UNIVERSITI PUTRA MALAYSIA

HABITAT, MORPHOLOGY, POPULATION GENETICS AND REPRODUCTIVE BIOLOGY OF HARD CLAM (BIVALVIA: VENERIDAE) FROM TWO LOCATIONS IN SARAWAK

HADI BIN HAMLI

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By

HADI BIN HAMLI

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

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairman: Associate Professor Mohd Hanafi Idris, PhD

Faculty: Agriculture and Food Sciences (Bintulu)

Biology and habitat characteristic of most common and abundance hard clam *Meretrix* spp. was investigated at two selected division of Sarawak namely Kuching and Betong from May 2013 to April 2014. Both areas comprised intertidal areas with sandy type sediment which able to support the growth of *Meretrix* spp. Environmental factor play important role to influence biology and physiology of *Meretrix* spp. for the present study. Habitat area for *Meretrix* spp. in the present study recorded the concentration of hydrogen ion (pH) range from 7.73–8.31, total dissolved solid 35.358–50.467 mg/L, salinity 22.5–31.8 psu, temperature 25.3–27.7 °C, turbidity 82.9–999 ntu, conductivity 3.473–4.683 S/cm, dissolved oxygen 3.74–5.58 mg/L, total rainfall 162.6–729.4 mm, ammonia 0.023–0.223 mg/L, nitrite 0.004–0.017 mg/L, nitrate 0.167–1.233 mg/L, phosphate 0.097–0.43 mg/L, total suspended solid 0.041–0.147 mg/L and chlorophyll *a* 0.419–0.147 µg/L.

A total of 3 hard clam species identified based on morphological characteristic were *Meretrix lyrata*, *M. meretrix* and *M. lusoria.* Pallial sinus scar profile was the main morphological characteristic used for the differentiation of these 3 *Meretrix* spp. While, a total of 13 morphometric characteristics were used to differentiate the three species of *Meretrix* recorded from Sarawak. Significant differences (ANOVA, $p<0.05$) on 7 morphometric characteristics (SW; Shell Width, AL; Anterior Length, LCT; Length of Cardinal Tooth, AW; Anterior Adductor Scar Width, PW; Posterior Adductor Scar Width, PS; Pallial Sinus Open Scar and LL; Ligament Length) based on proportion ratio with SL (Shell Length) were found among three *Meretrix* species recorded. Variation among *Meretrix* shell characteristic also was strengthen by results from clustering analysis, Principal Component Analysis (PCA) and genetic characteristic.

Genetic characteristic between *M. lyrata, M. meretrix* and *M. lusoria* were investigated based on universal marker cytochrome C oxidase subunit I (COI) with sequence LCO1490: 5'-ggtcaacaaatcataaagatattgg-3' and HCO2198: 5'-taaacttcagggtgaccaaaaaatca-3'. Genetic characteristic between this *Meretrix* spp. was able to be distinguished through phylogenetic analysis. The result was crucial to support the morphology and morphometric characterization. However, the present study using molecular approach was unable to verify the species of *Meretrix* sp. Therefore, additional genetic approaches are needed for further verification.

Present investigation on reproductive biology was able to determine the reproductive stages and spawning period of *Meretrix lyrata* within one year. Determination was made based on quantitative (Gonad Index and Condition Index) and qualitative approach (histological procedure). This study showed that the spawning period of *M. lyrata* is from May to September. Most of the *M. lyrata* in the studied samples undergoes continuous gonad development for 7 months from October 2013 to April 2014. This indicated *M. lyrata* only has one cycle of reproductive development in a year. Reproductive development of *M. lyrata* positively correlated to the abundance of phytoplankton in coastal area.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Doktor Falsafah

HABITAT, MORFOLOGI, POPULASI GENETIK DAN BIOLOGI PEMBIAKAN KERANG KERAS (BIVALVIA: VENERIDAE) DARI DUA LOKASI DI SARAWAK

Oleh

HADI HAMLI

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Biologi dan ciri habitat kerang keras *Meretrix* spp. yang paling biasa dan banyak telah dikaji di dua bahagian terpilih di Sarawak iaitu Kuching dan Betong bermula dari Mei 2013 hingg April 2014. Kedua–dua kawasan terdiri daripada kawasan pasang surut dengan mendapan jenis berpasir yang dapat menyokong pertumbuhan *Meretrix* spp. Faktor persekitaran memainkan peranan yang penting dalam mempengaruhi biologi dan fisiologi *M. lyrata* dalam kajian ini. Kajian ini merekodkan julat kepekatan ion hydrogen (pH) antara 7.73–8.31, jumlah pepejal terlarut 35.358–50.467 mg/L, kemasinan 22.5–31.8 psu, suhu 25.3–27.7 °C, kekeruhan 82.9–999 ntu, kekonduksian 3.473–4.683 S/cm, oksigen terlarut 3.74–5.58 mg/L, jumlah hujan 162.6–729.4 mm, nitrogen ammonia 0.023–0.223 mg/L, nitrit 0.004–0.017 mg/L, nitrat 0.167–1.233 mg/L, fosfat 0.097–0.43 mg/L, jumlah pepejal terampai 0.041–0.147 mg/L dan klorofil *a* 0.419–0.147 µg/L.

