



UNIVERSITI PUTRA MALAYSIA

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

HADI HAMLİ

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UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI

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By

HADI BIN HAMLİ

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

December 2015

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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

**HABITAT, MORPHOLOGY, POPULATION GENETICS AND
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HADI BIN HAMLİ

December 2015

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 strengthened 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'-ggtaacaaatcataaagatattgg-3' and HCO2198: 5'-taaacttcagggtgaccaaataatca-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 HAMLİ

Disember 2015

Pengerusi: Professor Madya Mohd Hanafi Idris, PhD

Fakulti: Sains Pertanian dan Makanan (Bintulu)

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 hingga 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'-ggcacaacaatcataaagattgg-3' and HCO2198: 5'-taaactcagggtagcaaaaaatca-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 spesies *Meretrix* sp. Oleh itu, pendekatan genetik tambahan diperlukan untuk mengesahkan spesies 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. Kebanyakan 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.

Members of the Thesis Examination Committee were as Follows:

Osumanu Haruna Ahmed, PhD

Associate Professor
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia
(Chairman)

Hishamuddin Bin Omar, PhD

Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Annie Christianus, PhD

Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Sukree Hajisamae, PhD

Associate Professor
Faculty of Science and Technology
Prince of Songkla University
Thailand
(External Examiner)

ZULKARNAIN ZAINAL, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 March 2016

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

Mohd Hanafi Idris, PhD

Associate Professor
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia
(Chairman)

Abu Hena Mustafa Kamal, PhD

Senior lecturer
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia
(Member)

Amy Halimah Rajae, PhD

Senior lecturer
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia
(Member)

BUJANG KIM HUAT, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 March 2016

TABLE OF CONTENTS

	Page
ABSTARCT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
LIST OF TABLES	xiv
LIST OF FIGURES	xvi
LIST OF ABBREVIATIONS	xix
CHAPTER	
1 GENERAL INTRODUCTION	1
1.1 Background	1
1.2 Problem Statement	2
1.3 Objectives	3
2 LITERATURE REVIEW	4
2.1 Veneridae Morphology	4
2.1.1 Classification	5
2.2 Habitat	5
2.2.1 Sediment Characteristic	5
2.2.2 Temperature	6
2.2.3 Dissolved Oxygen	7
2.2.4 Salinity	8
2.3 Feeding and Nutrient	8
2.4 Culture	9
2.4.1 Shellfish Production	10
2.5 Gonad Development	13
2.6 Embryonic and Larva Development	16
2.7 Genetic Characterization	18
2.7.1 Allozyme	18
2.7.2 Mitochondrial DNA Marker (mtDNA)	18
2.7.3 Nuclear DNA Marker	19
3 GENERAL METHODOLOGY	21
3.1 Description of Study Area	21
3.2 <i>Meretrix</i> spp. Habitat Study	22
3.2.1 Nutrient and Chlorophyll <i>a</i> Analysis	22
3.2.2 Sediment Structure Analysis	22
3.3 Collection and Identification of <i>Meretrix</i> spp.	22

4	NATURAL HABITAT CHARACTERISTIC OF <i>Meretrix</i>	24
4.1	Introduction	24
4.1.1	Objectives	25
4.2	Materials and Methods	25
4.2.1	Study Site Description	25
4.2.2	Seawater and Sediment Collection	27
4.2.3	Determination of Ammonia Nitrogen (NH_4^+)	27
4.2.4	Determination of Nitrite (NO_2^-)	27
4.2.5	Determination of Nitrate (NO_3^-)	28
4.2.6	Determination of Ortho-phosphate (PO_4^{3-})	29
4.2.7	Analysis of Total Suspended Solids (TSS)	29
4.2.8	Determination of Chlorophyll <i>a</i>	30
4.2.9	Analysis of Sediment Characteristic	30
4.2.10	Statistical Analysis	32
4.3	Results	32
4.3.1	Tidal Pattern	32
4.3.2	Physico-Chemical Parameters	33
4.3.3	Sediment Texture and Grain Size	37
4.4	Discussion	37
4.4.1	Tidal Pattern and Sediment Characteristic	39
4.5	Conclusion	40
5	MORPHOLOGY AND MORPHOMETRIC CHARACTERISTICS OF HARD CLAM <i>Meretrix</i>	41
5.1	Introduction	41
5.1.1	Objectives	42
5.2	Materials and Methods	42
5.2.1	Sample Preservation	42
5.2.2	Morphological Identification	43
5.2.3	Morphometric Study	43
5.2.4	Morphometric Data Analysis	44
5.3	Results	45
5.3.1	Morphological Characters of <i>Meretrix</i> Species	45
5.3.2	Exterior Shell Morphology Variation of <i>Meretrix</i> Species	52
5.3.3	Interior Shell Morphology Variation	53
5.3.4	Morphometric Study of <i>Meretrix</i> Species	53
5.3.5	Cluster Analysis	56
5.3.6	Principal Component Analysis (PCA)	57

