GENETIC CHARACTERIZATION, BREEDING STRATEGIES AND POND MANAGEMENT FOR ENHANCEMENT OF THE MALAYSIAN GIANT PRAWN, *MACROBRACHIUM ROSENBERGII* PRODUCTION

MOHAMED OMER ELSHEIKH SIDDEEG

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MOHAMED OMER ELSHEIKH SIDDEEG

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Name of Candidate: **Mohamed Omer Elsheikh Siddeeg**

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ABSTRACT

The giant Malaysian prawn, (*Macrobrachium rosenbergii de Man, 1879, 2n=59),* is an important species for aquaculture in the tropics and subtropics. Currently, it has been introduced into more than forty countries for this purpose. In the past half a century an accumulation of a reasonable amount of literature that definitely aided in the success of farming of this species was achieved. But after the decades extending from 1960s to this date unit per area production is approaching a plateau; this is attributed to the exhaustion of management improvement opportunities that typically contribute to crop increases only during the first few decades that follow the start of farming an undomesticated species. As a result, its aquaculture in Malaysia and other countries in Southeast Asia faced challenges that lead to decline in production. This research was conducted in order to dig reasons and find solutions for these challenges. So, investigations were conducted through genetic and management studies. Pond management investigations were conducted through an in-depth interviewing and visiting of GMP farmers at their farms in Negeri Sembilan States. Results of this portion of the study showed that many farmers are facing problems in obtaining seeds. In addition, feed cost was also found to increase tremendously. Moreover, farmers were found to ignore important steps such as proper pond bank repairing, grading, fertilization and some even do not discern the life cycle of the species, and consequently do not know the conditions required for its reproduction. Grow-out data for conventional genetic improvement study and tissue samples for molecular genetic study were collected from a farm owned by Department of Fisheries, Kg. Pulau Sayak at Tapah village. DNA extraction and molecular genetic analysis were conducted at the Animal Genetics and Genomics Laboratory, Division of genetics and Molecular biology, Institute of Biological Sciences, Faculty of Science, University of Malaya.

Regarding economically important characters, Kedah population showed significant (P<.05) superiority over the other populations for harvest weight, total length, carapace length and tail length. Moreover, heritability estimates for these characters range from high to moderate. So, using selection methods is recommended for future improvement of stocks. Harvest weight directly reflects product amount, therefore, multiple linear regression approach was applied to predict it through information of the rest of growth related traits. This model is more accurate than simple relations models. The predictors were significant (P<.05) factors for the multiple regression equations drawn. Significant differences between the equations according to age and sex was found. In studying the genetic diversity of these populations, the COI gene was sequenced for samples of the studied populations and their progenies. Forty three haplotypes were generated from 117 sequences of the parental populations; most which were unique. Genetic diversity for studied populations fall within the range of moderate to high (0.6575-0.9206) but nucleotide diversity was low (0.2511-1.1942%) showing subtle variation among them (Amova 5.45%, p<.00006). Networking the haplotypes and constructing a phylogenetic tree for only the unique ones revealed significant genetic structuring of these populations. Cyclical mating was found to be successful in securing genetic diversity as the genetic variability of progeny groups was high (0.7381 - 0.9412). Unique haplotypes may be useful in identifying breeding families and help in aquaculture improvement programs. Tajima's neutrality test indicated significant (p<.027) departure of Negeri Sembilan population from mutation- drift equilibrium; meaning that it may have been losing variability. Consequently, seasonal band of fishing to replenish the species populations in rivers of South Malay Peninsular is recommended.

