BIOLOGY AND DISTRIBUTION OF PEN SHELL (BIVALVIA: PINNIDAE) IN SELECTED AREAS OF PENINSULAR MALAYSIA

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DOCTOR OF PHILOSOPHY
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(BIVALVIA: PINNIDAE) IN SELECTED AREAS OF
PENINSULAR MALAYSIA

MOHD HANAFI BIN IDRIS

Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the Requirement for the
Degree of Doctor of Philosophy

July 2009
DEDICATION

I dedicated this work to my lovely wife, Mehpuzah Salim who has sacrificed so much for me to achieve my goal

To the memory of my late father and mother who are no longer to share with me for this moment

To my brothers and sisters

and

To all my friends who supported me all those past years that made me whom I am today is very much acknowledged
A study on taxonomy, biology and ecology of pen shells were conducted in Merambong shoal off the south western state of Johor, Peninsular Malaysia. Several pen shells specimens were also acquired from other sites in Peninsular Malaysia to allow wider scope of sample collection. The study was conducted from August 2005 to April 2007. The site was chosen due to ample abundance of pen shells that live associatedly with the seagrass. A total of seven species have been recorded from the study areas comprising five species of *Pinna* and two species of *Atrina*. Ten internal and external morphological characteristics have been used for the taxonomic identification of pen shells. *Pinna* species showed that of the ten characteristics analyzed, four characteristics were highly significant (P<0.01) (WL, DPML, PAMPDNL and WS). Similar result of four morphometric characteristics showed a highly significant (P<0.01) between the characters of three closely related species, *Pinna bicolor*, *P. deltodens* and *P. atropurpurea*. 
Adductor muscle tissue used for the isolation of DNA and RAPD successfully detected polymorphisms in the pen shells populations. The result showed 19 primers have produced various banding patterns and thus provided sufficient information for reliable discrimination of the analyzed samples. The results indicated that the primers used generated a total of 160 fragments with 70% to 100% of polymorphic fragments. The genetic distance among these seven species of pen shells was in the range of 0.0197 to 0.3190. The dendrograms constructed from RAPD markers data were able to reveal the relationships between the pen shells populations.

*Enhalus acoroides, Halophila ovalis, Halophila minor, Cymodocea serrulata* and *Thalassia hemprichii* were among the seagrasses associated with pen shells habitat in Merambong and Tanjung Adang shoals. However, pen shells can also be found in stony sand area of Merambong Island and live associated with zoanthid, *Zoanthus pulchellus*. The sediment types from four study areas were classified as sandy loam. Monthly in situ physico-chemical seawater parameters recorded showed no significant different (P>0.05) during the study period. Pen shell were recorded and classified into three classes of distribution i.e clumped, random and rare. Higher density was shown by *P. bicolor* in Merambong shoal (1) with the value of 0.83 ind/m² while *P. incurva* showed lower density with 0.03 ind/m² in Tanjung Adang shoal. *Pinna bicolor, P. deltodes* and *P. atropurpurea* were consistently found in these four study areas while, *P. deltodes* Menke and *P. incurva* Gmelin were both a new distribution record for Sungai Pulai seagrass beds. Merambong shoal population recorded higher diversity and richness as compared to the Tanjung
Adang shoal and Merambong Island, but the value of evenness was similar between Merambong shoal, Tanjung Adang shoal and Merambong Island. Five major phyla comprising 37 species of fouling organisms were recorded. Members from phylum Crustacea and Mollusca were higher in percentage of distribution when compared to the Annelida, Echinodermata and Chordata. Phylum Mollusca showed the highest diversity while phylum Annelida was recorded the highest species richness. A symbiotic adult alpheid shrimp, *Synalpheus carinatus* was recorded inhabiting the mantle cavity of the pen shells.

*Pinna bicolor* reaches sexual maturity at shell length of 170 mm. *Pinna bicolor* is dioecious and no hermaphrodite individual was found during the study period. Five stages of gonad development were observed and clearly been identified. *Pinna bicolor* showed a clear spent phase in the month of October 2006, December 2006 and March 2007 while the developing and spawning phases were observed throughout the whole study period. Monthly *in situ* physico-chemical parameters and rainfall recorded during the study did not significantly correlated with the reproductive activity of *P. bicolor* in Merambong Shoal. Pearson Correlation analysis also did not show any significant correlation between gonad index (GI) and physico-chemical parameters of seawater in Merambong shoal.