	5.3.7 Dichotomus Key for <i>Meretrix</i> spp.	59
5.4	Discussion	59
5.5	Conclusion	62
6	GENETIC CHARACTERISTIC OF HARD CLAM <i>Meretrix</i>	63
6.1	Introduction	63
	6.1.1 Objectives	63
6.2	Materials and Methods	64
	6.2.1 Sample Collection	64
	6.2.2 DNA Extraction	64
	6.2.3 DNA Quantitation and Electrophoresis	64
	6.2.4 Polymerase Reaction Chain (PCR) Amplification	65
	6.2.5 Purification and Sequencing	66
	6.2.6 Statistical Analysis	66
6.3	Results	67
	6.3.1 Sequences Characteristics	68
	6.3.2 Phylogenetic Analysis	69
6.4	Discussion	71
6.5	Conclusion	73
7	SOME ASPECT ON REPRODUCTIVE BIOLOGY OF <i>Meretrix lyrata</i>	74
7.1	Introduction	74
	7.1.1 Objectives	75
7.2	Materials and Methods	75
	7.2.1 Length-Weight relationship	76
	7.2.2. Histological Procedure	76
	7.2.3 Condition Index (CI) Analysis	79
	7.2.4 Wet and Dry Weight	79
	7.2.5 Statistical Analysis	79
7.3	Results	79
	7.3.1 Length-Weight Relationship	81
	7.3.2 Male Gonad Maturation Stages	81
	7.3.3 Female Gonad Maturation Stages	83
	7.3.4 Sex Ratio	85
	7.3.5 Monthly Variation in <i>Meretrix lyrata</i> Gamete Phases	86
	7.3.6 Monthly Gonad Index (GI) of <i>Meretrix lyrata</i>	87
	7.3.7 Monthly Condition Index (CI) of <i>Meretrix lyrata</i>	88
	7.3.8 Correlation of <i>Meretrix lyrata</i> Condition Index (CI) with Gonad Index (GI), Wet and Dry Weight	88

7.3.9	Correlation of <i>Meretrix lyrata</i> Gonad Index (GI) with Environmental Factors	91
7.3.10	Correlation of <i>Meretrix lyrata</i> Condition Index (CI) with Environmental Factors	92
7.4	Discussion	93
7.4.1	Size Distribution	93
7.4.2	Length-Weight Relationships	92
7.4.3	Monthly Gonad Maturation Stages of <i>Meretrix lyrata</i>	92
7.4.4	Sex Ratio	95
7.4.5	Monthly Gonad Index (GI) of <i>Meretrix lyrata</i>	96
7.4.6	Monthly Condition Index (CI) of <i>Meretrix lyrata</i>	97
7.4.7	Correlation between Condition Index (CI) with Gonad Index (GI) and Tissue Weight of <i>Meretrix lyrata</i>	97
7.4.8	Correlation of <i>Meretrix lyrata</i> Gonad Development with Environmental Factors	98
7.5	Conclusion	99
8	SUMMARY, CONCLUSION AND RECOMMENDATIONS	100
	REFERENCES	103
	APPENDICES	121
	BIODATA OF STUDENT	126
	LIST OF PUBLICATIONS	127

LIST OF TABLES

Table		Page
1.1	Shellfish landing in Malaysia from 2009 to 2013 in tone	2
2.1	Major marine cultured bivalve in the world (Garibaldi, 1996)	10
2.2	Classification stages of bivalve gonad	14
2.3	Clams species that have been propagate through artificial breeding	16
4.1	Grain size classification scheme	32
4.2	Monthly variation of different water parameters in the Buntal estuary from May 2013 to April 2014	34
4.3	Correlations among physico-chemical parameters by Pearson (r) correlation	35
4.4	Sediment texture and grain size	37
5.1	Morphometric characteristic abbreviation used for <i>Meretrix</i> spp identification	43
5.2	Analysis of one way ANOVA with Tukey's HSD test for 12 morphometric characteristic proportion with shell length of three <i>Meretrix</i> species	54
5.3	Loading of variables on the first four principal components <i>Meretrix</i> species	58
6.1	List of <i>Meretrix</i> accession sequences from the GeneBank (NCBI)	67
6.2	Similarity percentage of present study on <i>Meretrix</i> spp. with 16 selected <i>Meretrix</i> spp. mtDNA accession sequences from GenBank (NCBI)	68
6.3	Nucleotide base composition for accession sequences	69
7.1	Stages in dehydration and paraffin wax infiltration	77
7.2	Staining procedure	78
7.3	Monthly length-weight relationship of collected <i>Meretrix lyrata</i> for gonad development study	81
7.4	Monthly distribution of male and female of <i>Meretrix lyrata</i>	86

