



UNIVERSITI PUTRA MALAYSIA

***POPULATION GENETICS AND REPRODUCTIVE BIOLOGY OF BLUE
SWIMMING CRAB, *Portunus pelagicus* (LINNAEUS, 1758) IN THE WEST
COAST OF PENINSULAR MALAYSIA***

INTAN ROHAYU BINTI SUKIMIN

FP 2018 7



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By

INTAN ROHAYU BINTI SUKIMIN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fullfillment of the Requirements for the Degree of Master of Science**

August 2017

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DEDICATION

To the greatest father, Sukimin bin Selamat, and the best mother, Masitah binti Abdul Samad, this one for you. Thank for your never ending supports, prayers, time and energy, perseverance, and loving care for helping me completing my goal

To my brother and sisters;

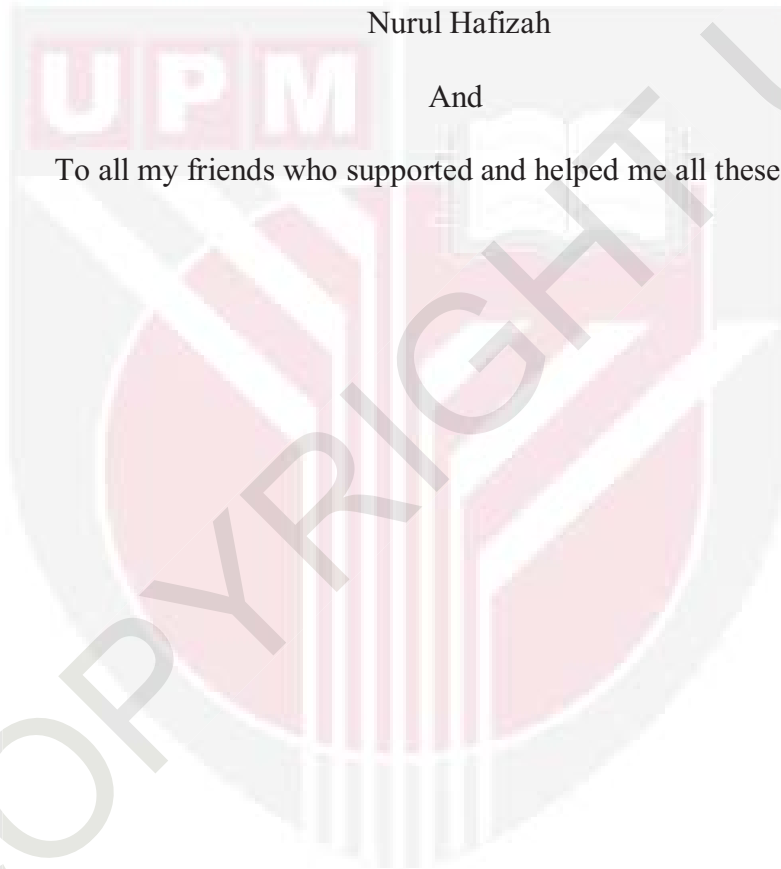
Mohd Amerul Razif

Rohanizah

Nurul Hafizah

And

To all my friends who supported and helped me all these years



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

POPULATION GENETICS AND REPRODUCTIVE BIOLOGY OF BLUE SWIMMING CRAB, *Portunus pelagicus* (LINNAEUS, 1758) IN THE WEST COAST OF PENINSULAR MALAYSIA