Growth rate in natural habitat has been found to be indeterminate and rapid when compared to *P. bicolor* in culture tank. For the length-weight relationships, the growth coefficient “b” was found to be significantly higher
than the isometric value (3.111) at 5% level and this is an indication of isometric growth in *P. bicolor* from Merambong shoal. The adductor muscle of *A. vexillum* was found the biggest in size when compared to other species.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

BIOLOGI DAN TABURAN SIPUT KIPAS (BIVALVIA: PINNIDAE) DI KAWASAN TERPILIH DI SEMENANJUNG MALAYSIA

Oleh

MOHD HANAFI BIN IDRIS

Julai 2008

Pengerusi : Profesor Madya Aziz Bin Arshad, PhD
Fakulti : Sains

Tisu otot pengatup yang digunakan bagi pengasingan DNA dan RAPD telah berjaya mengenalpasti polimorfik bagi populasi siput kipas. Keputusan mendapati 19 primer, telah menghasilkan corak ban yang pelbagai dan memberikan maklumat yang mencukupi untuk menjalankan analisis perbezaan. Keputusan mendapati primer yang telah digunakan menghasilkan sejumlah 160 sektor dengan 70% hingga 100% sektor polimorfik. Jarak genetik bagi tujuh spesis siput kipas adalah di antara 0.0197 hingga 0.3190. Pembentukan kelompok daripada data penanda RAPD memberikan petunjuk perhubungan di antara setiap populasi siput kipas.

*Enhalus acoroides, Halophila ovalis, Halophila minor, Cymodocea serrulata* dan *Thalassia hemprichii* merupakan rumput laut yang hidup bersama dengan siput kipas di beting Merambong dan beting Tanjung Adang. Walau bagaimanapun, siput kipas juga boleh dijumpai di kawasan pasir berbatu di Pulau Merambong yang hidup bersama zoanthid, *Zoanthus pulchellus*. Profil sedimen mendapati empat kawasan kajian dikelasifikasikan sebagai pasir berlumpur. Parameter fizikal-kimia air laut telah direkodkan secara rawak pada setiap bulan dan tiada perbezaan nyata (P>0.05) didapati sepanjang kajian dijalankan. Siput kipas yang direkodkan dikelasifikasikan kepada tiga bentuk taburan iaitu berkelompok, rawak dan jarang-jarang. Densiti tertinggi direkodkan oleh *P. bicolor* di beting Merambong (1) dengan nilai 0.83 ind/m² manakala *P. incurva* merekodkan densiti terendah iaitu 0.03 ind/m² di beting Tanjung Adang. *Pinna bicolor, P. deltodes* dan *Pinna atropurpurea* merupakan spesies yang sentiasa dijumpai di setiap kawasan kajian manakala, *P. deltodes* dan *P. incurva* merupakan taburan baru yang...

Kadar tumbesaran *P. bicolor* di kawasan semulajadi didapati pantas dan tidak tetap jika dibandingkan dengan yang diterukan di dalam tangki. Bagi perhubungan panjang-berat, angkali tumbesaran “b” didapati sangat nyata berbanding nilai isometrik (3.111) pada kadar 5% dan ini merupakan penanda aras pertumbuhan isometrik *P. bicolor* di beting Merambong. Saiz otot pengatup *A. vexillum* merupakan yang terbesar dibandingkan dengan spesies yang lain.
ACKNOWLEDGEMENTS

In the Name of ALLAH, The Merciful Benefactor, The Merciful Redeemer.
Praise goes to ALLAH Almighty for I am blessed with strength and ardour to finally accomplish this thesis.

I would like to express my deepest gratitude to my supervisor Assoc. Prof. Dr. Aziz Arshad for his guidance, and assistance during this study. Also, my sincere thanks to my committee members Professor Dr. Japar Sidik Bujang, Professor Dr. Mazlan Abdul Ghaffar and Assoc. Prof. Dr. Siti Khalijah Daud for all the advices given towards the completion of my study.