7.5	Reproductive stages of <i>Meretrix lyrata</i> from Buntal estuary, Sarawak	86
7.6	Monthly male and female Gonad Index (GI) for <i>Meretrix lyrata</i> from Buntal estuary	87
7.7	Pearson Correlation between Condition Index (CI) with other variables	89
7.8	Pearson correlation analysis of Gonad Index (GI) of <i>Meretrix lyrata</i> with different environment parameters at the Buntal estuary	91
7.9	Pearson correlation analysis of Condition Index (CI) of <i>Meretrix lyrata</i> with different environment parameters at the Buntal estuary	92
7.10	Previously published values of the coefficients <i>a</i> and <i>b</i> for <i>Meretrix</i>	94
7.11	Comparison of Condition Index (CI) of <i>M. lyrata</i> with those of other shellfish	98
1A	Maximum Likelihood fits of 24 different nucleotide substitution models	123

LIST OF FIGURES

Figure		Page
2.1	Comparison of cultured and wild captured bivalve for 1991, 1995, 2000 and 2005. Adapted from Globefish-FAO (2007) and Helm <i>et al.</i> , (2004)	10
2.2	World production of cultured bivalve in year 1999 (FAO, 2001a)	11
2.3	Malaysian bivalve production in year 2013 (Department of Fisheries Malaysia, 2013)	12
2.4	Stages of ovary development. Stage 0: rest, Stage 1: early development, Stage 2: late development, Stage 3: Mature (Duinker <i>et al.</i> , 2008)	15
2.5	Stage of testis development. Stage 0: rest, Stage 1: early development, Stage 2: late development, Stage 3: Mature (Duinker <i>et al.</i> , 2008)	15
2.6	Bivalve larval development stages after Reverol <i>et al.</i> (2004) and Costa <i>et al.</i> (2008)	17
3.1	Study area for natural habitat of <i>Meretrix</i> spp.	21
4.1	Map of <i>Meretrix</i> spp. distribution at two division of Sarawak	25
4.2	Habitat of <i>Meretrix</i> spp. at the Sungai Buntal estuary	26
4.3	<i>Meretrix lyrata</i> burrowed itself in sediment during the high tide	26
4.4	Triangular classification of soil texture, adapted from Hawver and Bassuk (2007)	31
4.5	Monthly high tide at the study area (Sarawak Marine Department Malaysia, 2013; Sarawak Marine Department, 2014)	33
5.1	Map of <i>Meretrix</i> spp. distribution at two division of Sarawak	42
5.2	Measurements of shell characters for <i>Meretrix</i> spp. Note: 1(SL), 2(SH), 3(SW), 4(LL), 5(PL), 6(AL), 7(UL), 8(LCT), 9(LPAS), 10(PW), 11(AW), 12(PVM), 13(PS)*	44

5.3	Morphological structure of <i>Meretrix lyrata</i> (Sowerby, 1851) A; Outer view, B; Inner view, C; Sketch of inner view	47
5.4	Morphological structure of <i>Meretrix meretrix</i> (Linnaeus, 1958), A; Outer view, B; Inner view, C; Sketch of inner view	49
5.5	Morphological structure of <i>Meretrix lusoria</i> (Roding, 1798), A; Outer view, B; Inner view, C; Sketch of inner view	51
5.6	Outer shell characteristic between <i>Meretrix</i> species. (A) <i>M. lyrata</i> ; (B) <i>M. Meretrix</i> ; (C) <i>M. lusoria</i>	52
5.7	Pallial sinus scar pattern for (A) <i>Meretrix lyrata</i> ; (B) <i>M. meretrix</i> ; (C) <i>M. lusoria</i>	53
5.8	Differences of pallial sinus open scar (PS) between three local hard clams. (A) <i>M. lyrata</i> , (B) <i>M. meretrix</i> (C) <i>M. lusoria</i>	55
5.9	Box plot of morphometric characteristic for <i>Meretrix</i> spp.	56
5.10	Hierarchical cluster between three local hard clams from Sarawak	57
5.11	Principal Component Analysis (PCA) for three local hard clams from Sarawak	58
6.1	Study area of <i>Meretrix</i> spp. for genetic study	64
6.2	Fragment size produce by <i>Meretrix</i> spp. using COI marker. 1: <i>M. lyrata</i> , 2: <i>M. lusoria</i> , 3: <i>M. meretrix</i>	67
6.3	Phylogenetic tree of <i>Meretrix</i> accession sequence inferred from the Maximum Likelihood analysis using Tamura three-parameter model. Only bootstrap score greater than 50% are shown	70
7.1	Study site for monthly <i>Meretrix lyrata</i> collection	75
7.2	Total length class of <i>Meretrix lyrata</i> used for gonad development study	80
7.3	Total body weight class of <i>Meretrix lyrata</i> used for gonad development study	80
7.4	Male gonad stages for <i>Meretrix lyrata</i> , A: Rest, B: Early development, C: Late development, D: Matured, E: Spawning, F: Spent, Scale: 50 μ m. Note: ct = connective tissue; dgs = degenerate spermatozoa/ spermatid/ spermatocyte; f = follicle; fw = follicle wall; lu = lumen; mt = muscular tissue; sz = spermatozoa; sd = spermatid; s = spermatocyte	83