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August 2017

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Portunus pelagicus is an important commercial species that widely spread in the Peninsular Malaysia. This species attain high demand in the market due to their delicacy. The aims of this study were to determine the genetic diversity of *P. pelagicus* between two study areas using sequences of cytochrome c oxidase I (COI) mitochondrial DNA (mtDNA) gene, sexual maturity and reproduction pattern of *P. pelagicus* in Batu Pahat, Johor and Teluk kemang, Port Dickson. The specimens of *P. pelagicus* were collected monthly started from September 2015 to August 2016. All samples were measured their carapace width (CW), carapace length (CL) and body weight (BW) while maturity stages of male and female crabs were observed morphologically. The muscle tissues were taken for molecular analysis. Genetic diversity of two populations were determined through the haplotype and nucleotide diversity. Overall, a total of 63 sequences (599 bp) were obtained with 6 mtDNA haplotypes were found. The sharing of haplotypes among populations and low pairwise genetic distances (Fst) (0.2- 1.9 %) among haplotypes suggested high gene flow among the two populations. A total of 402 *P. pelagicus* individuals were examined including 254 males and 148 female.

The result indicated that the overall sex ratio of males to females was 1: 0.36. The ratio shows that the numbers of females were lower than males which may be due to migration. The annual mean variation of GSI showed continuous breeding with the existence of four spawning peaks of different month (November, February, May and July) between September 2015 and August 2016 of the study area. The maximum GSI value 8.92 ± 5.096 was observed in the month of November 2015. While the minimum GSI value was 2.02 ± 1.918 observed in April 2016. The condition factors showed highest peak for male was 1.06 in August and female was 1.05 in May. The estimated

mean size of males and female reached sexually matured (Lm50) at 112.56 mm and 114.21 mm CW respectively and show slightly different with some estimates in some other coastal waters of Malaysia, Indonesia and Philippines. This discrepancy might reflect of disparate in biology of the species and genetic composition. The estimated mean fecundity of *P. pelagicus* was 719,307 (range, 512269- 1524990) eggs. The fecundity of *P. pelagicus* was positively and linearly correlated with CW and CL and BW, so larger females produced high number of eggs than smaller ones because larger females have large body cavities.

The high genetic similarities of the COI mtDNA analyses of *P. pelagicus* from Port Dickson and Batu Pahat obtained in this study concluded that this species belong to a same gene pool and be regarded as a single broodstock population. For sustainable use of crab at Teluk Kemang and Batu Pahat coastal waters, it is important to recommend a legal size and closure season based on spawning season and size at 50 % of sexual maturity. Thus, it is recommended the legal size of crab should be greater than 112.56 mm-CW for male and 114.21 mm-CW for female, while the closing season should be in November, February, May and July.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**POPULASI GENETIK DAN BIOLOGI PEMBIAKAN KETAM BIRU,
Portunus pelagicus (LINNAEUS, 1758) DI PANTAI BARAT SEMENANJUNG
MALAYSIA**

Oleh

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Portunus pelagicus adalah spesis komersial yang penting dan tersebar luas di semenanjung Malaysia. Spesis ini mendapat demand yang tinggi di pasaran keranarasanya yang enak (Maheswarudu *et al.*, 2008). Tujuan kajian ini adalah untuk menentukan kepelbagaian genetik *P. pelagicus* antara dua kawasan kajian dengan menggunakan jujukan mitokondria gen sitokrom C oksida I (COI), kematangan seksual dan corak pembiakkan *P. pelagicus* di Batu Pahat, Johor dan Teluk Kemang, Port Dickson. Specimen *P. pelagicus* dikumpul setiap bulan bermula daripada September 2015 sehingga Ogos 2016. Lebar karafase (CW), panjang karafase (CL), dan berat badan (W) semua sampel telah diukur manakala fasa kematangan ketam jantan dan betina telah diperhatikan secara morfologi. Tisu otot telah diambil untuk analisis molekul. Kepelbagaian genetik untuk dua populasi (Port Dickson dan Batu Pahat) telah ditentukan melalui kepelbagaian haplotip dan nukleotip. Secara keseluruhan, sejumlah 63 jujukan (599 bp) telah diperolehi dengan 6 mtDNA haplotip telah dijumpai. Haplotip yang dikongsi antara populasi dan jarak pasangan genetik yang rendah (*F_{st}*) (0.2- 1.9 %) antara haplotip mencadangkan pengaliran gene yang tinggi antara dua populasi. Sejumlah 402 individu *P. pelagicus* telah diperiksa daripada 254 jantan dan 148 betina.