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Lastly, to my wife and family for their encouragement, supports, patience, understanding and faith on me.
I certify that a Thesis Examination Committee has met on July 16, 2009 to conduct the final examination of Mohd Hanafi Idris on his thesis entitled "Biology and Distribution of Pen Shells (Bivalvia:Pinnidae) in Selected Areas of Peninsular Malaysia" in accordance with the Universities and University Colleges Act 1971 and the constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

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Date:
This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the supervisory Committee were as follows:

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Date: 11 September 2009
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

MOHD HANAFI BIN IDRIS

Date: 11 August 2009
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Mean and standard deviation of seawater temperature (°C) from Merambong shoal and aquarium tank from year 2005 to 2007</td>
<td>59</td>
</tr>
<tr>
<td>3.2</td>
<td>Mean and standard deviation of salinity (psu) from Merambong shoal and aquarium tank from year 2005 to 2007</td>
<td>60</td>
</tr>
<tr>
<td>3.3</td>
<td>Mean and standard deviation of dissolved oxygen (mg/l) from Merambong shoal and aquarium tank from year 2005 to 2007</td>
<td>61</td>
</tr>
<tr>
<td>3.4</td>
<td>Mean and standard deviation of hydrogen ion concentration (pH) from Merambong shoal and aquarium tank from year 2005 to 2007</td>
<td>62</td>
</tr>
<tr>
<td>3.5</td>
<td>Mean and standard deviation of conductivity (mS/cm) from Merambong shoal and aquarium tank from year 2005 to 2007</td>
<td>63</td>
</tr>
<tr>
<td>4.1</td>
<td>Morphometric data all taken accordance to Scheltema (1983) and (*) additional measurement used in the present study</td>
<td>74</td>
</tr>
<tr>
<td>4.2</td>
<td>Percentage occurrence of pen shells existing from study areas</td>
<td>78</td>
</tr>
<tr>
<td>4.3</td>
<td>Comparative descriptions of <em>Pinna</em> and <em>Atrina</em> existing in study areas</td>
<td>108</td>
</tr>
<tr>
<td>4.4</td>
<td>Range and mean ± standard deviation of morphometric characteristics in five populations of <em>Pinna bicolor</em></td>
<td>111</td>
</tr>
<tr>
<td>4.5</td>
<td>Range and mean ± standard deviation of morphometric characteristics in three populations of <em>Pinna muricata</em></td>
<td>111</td>
</tr>
<tr>
<td>4.6</td>
<td>Range and mean ± standard deviation of morphometric characteristics in two populations of <em>Pinna incurva</em></td>
<td>112</td>
</tr>
<tr>
<td>4.7</td>
<td>Range and mean ± standard deviation of morphometric characteristics in three populations of <em>Pinna deltodes</em></td>
<td>112</td>
</tr>
<tr>
<td>4.8</td>
<td>Range and mean ± standard deviation of morphometric characteristics in four populations of <em>Pinna atropurpurea</em></td>
<td>113</td>
</tr>
</tbody>
</table>
4.9 Range and mean ± standard deviation of morphometric characteristics in four populations of *Atrina pectinata* 113

4.10 Range and mean ± standard deviation of morphometric characteristics in two populations of *Atrina vexillum* 114

4.11 Range and mean ± SD of morphometric data of *Pinna* and *Atrina* 115

4.12 Variables and species population of *Pinna* and *Atrina* for proportion characters to total length (TL) 118

4.13 Value of first four components obtained through a PCA performed on proportions morphometric data of *Pinna* and *Atrina* 123

5.1 Sequences and CG content of Opreon RAPD 10-mer Kit A primers used 144

5.2 List of decamers used as arbitrary primers 149

5.3 Percentage of polymorphic loci and locus for each species and locations 150

5.4 Matrix of genetic distances among 19 populations of pen shell from Peninsular Malaysia. Abbreviation: Pa (MS)=*Pinna atropurpurea* (Merambong shoal); Pb (MS)=*P. bicolor* (Merambong shoal); Av (MS)=*Atrina vexillum* (Merambong shoal); Pm (MI)=*P. muricata* (Merambong Island); Ap (TAS)=*A. pectinata* (Tanjung Adang shoal); Ap (BP)=*A. pectinata* (Bagan Panchor); Pi (TAS)=*A. incurva* (Tanjung Adang shoal); Pb (TAS)=*P. bicolor* (Tanjung Adang shoal); Pa (MI)=*P. atropurpurea* (Merambong Island); Pd (MI)=*P. deltodes* (Merambong Island); Pd (MS)=*P. deltodes* (Merambong shoal); Pa (TAS)=*P. atropurpurea* (Tanjung Adang shoal); Pa (PT)=*P. atropurpurea* (Pulau Tinggi); Pb (PT)=*P. bicolor* (Pulau Tinggi); Pm (ML)=*P. muricata* (Merchang Lagoon); P sp (ML)=*Pinna* sp (Merchang Lagoon). 154