Tiga spesis kerang keras telah dikenalpasti dengan jelas berdasarkan ciri morfologi iaitu *Meretrix lyrata*, *M. meretrix* and *M. lusoria.* Bentuk parut sinus pallial adalah ciri utama morfologi yang boleh membezakan tiga spesis *Meretrix* yang direkod di Sarawak. Perbezaan ketara dikenalpasti antara tiga rekod spesis *Meretrix* (ANOVA, $p<0.05$) terhadap tujuh ciri morfometrik (SW; Lebar Cengkerang, AL; Panjang Anterior, LCT; Panjang Gigi Utama, AW; Lebar Parut Adductor Anterior, PW; Lebar Parut Adductor Posterior, PS; Bukaan Parut Sinus Pallial and LL; Panjang Ligament) berdasarkan nisbah perkadaran dengan SL (Panjang Cengkerang). Variasi antara ciri cengkerang *Meretrix* juga telah disokong oleh keputusan analisis kelompok, Analisis Komponen Utama (PCA) dan ciri genetik.

Ciri genetik di antara *M. lyrata, M. meretrix* dan *M. lusoria* telah dikaji berdasarkan penanda umum "cytochrome C oxidase" subunit I (COI) dengan jujukan LCO1490: 5'-ggtcaacaaatcataaagatattgg-3' and HCO2198: 5'-taaacttcagggtgaccaaaaaatca-3'. Ciri genetik di antara *Meretrix* spp. telah dapat dibezakan melalui analisis phylogenetik. Hasil keputusan adalah penting untuk menyokong pencirian morfologi dan morfometrik. Walau bagaimanapun, kajian ini menggunakan pendekatan molekular tidak dapat mengesahkan spesis *Meretrix* sp. Oleh itu, pendekatan genetik tambahan diperlukan untuk mengesahkan spesis tersebut.

Kajian semasa keatas biologi pembiakan telah menentukan peringkat gonad dan tempoh bertelur untuk *Meretrix lyrata* dalam setahun. Penentuan adalah berdasarkan pendekatan kuantitatif (Index Gonad dan Index Keadaan) dan pendekatan kualitatif (tatacara histologi). Kajian menunjukkan *M. lyrata* dalam sample yang dikaji mempunyai tempoh bertelur dari Mei hingga September. Kebanyakkan individu menjalani perkembangan gonad yang berterusan selama 7 bulan bermula dari Oktober hingga April 2014. Hasil kajian menunjukkan *M. lyrata* mempunyai satu kitaran pembiakan dalam setahun. Perkembangan pembiakan *M. lyrata* menunjukkan kaitan positif terhadap kelimpahan fitoplankton di perairan tersebut.

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I certify that a Thesis Examination Committee has met 10 December 2015 to conduct the final examination of Hadi Bin Hamli on his thesis entitled "Habitat, Morphology, Population Genetics and Reproductive Biology of Hard Clam (Bivalvia: Veneridae) from Two Locations in Sarawak" 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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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AL	Anterior length
AW	Anterior adductor scar width
Bp	Base pair
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organization
LCT	Length of cardinal tooth
LL	Ligament length
LPAS	Length of posterior adductor scar to anterior adductor scar
NA	Not available
Ns	No significant difference
PL	Posterior length
PCA	Principal Component Analysis
PCR	Polymerase Reaction Chain
PRIMER	Plymouth Routines In Multivariate Ecological Research
PS	Distance of the pallial sinus opening
PVM	Pallial line to ventral margin
PW	Posterior adductor scar width
SAS	Statistical Analysis of Software
SH	Shell height
SL	Shell length
SW	Shell Width
UL	Umbone length
µL	Microliters
mL	Mililiters
Ng	Nanogram
M	Meter
mm	Millimeter
µg/L	Microgram per liter
mg/L	Miligram per liter
g/L	Gram per liter
mg/m^3	Miligram per cubic mater
psu	Particle salinity unit
NTU	Nephelometric turbidity units
S/cm	Siemens per centimeter
℃	Degree Celsius

CHAPTER 1

INTRODUCTION

1.1 Background

Veneridae (bivalve) is listed under mollusca phylum and as one of the important invertebrate that generates the source of animal protein for human consumption in the modern world. Veneridae is among of 82 families of bivalve and approximately 10,000 species, included of oyster, clams, scallop and mussels (Okutani, 2000; Wye, 2007). Veneridae generally found inhabit at marine area particularly intertidal area such as coastal and estuary. Favourable habitat condition can increase number and diversity of bivalve for instant, Southeast Asia turn out to be the utmost diversity bivalve faunas compared with other 29 regions around the world (Crame, 2000). Furthermore, they can be found distributed around the Western Central Pacific area (Poutiers, 1998). Slight changes to the environment condition will definitely affect distribution and diversity pattern of mollusc that occupied the habitat.