ABSTRAK

Pembiakan Udang galah Malaysia, (*Macrobrachium rosenbergii de Man, 1879, 2n=59*) terkenal dengan kegunaannya di dalam akuakultur. Tambahan pula, hasilannya lebih menguntungkan berbanding akukultur lain kerana oermintaannya yang tinggi serta risiko yang rendah. Tetapi, pengelibatan sektor swasta di dalam pengeluarannya tidak memberangsangkan. Jesteru, maklumat mengenai struktur genetik, variasi dan hubungan di antara populasi serta strategi penambahbaikan genetik amat bermanfaat bagi Malaysia. Sehingga kini, tidak banyak pihak yang mengambil berat mengenai isu genetik ini. Berdasarkan temu-ramah yang dijalankan, kebanyakan pengusaha *hatchery* kebanyakannya mengunakan baka yang sama tanpa mencampuran bagi meningkatkan variasi genetik. Ini menyebabkan *inbreeding* yang merendahkan kadar hidup dan tumbesaran larva serta merendahkan hasil. Analisa variasi berat badan, panjang badan, panjang *carapace* dan panjang ekor menunjukkan signifikasi ($p<.5$) berbanding populasi lain. Corak yang sama diperhati apabila membandingkan kumpulan progeni. Nilai pewarisan dijangka dalam kitar sederhana ke tinggi. Ini meningkatkan potensi aplikasi kaedah pemilihan di dalam program penambah-baikan genetik. Berat badan adalah ciri yang paling penting dari segi pendapatan pengusaha. Maka, data morphometrik digunakan untuk menjangka berat badan melalui *linear multiple regression model*. Covariat yang diguna untuk menjangka sebagai respon menerusi model ini dianggap bermakna bagi lebih 80% kes. Setahu saya, kajian ini merupakan yang pertama yang mengunakan hubungan persamaan untuk mengkaji data morphometrik hidupan aquatik. Kaedah ini boleh digunakan bagi data yang serupa bagi semua jenis udang. Tambahan pula, kebanyakan halotip yang diperoleh adalah unik ke populasi masing-masing memblehkan ia diguna sebagai penanda bagi mereka bentuk program penternakan. Bagi kumpulan ibubapa dan progeni yang dikaji, lapan puluh halotip dihantar ke NCBI dan akan dipamerkan kepada orang ramai tidak lama lagi.

Variasi genetik sederhana hingga tinggi dikaitkan dengan diversiti nukleotid rendah bagi kebanyakan populasi. Hasil kajian analisa variasi molecular ($p<.05$; FST = 0.05451), perangkaian dan pokok filogenetik yang dibina dari haplotip yang unik menunjukkan struktur genetik yang jelas bagi populasi-populasi tersebut. Ujian neutral Tajima, ujian yang paling sensitif bagi variasi genetik sederhana, menunjukkan perbezaan yang bermakna satu populasi dari garis tengah mutasi, mencadangkan kehilangan variabiliti. Ini menyebabkan saranan pengharaman pemancingan atau penangkapan ikan bermusim di selatan Wilayah Persekutuan Kuala Lumpur. Tetapi ujian Fs Fu dan ujian *mismatch* yang dilakukan tidak dapat menunjukan apa-apa bukti bagi perbezaan dari neutral. Tambahn pula, kitaran persenyawaan terbukti berkesan dalam meningkatkan variasi genetik di dalam kumpulam progeni.

بسم الله الرحمن الرحيم

In the Name of Allah, the most gracious, the most merciful

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I first say "All the praises and thanks are to Allah, the Lord of the Âlalamîn". Allah almighty said: "And not alike are the two bodies of water. One is fresh and sweet, palatable for drinking, and one is salty and bitter. And from each you eat tender meat and extract ornaments which you wear, and you see the ships plowing through [them] that you might seek of His bounty; and perhaps you will be grateful, (Surat Fatir- the Holly Quran).

Then, I would like to thank University of Malaya that offered me a chance to pursue my studies after a long pause. I also thank Associate professor Dr. Subha Bhassu who chose me within her crew and kept encouraging me without losing faith in me although I fall in so many pitfalls. Her confidence in my potentials has never been shaken. She continued her guidance till my ship finally reached the shore. I also thank Dr. Firuza Begham Binti Mustafa, who also supported this work especially the part concerning social science. My thanks are extended to the director of the Institute of Marine Science, Kedah, Kampung Pulau Sayak, of the fisheries department, Dr. Mohd Fariduddin Bin Othman who allowed me to be part of the genetic improvement program of the giant Malaysian prawn at the Institute just mentioned.