6.1 Classes of dominance used to record cover 177

6.2 Mean density (±SD) (N/m²) and variance of pen shells from study areas (N equivalent to number of individual/m²) 182

6.3 List of seagrasses, seaweeds and zoanthid and their percentage covers at study areas 190

6.4 Fouling organism’s communities: species frequency, mean abundance and dominance values 194
6.5 Univariate analysis of fouling organisms recorded from living pen shells and groups based on phylum categories

7.1 Stepwise dehydration with a series of alcohol and subsequent paraffin wax infiltration

7.2 Step-by-step staining procedure as practiced at the Anatomy Laboratory at Department of Biology

7.3 Monthly gonad stages for male and female of *P. bicolor* in Merambong shoal

7.4 Pearson Correlation analysis of gonad index (GI) of *P. bicolor* with different physico-chemical parameters of seawater in Merambong shoal

8.1 Length-length relationships between total length (TL), width length (WL), nacreous length (NL) and posterior adductor muscle diameter (PAMD) of *P. bicolor* from Merambong shoal

8.2 Length-length relationships between total length (TL) and posterior adductor muscle diameter (PAMD) of different species of pen shells from Merambong shoal

8.3 Average increase in shell length (mm/month) for 1 year old *Pinna bicolor, P. nobilis* and *P. rugosa* in this study and other studies

8.4 Previously published values of the coefficient “a” and “b” from *Pinna bicolor* and other bivalve from various locations

8.5 Range size of adductor muscle and total length of seven pen shells species in Merambong shoal

1A Result of one way ANOVA for physico-chemical parameters of seawater in Merambong shoal; (A) temperature, (B) salinity, (C) dissolved oxygen, (D) pH and (E) conductivity

2A Result of one way ANOVA for physico-chemical parameters of seawater in aquarium tank; (A) temperature, (B) salinity, (C) dissolved oxygen, (D) pH and (E) conductivity

1B Particle size distribution from Merambong shoal

2B Particle size distribution from Tanjung Adang shoal

3B Particle size distribution from Merambong Island, Johor
<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>4</td>
</tr>
<tr>
<td>2.0</td>
<td>14</td>
</tr>
<tr>
<td>2.1</td>
<td>26</td>
</tr>
<tr>
<td>3.0</td>
<td>45</td>
</tr>
<tr>
<td>3.1</td>
<td>50</td>
</tr>
<tr>
<td>3.2</td>
<td>64</td>
</tr>
<tr>
<td>4.1</td>
<td>72</td>
</tr>
<tr>
<td>4.2</td>
<td>75</td>
</tr>
</tbody>
</table>

1.0 Annual changes of pen shell fishing production in Japan coastal Waters

2.0 Semi diagrammatic drawing of the anatomy of *Atrina* (A) and *Pinna* (B). The right half shows only the muscles and characteristic features of the mantle (from Johnsonia, vol.3, no. 38, pl. 152 in Rosewater, 1961 (A) and Winckworth (1929 (B). Abbreviation : m.g-mantle gland; c.c-Ciliated channel; a-anus; g-Outer demibranch of left gill; p.c-Pericardial sinus; b.r-Byssal retractor; p.a-Poterior adductor; h-heart; v.m-Visceral mass (gonad); k-Kidney; i-Intestine; l.p-Labial palp; l-Liver; s.v-Stomach valve; s-Stomach; b-Byssus; m-Mouth; f-Foot; a.a-Anterior adductor)

2.1 Histological observation of gonads of pen shells stained with Hematoxylin and Eosin. Bars 50 µm

3.0 The sampling areas. Merambong shoal (A), Tanjung Adang shoal (B) and Merambong Island (C) of south western of Johor coast, (D) Merchang Lagoon of Terengganu, (E) Bagan Panchor of Perak and (F) Pulau Tinggi of eastern Johor, Malaysia

3.1 A quadrat divided into a grid for estimation of percentage coverage

3.2 Percentage cover of sediment profile from Merambong shoal, Tanjung Adang shoal and Merambong Island

4.1 Diagrammatic sketch of the valves of the genera *Pinna* and *Atrina* to show diagnostic characters. 1 – 2 : External and internal surfaces of the valves of *Pinna* and 3 – 4 : The same of the valves of *Atrina*

