STUDY ON REPRODUCTION OF RAZOR CLAM, *Solen regularis* DUNKER, 1862 (BIVALVIA: SOLENIDAE) IN THE WESTERN PART OF SARAWAK, MALAYSIA

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Declaration

I declare that, except as acknowledgement in the text, the work presented in the thesis is entirely my own work and has not been submitted, either in part or in whole for a degree at this or any other university.

Sincerely,

Aileen May anak Ridis Rinyod.
Dedicated to my dearest beloved parents and family members...
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Aileen May anak Ridis Rinyod
ABSTRACT

Razor clam or ‘ambal’ is a highly priced marine bivalve collected using traditional methods from several intertidal sandy beaches and mudflats by local people of Sarawak. ‘Ambal’ of Sarawak belongs to Family Solenidae with three different species namely i) Solen regularis Dunker, 1862, ii) S. lamarckii Chenu, 1843 and iii) S. sarawakensis von Cosel, 2002. In this study, the reproduction of S. regularis was monitored for a duration of two years (March 2007 until February 2009) at Asajaya Laut and Buntal, located in the western part of Sarawak. Samples were taken at a two-week interval or monthly with in-situ physico-chemical parameters of sea water (temperature, dissolved oxygen, pH and salinity) measurement and analysis of chlorophyll-a concentration in the water. This study has two main parts which were gonadal condition index (GCI) study and histological study on the gonad. The objectives of this study were to determine the reproductive cycle of S. regularis, to observe the possible effect of environmental factors on the clam’s reproduction and to elucidate the stages of gonadal development through drawing up a gametogenic scale using histological methods.

GCI study was monitored for 24 months (March 2007 to February 2009) as an initial approach to sexual development through monitoring the reproductive cycle of the razor clam and to observe the effect of environmental factors towards the cycle. A maximum of 30 specimens were dissected from each sampling for sex identification and GCI. The male gonad appeared beige in colour while female gonad is whitish. Throughout the study, the mean GCI ranged from 0.000 to 0.247 (± 0.077) at Asajaya Laut and 0.000 to 0.253 (± 0.079)
at Buntal. Based on the mean GCI pattern, the reproductive cycle for both study sites showed similar pattern with five stages as follows; i) gonadal development (during the increment of GCI value) ii) maturation (during the maxima GCI value), iii) spawning (indicated by the decreasing GCI value after the maxima), iv) spent (occurred after the last spawning which indicate the end of active phase of reproduction) and v) resting period (during the minima GCI value). Spawning period was observed from end of March-April to September and followed by a resting period from end of October to January in the following year. Throughout the study, there were slight differences in the timing of the reproductive cycle stages occurred at each site whereas only at Buntal that chlorophyll-a showed moderate correlation with the mean GCI ($r = 0.513, p = 0.001$).

The gametogenic cycle was determined using histological methods together with GCI data. In Asajaya Laut, study was carried out starting from February 2008 until February 2009 while the study in Buntal started one month later. Results showed that the histological structures of males and females S. regularis gametes can be characterized into seven gametogenic cycle stages as follows; i) sexual rest (Stage 0), ii) start of gametogenesis (Stage I), iii) advance gametogenesis (Stage II), iv) ripe (Stage IIIA), v) spawning (Stage IIIB), vi) restoration (Stage IIIC) and vii) spent (Stage IV). The result of histological study validates the GCI findings. It was noted that during the maxima GCI value, majority of the individuals were in Stage III while during the minima GCI value, they were in their sexual rest (Stage 0). At both locations, S. regularis populations were in their Stage 0 from November to December and gametogenesis (Stage I) began in January. S. regularis showed prolonged
spawning activity indicated by their active phase of reproduction (Stage IIIA, IIIB and IIIC) from February to September and January to October for Asajaya Laut and Buntal, respectively. High percentage of razor clam individuals entered their spent stage in October for both sites which complete the whole gametogenic cycle. However, towards the end of spawning period and early part of the gametogenesis, individuals collected within the same month did not show a uniform gametogenic cycle stage.

Infestation of endoparasites in the gonadal cavity was also observed in *S. regularis* involving two types of endoparasites (nematodes and trematode). However, detail investigation was not carried. Therefore, study on the infections of those endoparasites is crucial in order to investigate the effect of the endoparasites infection to the razor clam reproduction and food safety status for human consumption.

The outcomes of this study had provided valuable information on the reproduction of *S. regularis*. Findings from this can be used for the conservation purpose, establishment of sustainable razor clam fishery management and for future aquaculture in Sarawak.