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DEDICATION

To my sincere wife Ihlam,

My mother &

Soul of my father

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbols

σ^2	Variance
<	Less than
>	Greater than
µl	Microliter (10^{-6} Litre)
♀	Female
♂	Male

Abbreviations

AMOVA	Analysis of molecular variance.
ANOVA	Analysis of variance
bp	Base pair
Bumiputera	Malaysian term to describe the Malay race and other indigenous peoples of Southeast Asia.
cm	Centimetre
ºC	Degree Celsius.
d.f.	Degrees of freedom.
DESA	Department of Economics and Social Affairs of United Nations
DNA	Deoxy-ribonucleic acid.
DO	Dissolved Oxygen.
DOF	Department of Fisheries- Malaysia.
FAO	Food and Agricultural Organization of United Nations
FST	Fixation Index
g	Gram
GLM	General linear model

ha	Hectare
Kg	Kilogram
L	Litre
M	Metre
MANOVA	Multivariate analysis of variance
Metric ton	1000 kg
Mg Cl_2	Magnesium chloride.
MGP	Malaysian giant prawn.
mM	Millimole.
Nucleotide A	Adenine
Nucleotide C	Cytosine
Nucleotide G	Guanine
Nucleotide T	Thymine
NACA	National Advisory committee for aeronautics
p.p.m	part per million
p.p.t	part per thousand
PCR	Polymerase Chain Reaction
PL	Postlarvae.
USD	United States Dollar
WSSV	White Spot Syndrome Virus

CHAPTER ONE: INTRODUCTION

The Food and Agricultural Organization of the United Nations (FAO) has anticipated that about 800 million people are suffering from chronic malnutrition around the globe (Collier & Dercon 2009), with a population that has already reached 7.2 billion (DESA, 2013). This population is expected to hit 9.6 billion people by 2050 (United Nations News Centre, 2013; Cohen, 2001). Nearly all of this population increase will occur in developing countries. On another hand, United Nations Economic and Population Division has reported that urbanization will continue at an accelerated pace, thus estimating that about 70% of the world's population will become urban and income levels will be many multiples of what they are now (DESA, 2013). In order to feed this larger, more urban and richer population, food production must increase by 70%; annual cereal production will need to rise to about 3 billion metric tons from 2.1 billion of today and annual meat production will need to rise by over 200 million metric tons to reach 470 million metric tons, (Collier & Dercon, 2009).

A relatively unacknowledged, nevertheless promising, fact is that fish can participate intensely in satisfying the needs of the growing middle class income as well as meeting the food security needs of the poorest. Currently, fish globally provides more than one and a half billion people with almost 20 per cent of their average per capita intake of animal protein, and three billion people with at least 15% of high quality protein (FAO, 2014). Hence fish is termed the rich food for poor people.

Other than proteins, fish is rich in lipids, vitamins and minerals especially those whole eaten types. Actually, fish consumption in the word has doubled since 1973, with the developing world, which has also high population growth, being responsible for nearly all of this growth (FAO, 2013), resulting in over-utilization and decline of natural fisheries that has led unsurprisingly to a need for alternative sources of seafood (Naylor et al., 2000).

Currently, aquaculture is globally an established important means of food production resulting in its contribution to global seafood production to overtake wild fisheries for the first time (Fotedar & Philips, 2011). In spite of this admirable pace of growth, still the sector depends on genetically unimproved stocks as only 10% of aquatic products are produced using stocks with systematic genetic improvement programs; this is true for all aquatic species under culture, with exception of only salmons and marine shrimp (*Penaeus. vannamei, P. stylirostris, and more recently P. monodon*) stocks which were genetically improved in developed countries (namely Norway and USA) and are farmed there or elsewhere (Hulata, 2001; Benzie, 2009; Solar, 2009).