4.2 Morphometric characters used from three different shape of Malaysian pen shells. A = *Pinna bicolor*; B = *Atrina vexillum*; C = *Atrina pectinata*. Abbreviations: (1) Anterior to posterior adductor muscle; (2) Posterior adductor muscle to posterior shell margin, (3) Dorsal posterior margin, (4) Dorsal margin length, (5) Width length, (6) Total length, (7) Width of sulcus, (8) Posterior adductor muscle to posterior dorsal nacreous layer, (9) Dorsal nacreous length and (10) Posterior adductor muscle diameter
4.3 Exterior of right valve of *Pinna bicolor* showing outer and inner surface (A), Internal view of left valve (B) and diagrammatic sketch of internal part of *P. bicolor* and their characteristics view (C)

4.4 Exterior of right valve of *Pinna muricata* showing outer and inner surface (A), Internal view of left valve (B) showing position of posterior adductor muscle scar protruded beyond ventral nacreous lobe and diagrammatic sketch of internal part of *P. muricata* and their characteristics view (C)

4.5 Exterior of right valve of *Pinna incurva* showing outer and inner surface (A), Internal view of left valve (B) showing the deep ‘V’ shape on dorsal and ventral lobes of nacreous layer and diagrammatic sketch of internal part of *P. incurva* and their characteristics view (C)

4.6 Exterior of right valve of *Pinna deltodes* showing outer and inner surface (A), Internal view of left valve (B), showing the posterior adductor muscle protruded on posterior margin of dorsal lobe and the wide of sulcus between dorsal and ventral lobe of nacreous layer and diagrammatic sketch of internal part of *P. deltodes* and their characteristics view (C)

4.7 Exterior of right valve of *Pinna atropurpurea* showing outer and inner surface (A), Internal view of left valve (B), showing dorsal and ventral lobes of nacreous layer forms posteriory oblique truncated and sloping from sulcus and the nacreous lobes forming deep ‘V’ shape and diagrammatic sketch of internal part of *P. atropurpurea* and their characteristics view (C)

4.8 Exterior of right valve of *Atrina pectinata* showing outer and inner surface (A), Internal view of left valve (B), showing posterior adductor muscle not protruded beyond to posterior margin of nacreous layer and diagrammatic sketch of internal part of *A. pectinata* and their characteristics view (C)

4.9 Exterior of right valve of *Atrina vexillum* showing outer and inner surface (A), Internal view of left valve (B) showing posterior adductor muscle protruded beyond to posterior margin of nacreous layer and diagrammatic sketch of internal part of *A. vexillum* and their characteristics view (C)

4.10 Photo on posterior margin of the shell (A) *P. bicolor*; (B) *P. deltodes*; (C) *P. atropurpurea* and their morphometric characters, DPML = dorsal posterior margin length (no.3) and WL = width length (no.5)
4.11 Photo and diagrammatic sketch on different characters on width of sulcus. A – C = Width of sulcus (no. 7), D – F = difference on shape of nacreous lobe of three local *Pinna* species (A and D – *P. bicolor*, B and E – *P. deltodes*, C and F – *P. atropurpurea*)

4.12 Photo and diagrammatic sketch on difference characters on posterior adductor muscle scar located. A – C = Posterior adductor muscle scar located to posterior dorsal nacreous layer (no. 8) (A – *P. bicolor*, B – *P. deltodes*, C – *P. atropurpurea*)

4.13 Dendrogram plot showed the three difference groups of *Pinna* and *Atrina* species from the proportions morphometric measurement and were significantly different at 95% of similarity level

4.14 Plots of the coordinates of individuals of *Pinna* and *Atrina* according to the first two discriminate functions, obtained from proportions morphometric data

5.1 RAPD profile of *Pinna* and *Atrina* species and populations generated by primer OPA-03. (1 and 2) – *P. atropurpurea* from MS; (3 and 4) – *P. bicolor* from MS; (5 and 6) – *P. deltodes* from MS; (7 and 8) – *A. vexillum* from MS; (9) – *P. atropurpurea* from TAS; (10 and 11) – *P. bicolor* from TAS; (12 and 13) – *P. incurva* from TAS; (14 and 15) – *A. pectinata* from TAS; (16 and 17) – *P. atropurpurea* from MI; (18 and 19) – *P. deltodes* from MI; (20 and 21) – *P. muricata* from MI; (22 and 23) – *A. pectinata* from BP; (24) – *P. atropurpurea* from PT; (25) – *P. bicolor* from PT; (26 and 27) – *P. muricata* from ML; (28 to 31) – *Pinna* sp. from ML; (M) – 100 bp ladder.