7.5	Female gonad stages for <i>Meretrix lyrata</i> , A: Rest, B: Early development, C: Late development, D: Matured, E: Spawning, F: Spent, Scale: 50 μ m. Note: dgs = degenerate oocyte; do= developing oocyte; gv=germinal vesicle; mo= matured oocyte; mt= muscular tissue; n=nucleus; ct=connective tissue; lu= lumen; f=follicle; fw=follicle wall	85
7.6	Gonad Index (GI) values for <i>Meretrix lyrata</i> from Buntal estuary	87
7.7	Temporal Condition Index (CI) for <i>Meretrix lyrata</i> from Buntal estuary (Mean \pm standard error)	88
7.8	Monthly correlation graph between Condition Index (CI) and wet weight of <i>Meretrix lyrata</i>	89
7.9	Monthly correlation graph between Condition Index (CI) and dry weight of <i>Meretrix lyrata</i>	90
7.10	Monthly correlation graph between Condition Index (CI) and Gonad Index (GI) of <i>Meretrix lyrata</i>	90
7.11	Monthly correlation graph between <i>Meretrix lyrata</i> Gonad Index (GI) and chlorophyll <i>a</i>	92
7.12	Monthly graph pattern between <i>Meretrix lyrata</i> Condition Index (CI) and chlorophyll <i>a</i> concentration in the seawater	93

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
$\mu\text{g/L}$	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
$^{\circ}\text{C}$	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.

Coastal development in Sarawak will eventually expose coastal area and estuarine to destruction. Pollution from the urban area and factories will damage the natural habitat of *Meretrix* spp. Pollutant commonly derived from sawmills, wood chip and sago factories may destroy the natural habitat of mollusc and other biodiversity (Davy and Graham, 1982). Disturbance on the habitat will alter the nutrient and abiotic composition which eventually affect the survival of *Meretrix* spp.

Present study was conducted to establish baseline information on the biological and habitat aspect of *Meretrix* spp. which can be used as guide to culture this species as solution to reduce over exploitation and depletion of shellfish population from its natural habitat. Documentation of *Meretrix* spp. can provide scientific importance as a record and reference for future research activities toward aquaculture development, conservation and proper management of this natural resource in Sarawak. In addition, the findings of this study will provide documentation on habitat, morphology, genetic, and reproductive cycle of *Meretrix* spp. from Sarawak, Malaysia.

1.3 Objectives

Objectives of the study were:

1. To identify physico-chemical characteristics of the hard clam habitat from two sampling locations.
2. To observe and describe the morphology and morphometric of hard clam from two sampling locations.
3. To describe the genetic characteristic of hard clam from two sampling locations.
4. To describe some aspect of the reproductive biology of *M. lyrata* based on histology, Gonad Index, and Conditioning Index and its relationship to the water parameter of its habitat.

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BIODATA OF STUDENT

Author, Hadi Bin Hamli was born on 9th August 1987 at the Kampung Spaoh, Betong, Sarawak. He received primary education at the Sekolah Rendah Kebangsaan Kidurong 1 from 1994 to 1997 and Sekolah Rendah Kebangsaan Kidurong 2 from 1998 to 1999. He then received secondary education at Sekolah Menengah Kebangsaan Kidurong on 2000 and Sekolah Menengah Sains Miri from 2000 to 2004. After finished Sijil Pelajaran Malaysia on 2004, He continued his study from 2005 to 2006 at the Labuan Matriculation College. Starting from the middle year of 2006, he furthers his higher education at the Universiti Putra Malaysia Bintulu Sarawak Campus and obtained Bachelor Science of Bioindustry degree on 2010. After awarded with degree, he continued his study in master and awarded with Master Science in Aquatic Biology on 2013. He furthers his study in Doctor of Philosphy at Department of Animal Science and Fishery, Universiti Putra Malaysia Bintulu Sarawak Campus.

LIST OF PUBLICATIONS

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