Keywords: *Solen regularis*, Gonadal Condition Index (GCI), histological, reproductive and gametogenic cycle, Asajaya Laut, Buntal.
KAJIAN TENTANG PEMBIAKAN AMBAL *Solen regularis* DUNKER, 1862 
(BIVALVIA: SOLENIDAE) DI BAHAGIAN BARAT SARAWAK, MALAYSIA

Di Sarawak, ambal adalah sejenis bivalvia marin yang mempunyai nilai 
pasaran yang tinggi, dikutip secara kaedah tradisional oleh penduduk tempatan 
di beberapa pesisir pantai berpasir dan berlumpur. Terdapat tiga spesies ambal 
dari Famili Solenidae iaitu i) *Solen regularis* Dunker, 1862, ii) *S. lamarckii* Chenu, 1843 dan iii) *S. sarawakensis* von Cosel, 2002. Dalam kajian ini, 
pembiakan *S. regularis* telah dipantau untuk tempoh dua tahun (Mac 2007 
hingga Februari 2009) di Asajaya Laut dan Buntal yang terletak di bahagian 
barat Sarawak. Sampel dikutip pada selang tempoh dua minggu atau sebulan 
sekali dan bacaan parameter fiziko-kimia in-situ air laut (suhu, oksigen terlarut, 
P&H dan kemasinan) dan analisis kepekatan klorofil-a untuk sampel air turut 
direkodkan. Kajian ini mempunyai dua bahagian utama iaitu berdasarkan 
Indek Kondisi Gonad (GCI) dan histologikal gonad. Objektif kajian ini adalah 
untuk mengetahui kitar pembiakan *S. regularis*, mengkaji adakah faktor 
persekitaran mempengaruhi pembiakannya dan memperincikan peringkat 
perkembangan gonad secara skala gametogenik menggunakan kaedah 
histologikal.

Kajian GCI telah dilakukan selama 24 bulan (Mac 2007 hingga Februari 
2009) sebagai langkah permulaan untuk perkembangan pembiakan dengan 
memantau kitar pembiakan ambal dan kesan persekitaran yang 
mempengaruhinya. Sebanyak 30 spesimen telah dibedah pada setiap 
pensampelan untuk identifikasi jantina dan GCI. Gonad jantan kelihatan
berwarna perang pasir manakala gonad betina pula keputihan. Sepanjang kajian dijalankan, nilai min GCI adalah dalam julat 0.000 hingga 0.247 (± 0.077) di Asajaya Laut dan dari 0.000 hingga 0.253 (± 0.079) di Buntal. Berdasarkan corak nilai min GCI, kitar pembiakan ambal di kedua-dua lokasi menunjukkan corak yang serupa dengan lima peringkat iaitu, i) pembentukan dan perkembangan gonad (semasa peningkatan nilai GCI), ii) kematangan gonad (semasa nilai GCI maksima), iii) pelepasan gamet dari gonad yang matang (semasa penurunan nilai GCI selepas maksima), iv) pelepasan sisa gamet dari gonad yang matang (semasa penurunan berterusan nilai GCI yang menunjukkan berakhirnya fasa aktif pembiakan), v) tempoh berehat (nilai GCI paling minima). Pelepasan gamet dari gonad yang matang dijangka bermula dari hujung Mac-April hingga September dan diikuti oleh tempoh berehat dari hujung Oktober hingga Januari pada tahun berikutnya. Sepanjang tempoh kajian, terdapat sedikit perbezaan dari segi tempoh berlakunya peringkat kitar pembiakan di setiap lokasi di mana hanya di Buntal kepekatan klorofil-a mempunyai korelasi sederhana terhadap nilai min GCI ($r = 0.513, p = 0.001$).

Kajian kitar gametogenik ditentukan secara kaedah histologikal bersama GCI. Di Asajaya Laut, kajian bermula dari Februari 2008 hingga Februari 2009 manakala kajian di Buntal bermula lewat sebulan. Keputusan menunjukkan bahawa struktur histologikal untuk gamet jantan dan betina $S. regularis$ boleh dicirikan kepada tujuh peringkat kitar gametogenik iaitu i) rehat seksual (Peringkat 0), ii) permulaan gametogenesis (Peringkat I), iii) gametogenesis lanjutan (Peringkat II), iv) kematangan (Peringkat IIIA), v) perlepasan gamet matang (Peringkat IIIB), vi) pemulihan (Peringkat IIIC) dan vii) kemerosotan (Peringkat IV). Hasil kajian histologikal telah mengesahkan hasil kajian GCI.
Didapati bahawa semasa nilai GCI maksima, kebanyakkan individu adalah dalam Peringkat III manakala ketika nilai GCI minima, majoriti berada dalam rehat seksual (Peringkat 0). Dari November hingga Disember, populasi *S. regularis* di kedua-dua lokasi berada dalam Peringkat 0 dan dari Januari, permulaan gametogenesis (Peringkat I) bermula. *S. regularis* mempunyai tempoh aktiviti perlepasan gamet yang panjang (Peringkat IIIA, IIIB dan IIIIC) iaitu dari Februari hingga September di Asajaya Laut manakala dari Januari hingga Oktober di Buntal. Pada Oktober, populasi di kedua-dua lokasi mula didapati berada di peringkat kemerosotan (Peringkat IV). Walau bagaimanapun, pada penghujung musim pembiakan dan peringkat awal gametogenesis berlaku, individu-individu yang dikutip dalam bulan yang sama tidak menunjukkan peringkat kitar gametogenesis yang seragam.