5.2 RAPD profile of *Pinna* and *Atrina* species and populations generated by primer OPA-04. (1 and 2) – *P. atropurpurea* from MS; (3 and 4) – *P. bicolor* from MS; (5 and 6) – *P. deltodes* from MS; (7 and 8) – *A. vexillum* from MS; (9) – *P. atropurpurea* from TAS; (10 and 11) – *P. bicolor* from TAS; (12 and 13) – *P. incurva* from TAS; (14 and 15) – *A. pectinata* from TAS; (16 and 17) – *P. atropurpurea* from MI; (18 and 19) – *P. deltodes* from MI; (20 and 21) – *P. muricata* from MI; (22 and 23) – *A. pectinata* from BP; (24) – *P. atropurpurea* from PT; (25) – *P. bicolor* from PT; (26 and 27) – *P. muricata* from ML; (28 to 31) – *Pinna* sp. from ML; (M) – 100 bp ladder.
5.3 RAPD profile of Pinna and Atrina species and populations generated by primer OPA-07. (1 and 2) – P. atropurpurea from MS; (3 and 4) – P. bicolor from MS; (5 and 6) – P. deltodes from MS; (7 and 8) – A. vexillum from MS; (9) – P. atropurpurea from TAS; (10 and 11) – P. bicolor from TAS; (12 and 13) – P. incurva from TAS; (14 and 15) – A. pectinata from TAS; (16 and 17) – P. atropurpurea from MI; (18 and 19) – P. deltodes from MI; (20 and 21) – P. muricata from MI; (22 and 23) – A. pectinata from BP; (24) – P. atropurpurea from PT; (25) – P. bicolor from PT; (26 and 27) – P. muricata from ML; (28 to 31) – Pinna sp. from ML; (M) – 100 bp ladder.

5.4 RAPD profile of Pinna and Atrina species and populations generated by primer OPA-08. (1 and 2) – P. atropurpurea from MS; (3 and 4) – P. bicolor from MS; (5 and 6) – P. deltodes from MS; (7 and 8) – A. vexillum from MS; (9) – P. atropurpurea from TAS; (10 and 11) – P. bicolor from TAS; (12 and 13) – P. incurva from TAS; (14 and 15) – A. pectinata from TAS; (16 and 17) – P. atropurpurea from MI; (18 and 19) – P. deltodes from MI; (20 and 21) – P. muricata from MI; (22 and 23) – A. pectinata from BP; (24) – P. atropurpurea from PT; (25) – P. bicolor from PT; (26 and 27) – P. muricata from ML; (28 to 31) – Pinna sp. from ML; (M) – 100 bp ladder.

5.5 RAPD profile of Pinna and Atrina species and populations generated by primer OPA-10. (1 and 2) – P. atropurpurea from MS; (3 and 4) – P. bicolor from MS; (5 and 6) – P. deltodes from MS; (7 and 8) – A. vexillum from MS; (9) – P. atropurpurea from TAS; (10 and 11) – P. bicolor from TAS; (12 and 13) – P. incurva from TAS; (14 and 15) – A. pectinata from TAS; (16 and 17) – P. atropurpurea from MI; (18 and 19) – P. deltodes from MI; (20 and 21) – P. muricata from MI; (22 and 23) – A. pectinata from BP; (24) – P. atropurpurea from PT; (25) – P. bicolor from PT; (26 and 27) – P. muricata from ML; (28 to 31) – Pinna sp. from ML; (M) – 100 bp ladder.

5.6 Dendrogram based Nei’s (1978) genetic populations among 16 populations of pen shells based on RAPD makers genetic distance: Method = UPGMA Modified from NEIGHBOR procedure of PHYLIP Version 3.5. Abbreviation: (MS=Merambong shoal; TAS=Tanjung Adang shoal; MI=Merambong Island; PT=Pulau Tinggi; BP=Bagan Panchor; ML=Merchang Lagoon).