Keputusan menunjukkan keseluruhan nisbah seks jantan kepada betina adalah 1: 0.36. Nisbah tersebut menunjukkan bahawa betina lebih rendah daripada jantan dan dipercayai berkaitan dengan penghijrahan. Perubahan purata tahunan GSI menunjukkan pembiakkan berterusan dengan kewujudan empat puncak bertelur di bulan yang berbeza (November, Februari, Mei dan Julai) antara September 2015 dan Ogos 2016 di kawasan kajian tersebut. Nilai GSI yang tertinggi ialah 2.02 ± 1.918 diperhatikan pada April 2016. Faktor keadaan menunjukkan puncak tertinggi untuk

jantan ialah 1.06 pada Ogos dan betina ialah 1.05 pada Mei. Anggaran saiz min untuk jantan dan betina yang mencapai kematangan seksual (Lm50) masing-masing ialah 112.56 mm dan 114.21 mm CW dan menunjukkan sedikit perbezaan dengan anggaran pada sesetengah pantai yang lain di Malaysia, Indonesia dan Philippines. Percanggahan ini berkemungkinan oleh perbezaan pada biologi bagi spesis dan komposisi genetik. Dianggarkan min kesuburan *P. pelagicus* adalah positif dan berkait rapat secara menegak dengan CW, CL dan BW, jadi semakin besar betina akan menghasilkan jumlah telur yang lebih banyak berbanding betina yang lebih kecil kerana betina yang lebih besar mempunyai rongga badan yang besar.

Persamaan genetic yang tinggi pada analisis COI mtDNA *P. pelagicus* dari Port Dickson dan Batu Pahat yang diperoleh dalam kajian ini merumuskan bahawa spesis ini daripada kolam gen yang sama dan boleh diambil kira sebagai satu populasi induk tunggal. Biologi pembiakan untuk dua tempat menunjukkan tiada perbezaan ketara. Oleh kerana tidak ada perbezaan yang ketara antara dua populasi, biologi pembiakan *Portunus pelagicus* untuk dua tempat hendaklah digabungkan. Untuk penggunaan ketam yang lestari di pantai Teluk Kemang dan Batu Pahat, ia adalah penting untuk mencadangkan saiz yang dibenarkan dan musim penutupan berdasarkan pada musim bertelur dan saiz pada 50 % kematangan seksual. Oleh yang demikian, cadangan saiz yang dibenarkan untuk ketam adalah lebih besar dari 112.56 mm-CW untuk jantan dan 114.21 mm-CW untuk betina, manakala musim penutupan adalah antara November, Februari, Mei dan Julai.

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

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LIST OF ABBREVIATIONS

Cm	Centimeter
M	meter
mm	Millimeter
CW	Carapace width
Lm50	50 % of size at maturity
GSI	Gonad somatic index
DNA	Deoxyribonucleic acid
mtDNA	Mitochondrial DNA
CL	Carapace length
BW	Body weight
Mg	Milligrams
°C	Degree Celsius
X ²	Chi square
g	gram
Promega	Promega Genomic DNA Purification Kit Protocol
PCR	Polymerase Chain Reaction
COI	Cytochrome Oxydase I
μl	Micro litre
5X	Five time concentration
%	Percent
dNTPs	Deoxynucleotide
ddH ₂ O	Double distilled water
V	Volt

UV	Ultra Violet
Bp	Base pair
NJ	Neighbor-joining
MP	Maximum-Parsimony
ME	Minimum Evolution
ML	Maximum Likelihood
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
Km	Kilometer
DOF	Department of Fisheries
Kn	Condition factor
W	Weight
ppt	Part per thousand

CHAPTER 1

INTRODUCTION

1.1 Background

Portunus pelagicus inhabits the shallow, tropical and temperate coastal waters throughout Indo-West Pacific from Africa to India, Australia and Southeast Asia (Smith and Sumpton, 1989). In tropical regions, they are representing a valuable component of small-scale coastal fisheries (Batoy *et al.*, 1987). Normally, *P. pelagicus* were caught using traps, beach seine nets and bottom-set gillnets (Haefner, 1985). They are commonly prey on slow moving invertebrate such as molluscs, bivalve, worms and crustaceans, but also feed on scavenge such as dead fish and squid (Wassenberg and Hill, 1987).