Penemuan tentang jangkitan dua jenis endoparasit (nematod dan trematod) pada kaviti gonad *S. regularis* turut diperhatikan. Walau bagaimanapun, kajian yang mendalam tidak dilakukan. Maka, kajian lanjut untuk menyiasat kesan jangkitan endoparasit ini kepada pembiakan ambal dan status keselamatannya bagi pemakanan manusia adalah amat perlu.

Hasil daripada keseluruhan kajian ini telah memberikan maklumat yang amat berguna tentang pembiakan *S. regularis*. Hasil kajian ini harus digunapakai untuk tujuan pemuliharaan ambal, pembentukan pengurusan perikanan ambal yang mampun dan bagi tujuan akuakultur di Sarawak pada masa hadapan.

*Kata kunci: Solen regularis, Index Kondisi Gonad (GCI), histologikal, kitar pembiakan dan gametogenik, Asajaya Laut, Buntal*
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**Figure 5.10** Monthly oocytes diameter (µm) (mean ± S.D) of *S. regularis* with gonadal condition index values for (a) both males and females and (b) females only obtained from Asajaya Laut. OD = mean oocytes diameter.

**Figure 5.11** Monthly oocytes diameter (µm) (mean ± S.D) of *S. regularis* with gonadal condition index values for (a) both males and females and (b) females only obtained from Buntal. *Notes: ● = no sample; OD = mean oocytes diameter.

**Figure 6.1** Photomicrographs of nematodes (*Gnathostoma* sp.) found in the gonadal cavity tissues of *S. regularis* taken from Asajaya Laut (a-d) and Buntal (e-h). Scale bar = 100 µm.

**Figure 6.2** Photomicrograph of cross sections in healthy *S. regularis*, (a) male gonadal tissue taken from Asajaya Laut (February 2008) and (b) female gonadal tissue (April 2008); Replacement of almost all gonad content (oocytes (Oc) still can be observed) by the trematode sporocyst (S) taken from (c) Asajaya Laut (March 2008) and (d) Buntal (October 2008); Replacement of gonad content by the trematode sporocyst (S) taken from (e) Asajaya Laut (May 2008) and (f) Buntal (June 2008). Scale bar a, b, d, f = 50 µm; c, e = 100 µm.
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CT</td>
<td>interfollicular connective tissues</td>
</tr>
<tr>
<td>DO</td>
<td>dissolved oxygen</td>
</tr>
<tr>
<td>Fo</td>
<td>follicles</td>
</tr>
<tr>
<td>GCI</td>
<td>gonadal condition index</td>
</tr>
<tr>
<td>GE</td>
<td>germinal epithelium</td>
</tr>
<tr>
<td>GSz</td>
<td>group of spermatozoa</td>
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<tr>
<td>IOc</td>
<td>immature oocytes</td>
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<tr>
<td>MOc</td>
<td>small mature oocytes</td>
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<tr>
<td>MT</td>
<td>muscular tissue</td>
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<tr>
<td>NC</td>
<td>nutritive cells</td>
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<tr>
<td>OD</td>
<td>oocytes diameter</td>
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<tr>
<td>Og</td>
<td>oogonia</td>
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<tr>
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<td>primary oocytes</td>
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<tr>
<td>ROc</td>
<td>residual oocytes</td>
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<tr>
<td>RG</td>
<td>residual gametes</td>
</tr>
<tr>
<td>RSz</td>
<td>residual spermatozoa</td>
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<tr>
<td>Sc</td>
<td>spermatocytes</td>
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<tr>
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<tr>
<td>SOc</td>
<td>secondary oocytes</td>
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<tr>
<td>St</td>
<td>spermatid</td>
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<tr>
<td>TSL</td>
<td>total shell length</td>
</tr>
<tr>
<td>TWW</td>
<td>total wet weight</td>
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CHAPTER 1