6.1 Line transects and quadrat devised by Wolff et al. (1993) and English et al. (1994) were laid at the study site
6.2 Percentage occurrence of pen shells in study areas

6.3 Densities (ind/m²) of pen shells in the study areas

6.4 Graph of univariate analysis of the three indices. (A) Total number of individuals; (B) Richness Index; (C) Evenness Index and (D) Diversity Index

6.5 Dendrogram plot for pen shells at Merambong shoal (1), Merambong shoal (2), Tanjung Adang shoal and Merambong Island

6.6 MDS ordination of pen shells community in Sungai Pulai seagrass beds

6.7 MDS plot for pen shells distribution from Sungai Pulai seagrass beds as surveyed during study period. A – P. bicolor; B – P. muricata; C – P. deltodes; D – P. atropurpurea; E – P. incurva; F – A. vexillum and G – A. pectinata

6.8 A few example of different habitat provided for pen shell at the study areas. A – Pen shells (a and b) from Merambong shoal associated with seagrasses and seaweed. B – Pen shell from Merambong Island associated with some tunicate. C – Pen shell from Merambong Island buried in hard substrate. D – Pen shell from Tanjung Adang shoal buried in soft substrate and associated with some seagrasses

6.9 Fouling organism’s community: species richness versus shell size of pen shell

6.10 Fouling organism’s community: Shannon-Wiener diversity versus the size of pen shells valve

6.11 Percentage of fouling organisms from five major phyla to be found attached on the surface of live pen shells

6.12 Univariate analysis on fouling organisms attached on surface of living Pen shells. A – Graph of Number of Individuals (N); B – Diversity Index (H); C – Richness Index (d) and D – Evenness Index (J) for five major phyla

6.13 Synalpheus carinatus (De man) found in the mantle cavity of the pen shells. A and B are female with (A) showing the presence of eggs attached to the pleopods and overlaying abdominal pleura (arrow). (C) Male showing pleopods with no overlying pleura and the difference in size of the chalea of the second periopods. Female (B) and male (D), dorsal view. Scale (5 mm)

xxvii
6.14 Snapper shrimp *Synalpheus carinatus* (De man), a – first pereiopod (cheliped); a’ – mesial view of chela and carpus; b – second pereiopod (left chela); b’ – same, view of chelate propodus and dactylus; c – second pereiopod (right chela); c’ – same, view of chelate propodus and dactylus; d – third pereiopod; d’ – same view of dactylus; e – forth pereiopod; e’ – same, view of dactylus; f – fifth pereiopod; f’ – same, view of dactylus; g – telson; h – uropod; i – uropod and telson; j – hypothetical cephalothorax, dorsal view; k – carapace, lateral view; l – frontal region view. Scale : 1 mm (b,j,k,l,m), 0.5 mm (a,c-g, h, i)

6.15 The relationship between the width and length of the cephalothorax of female and male *Synalpheus carinatus* (A). The relationship between the length of the right chela (close symbols) and left chela (open symbols) of the second pereiopod and total body length of female and male inhabiting pen shells (B). Comparison of shell length and total length of female and male *Synalpheus carinatus* (C)

7.1 Length classes of *Pinna bicolor* specimens for gonad development

7.2 Length-Weight relationships curve of *Pinna bicolor* from Merambong shoal for gonad study. (A)-Exponential length-weight relationships, (B)-Log$_{10}$ transformed length-weight of *P. bicolor*

7.3 Rainfall (mm) data collected taken from Merambong Shoal during the sampling period from April 2006 to April 2007

7.4 Histological cuts of gonads *P. bicolor*, Female. A – Stage I (Rest); B – Stage II (Early development); C – Stage II (Late development); D – Stage III (Mature); E – Stage IV (Spawning); F – Stage V (Spent). ct – conjunctive tissue; po – polygonal oocyte; ov – ovules; L – Lumen

7.5 Histological Cuts of Gonads *P. bicolor*, Male. A – Early active stage; B – Late active stage; C and D – Mature; E – Spawning; F – Spent. ec – Spermatocytes; eg – Spermatogonia; ez – Spermatozoa; em – empty acini

7.6 Reproductive stages of *Pinna bicolor* in Merambong shoal, Johore (n=65)

7.7 Gonad Index (GI) values for both male and female (A) and for pooled values (B) of *P. bicolor*

8.1 *Pinna bicolor* growth study, (A) : Schematic view of “in situ” measurement of *P. bicolor*, (B) : Biometry of *P. bicolor* – w = maximum width, a = minimum width, Hs = unburied length, h