Portunus pelagicus is an important recreational and commercial species (Sukumaran and Neelakantan, 1996) and posses high fishing pressure with fishing mortality estimated to be greater than natural mortality (Lai *et al.*, 2010). This species posse high demand due to unique taste and availability all year round make it become the valuable target in the fishery sectors (Sumpton *et al.*, 1994; Lai *et al.*, 2010). There are several countries around the world that are actively involved in *P. pelagicus* and other portunid research and fisheries such as China (Lai *et al.*, 2010; Liu *et al.*, 2014), Japan (Hamasaki *et al.*, 2011), India (Sukumaran, 1997; Soundarapandian *et al.*, 2007; Soundarapandian and Singh, 2008), Australia (Potter *et al.*, 1983; Johnson *et al.*, 2010), Thailand (Tongdee, 2001), Indonesia (Rejeki, 2007; Zairion *et al.*, 2015) and Malaysia (Ikhwanuddin *et al.*, 2012; Efrizal *et al.*, 2015).

Reproduction is important in order to maintain the species proliferation and survivorship of offspring. The study of reproduction biology in crustacean has been widely studied especially for species that important in term of commercial and ecological values (Pinheiro and Fransozo, 2002). Mainly the studies on *P. pelagicus* have been focused on size at sexual maturity (Zairion *et al.*, 2015; Ikhwanuddin *et al.*, 2009; Johnson *et al.*, 2010; Kamrani *et al.*, 2010) fecundity (Zairion *et al.*, 2015; Johnson *et al.*, 2010; Rasheed and Mustaqim, 2010) and other reproduction biology attributes such as mating and spawning duration or time (Robertson and Kruger, 1994; Rasheed and Mustaqim, 2010) of crab. The information of the study is important in order to manage the crab fisheries in sustainable basis (Soundarapandian *et al.*, 2013).

1.2 Statement of problem

Currently, no study have been conducted on the genetic diversity of *Portunus pelagicus* in water bodies of Malaysia although it important for broodstocks selection and breeding programs. On the other hand, the information about the reproduction biology of *P. pelagicus* in Malaysian coastal waters is very limited and there have no

studies on its reproductive biology although it supports substantial artisanal fisheries. Thus two study areas were selected which are Teluk Kemang, Port Dickson and Batu Pahat, Johor. At Teluk Kemang, the area is located near to Center of Marine Science, Universiti Putra Malaysia (CoMAS, UPM) and becomes popular among researchers and tourist especially for research and recreational activities. While at Batu Pahat, the area is surrounded by many small villages and swamp areas and less focused for recreational activities and no research study have been recorded. The shipping activities are heavier at Teluk Kemang compared to Batu Pahat. Although both areas are near to each and within the same fishing line, are there any differences in term of genetic variation and reproduction biology for *P. pelagicus* species. Thus information on genetic study and reproduction biology such as sex ratio, gonad development, and fecundity *P. pelagicus* is needed to help determine the appropriate management strategies for this species in Teluk Kemang, Port Dickson and Batu Pahat, Johor.

1.3 Objectives

Thus, this study was established to address for the following objectives:

1. To compare the genetic variation of *Portunus pelagicus* in Teluk Kemang, Port Dickson and Batu Pahat, Johor
2. To describe the reproduction cycle and determine the spawning season, sex ration and fecundity of *Portunus pelagicus*.

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