Mollusc diversity and behaviour highly correlated to nutrients cycle (Thakur *et al.,* 2012), physico-chemical variable (Khade and Mane 2012), and sediment properties (Suresh *et al.,* 2012). This environmental property discussed in Chapter 4 for Buntal estuary of Kuching Division and Kabong of Betong division which are suitable habitat area for *Meretrix* spp. Abundance and distribution of *Meretrix* spp. is corresponding to the habitat condition and different between *Meretrix* spp. East Asia region such as China, Japan and Korea are preference habitat for native *M. petechialis* and *M. lusoria* (Yamakawa and Imai, 2012). Species differences can be identified through the external feature such as shell morphology (Rosewater, 1961). However, two similar species that inhabit at the similar habitat area probably can cause confusion through morphology identification particularly when involve hybrid species. Therefore, extra approach such as molecular work will facilitate a lot for species identification. This molecular method has been widely practiced for many metazoan species included *Meretrix* spp. Torii *et al.* (2010) has used molecular work to study the phylogentic among *Meretrix* spp. from the Japan, Korea and China locality. Moreover, *Meretrix* of similar species from different locality or habitat areas will have different genetic sequences. Hence, both morphology and molecular work has significant influence in determination of *Meretrix* spp. and both methods have been discussed detail in Chapter 5 and 6 of this dissertation.

Habitat area such as coastal area and mangrove comprised of flora and fauna to sustain each other to form substantial ecosystem. Sarawak itself sustains large area of wetland approximately 1.24 million ha or 13% of the total land area (Page, 2011). This large area of wetland can support large number of fauna included *Meretrix* spp. with proper and adequate supplies of nutrient to help prolong heredity. Suitable of habitat condition such as salinity, temperature, nutrient and food are significantly important for the reproductive development of bivalve (Saxby, 2002; Chu and Kumar, 2008; Enriquez-Diaz *et al*., 2009). Reproductive development of bivalve only can be determined based on histological procedure, Gonad Index (GI) and Condition Index (CI). Based on these

approaches, *M. lyrata* has been selected for reproductive development study due to its abundance found at the Buntal estuary. Detail on reproductive development study and environmental factor that influence the gonad development has been elaborated in Chapter 7.

1.2 Problem Statement

Mariculture on bivalve are widely applied at Western coast of Peninsular Malaysia. Most of bivalve cultures are *Anadara granosa*, *Paphia undulata, Perna viridis*, and *Crassostrea* spp. (Vakily, 1989; Poutiers, 1998). In Sarawak *A. granosa* and *Crassostrea* spp. are only culture in small scale. While *Meretrix* sp., *Placuna* spp., *Polymesoda* spp., *Pinna* spp. and *Modolus* spp. are collected from its natural environment to meet local market demand (Lovatelli, 1988).

Shellfish contribute to the source of Malaysian fish landing and on year 2009 shellfish landing was 23,746 tonne (Department of Fisheries Malaysia, 2013) (Table 1.1). Surf clam became the largest contributor during this year with 22,039 tonne. The lowest shellfish landing was recorded on year 2010 with 2,458 tonne. Total of shellfish landing was started to increase from year of 2011 to 2012 with 2,694 and 5,038 tonne respectively. However, the total shellfish landing decrease on year of 2013 with 4,910 tonne. High shellfish landing on year of 2009 may due to the high demand on surf clam. Therefore, this cause over exploitation on surf clam which was lead to significant decrease on total shellfish landing for the consequent year.

Table 1.1. Shellfish landing in Malaysia from 2009 to 2013 in tone

Species	2009	2010	2011	2012	2013
Oyster	11	14	13	10	9
Mussels	179	129	100	79	30
Sea-green mussel	11	0	1	1	2
Surf clam	22,039	623	686	1,501	1,548
Hard clam	176	132	76	100	52
Miscellaneous	1,330	1,560	1,818	3,347	3,269
Total	**23,746**	**2,458**	**2,694**	**5,038**	**4,910**

(Source: Department of Fisheries Malaysia, 2013)

In Sarawak, *Meretrix* spp. (Veneridae) are widely exploited as alternative meat source especially community that live close to coastal area. This bivalve was only sold at two divisions in Sarawak (Hamli *et al.,* 2012). However, there is no record for *Meretrix* spp. farming practice in Malaysia particularly Sarawak. Thus, fishermen need to collect it from its natural habitat in the mud flat area during the low tide.

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