturity and spawning, and a phase of exhaustion at the end of the reproductive cycle (Darriba et al., 2005). This index also proved that GCI was highly efficient in distinguishing between the different stages of gametogenic development in E. arcuatus, despite the size of the animal where histology study had been conducted (Darriba et al., 2004). However, based on the present GCI results, there were differences in the timing and gametogenesis of razor clams from different latitudes: for tropical Solen species, S. strictus Gould, 1861, S. thailandicus Cosel, 2002, S. corneus Lamarck, 1818 and S. regularis Dunker, 1861 of Thailand, without specifically mentioning the species, were having twicespawning period during December to April and June to October (Narong, 2007); temperate S. marginatus at three different intertidals in Spain (Eo Estuary, Santander Bay and Terrón Estuary) showed different timing in spawning event which was observed from May to June, June to August and May to July, respectively (Remacha-Triviño and Anadón, 2006); E. arcuatus of northwest Spain has one spawning period which is from December-January to May-June (Darriba et al., 2004), E. siliqua of northwest Spain was from April to May (Darriba et al., 2005); E. siliqua of southern Portugal was from March to May (Gaspar and Monteiro, 1998); Z. acinaces of New Zealand was from September to October (Gribben, 2005); while for E. macha of southern Chile (Avellanal et al., 2002) and of Argentina (Barón et al., 2004), the species possess a continuous gametogenic cycle with no resting period as the razor clam recover quickly after spawning and remain in advanced stages of maturity during the most of the year. This showed that geographical location does influence the reproductive cycle of the razor clams species. Variation on the length of period for each stage could also be due to other environmental factors such as food availability, feeding, temperature, salinity and photoperiod (Remacha-Triviño and Anadón, 2006).

GCI is useful predict to gonadal development, spawning and resting period but with some limitations to monitor the exact stages in gametogenic development. Grant and Tyler (1983) stated that, an histological study of the sexual cycle should be done in order to identify each of the development stages more specifically and pinpoint the most likely periods for gamete release. Besides, histological technique is also the most reliable method for determining the seasonal reproductive development and spawning patterns of infaunal bivalves (Gribben, 2005).

Conclusion

The reproductive cycle of *S. regularis* at Asajaya Laut and Buntal, Sarawak is an annual cycle. They spawned at least three times during the spawning period from end of March-April to September followed by a resting period from end of October-November to January in the next year. It is very fortunate that the razor-clamming period does not coincide with the spawning season of *S. regularis* because the clams are being collected during their resting period. However, action should be taken regarding the pressure of fishing. Further study on the histological aspect

of *S. regularis* gonad tissues is needed in order to determine the accurate stages of gonadal development.

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