INTRODUCTION

1.1 Background research

Phylum Mollusca is the second largest phylum in the animal kingdom (Hyman, 1967) which includes at least 50,000 species and as many as 120,000 living species (Pechenik, 2010). They are among the most abundant and observable groups of marine animals because they have adapted to all major marine habitats (Sumich and Morrissey, 2004). The phylum consists of seven classes which are Aplacophora, Polyplacophora, Monoplacophora, Gastropoda, Cephalopoda, Scaphopoda and Pelecypoda (Bivalvia or Lamellibranchia) (Adiyodi and Adiyodi, 1983; Pechenik, 2010). Among these classes, Bivalvia consists about 15,000 contemporary species which includes many species of clams, oysters, mussels and scallops (Pechenik, 2010). Most of the bivalves are marine with only 10 to 15 percent can be found in freshwater and no bivalves are terrestrial (Pechenik, 2000).

Razor clam or commonly known as ‘ambal’ by local people in Sarawak is one of the well known bivalve seafood especially in the western part of the state. There are three different species of razor clams that belong to Family Solenidae namely i) Solen regularis Dunker, 1862 (Ambal Biasa/ Ambal Kapur), ii) S. lamarckii Chenu, 1843 (Ambal Jernang/ Ambal Merah) and iii) S. sarawakensis von Cosel, 2002 (Ambal Riong/ Ambal Penguris). The third one is most probably a new endemic species in Borneo (Hung and Ruhana, 2007). They could be
distinguished vividly based on their distinctive arrangements of adductor muscle scars, pallial muscle scars, and foot colouration (Ruhana and Hung, 2006; Hung and Ruhana, 2007).

Razor clams (*Solen* spp.) are found abundantly in the intertidal mudflats and sandy beaches of Kuching and Samarahan Divisions (Pang, 1992) namely at Asajaya Laut, Buntal, Bako, Muara Tebas, Moyan Laut, Sambir, Sebandi and Serpan (Rahim and Tan, 2008). In addition, razor clams have a great commercial value in Sarawak local market with price ranging from RM12.00 to RM20.00 (USD3.79 to USD6.33 with exchange rate USD1.00 = RM3.16) per kg depending on the demand, species and seasons (Ruhana et al., 2007; Rinyod and Rahim, 2009). Compared to 15 years ago, the price was RM7.00 per kg (Pang, 1994) and now had increased by RM5.00 to RM13.00 per kg. As a delicacy at seafood restaurants, one kilogram of razor clams was sold for RM50.00 to RM60.00 per dish.

In Kuching major markets outlet, razor clam was first marketed in the early seventies and razor clam fishery in Sarawak recorded high annual landing about 80 to 100 metrics tonnes in 1991/1992 and 1992/1993 seasons, respectively (Pang, 1994). During the 1991 to 1992 production, the highest collection of razor clam was about 2.5 metric tonnes in November 1991 but the production decreased in the next months onwards due to substantial decline in total effort level which was due to rumours about the usage of pesticides for collecting razor clam (Pang, 1994). The current status of the market demand on razor clam is still high with increment in price.

Besides Sarawak, razor clams can also be found in numerous intertidal localities along the west coast of Peninsular Malaysia, Labuan Territory
Federation and sandy beaches of Teluk Mengakong, Pulau Banggi and Kudat, Sabah (Ridzwan, 1993). As reported in Sabah Fisheries Annual Report 1983, about 5.5 metrics tonnes of razor clams (Solen spp.) were landed in Sabah fisheries market (Ridzwan, 1993). In Peninsular Malaysia, razor clam can be found along the mudflats of Kuala Langat, Selangor and Tanjung Lumpur beach, Pahang (Rahim and Tan, 2008).

The harvesting season of razor clam in Sarawak is about eight months starting from end of August to early March in the following year which is related to the occurrence of lowest low tide of spring tides during the day time (Ruhana et al., 2007; Rahim and Tan, 2008). They were collected by the local people using traditional method where the surface of mudflat is tapped with a wooden stick which has one sharpened end ('penugal', about 1 m in length) in order to detect the razor clam burrow (Rahim and Tan, 2008). Once the burrow was detected, a slender, elongated stick ('lidi' about 15 cm in length) coated with a paste made of salt, ash and slaked lime will be inserted into the burrow. The paste that acts as an irritant to the razor clams will force them to come out from their burrows (Rinyod and Rahim, 2009).

Among the three species, S. regularis was chosen in this study because it is the most common species being collected from the intertidal sandy beaches and mudflat of Asajaya Laut and Buntal throughout the clamming season (Rahim and Tan, 2008). Besides, it has a wide distribution across the mudflat which is from middle tide area towards the low tide area where another two species inhabit (Rinyod and Rahim, 2009). Due to this reason, the species can be easily accessed and collected throughout the whole year.