Malaysia possesses a long coastal land; hence brackish water aquaculture dominates its fishery sector. Fresh water aquaculture also has a bright future, as Malaysia is blessed by copious rainfall that averages at 250 mm per year with a larger range for the monthly spatial variation in the west coast region (Wong et al., 2009).

The giant Malaysian prawn (GMP), *Macrobrachium rosenbergii (de Man 1879)*, is native to east coast of India, Bay of Bengal, Gulf of Thailand, Malaysia and the northern Indonesian islands of Sumatra, Java and Kalimantan, (Wowor & Ng, 2007).

It is a commercially important aquaculture species in many tropical and subtropical zones as an endemic or exotic candidate cultured all year round. It could also be cultured seasonally in some temperate regions. As, to date, it has been introduced to more than 40 countries (Iketani et al., 2011). There has been a very rapid global expansion of GMP farming since 1980s. In 2009 the word production of all farmed freshwater prawns has reached 444000 metric tons, with a value of USD 2.2 billion; with GMP contributing by 51.7% of this amount (New & Nair, 2012). This indicates that the product is highly valuable for both local and export markets. This prawn can easily be produced by people of fishing and rural societies, amenable to integration with crop production and has no religious constrains. It is particularly well-suited to small-scale family businesses.

Therefore, its culture can participate effectively in poverty alleviation programs and empowerment of rural women. In addition, its culture has not been proved to cause any detrimental impacts on the environment, most likely because these prawns cannot be reared at densities as high as those commonly used in marine shrimp farming. Moreover, its productivity is generally lower, management is less labour intensive, potential for the abuse or waste of resources is minimal, and the grow-out of *Macrobrachium rosenbergii* , being freshwater organism, does not make agricultural land saline (Wahab et al., 2012). Furthermore, its producers can easily adhere to the FAO Code of Conduct for Responsible Fisheries would ensure that makes it remains sustainable and responsible.

Recently in Malaysia and some other countries in the region, GMP farmers were complaining of low yields as a result of low survival of postlarvae (PL) and slow growth that lead to ultimate decline in production. This decrease was noticed although prawns were

healthy and alert. The current study was conducted to check if inbreeding depression as a result of recruiting a few broodstock by hatchery operators in the process of producing PL was responsible for low juvenile survival that could ultimately lead to low production.

The advantage of in depth interview, which is one of the useful techniques of unstructured qualitative data collection, is deep discussion that increases insight into thought of target people and behaviour on important issues (Guion et al., 2011). Thus it was used to get a quick clue of reasons behind GMP produce decline in Malaysia as a result of low PL survival rate.

Economically important traits of the species namely, harvest weight; total body length, carapace length and tail length were assessed. These morphometric traits are controlled by several genes located at different loci, on different chromosomes, each with small additive effect; they can easily be measured phenotypically with both genetic an environment effects as well as their interactions. Accordingly, conventional quantitative genetics and animal breeding methods, heavily based on statistical approaches, were applied in the study. These conventional approaches to genetic improvement of livestock, plants and aquatic organisms were found to be very successful (Thornton, 2010; Kadarmideen, 2010; Jonas & de Koning, 2013; Jung, 2013). They namely change frequencies of required phenotypes such as tail weight in GMP. Modern molecular methods such as quantitative trait loci could be used to improve such economically important traits as well. In addition, as harvest weight is the trait that dictates harvesting time, its estimation as a natural response to the rest of other morphometric traits is of crucial commercial interest; therefore, multiple regression approach was used for this purpose. This approach unlike, simple regression approach performed between two variables at a time, is more comprehensive and more accurate

tackling all the covariates to estimate the response variable at a time. Yet the application of multiple regression approach on aquatic animals is still rare.

On another hand, modern molecular approach was applied to study genetic variability and structure in both parental ecotypes and their progeny families. The progenies were produced under cyclical mating of the parental ecotypes; hence molecular analysis was also used to evaluate the effectiveness of this mating system in securing genetic diversity and producing better growing progenies. Precisely, the cytochrome c oxidase subunit locus I gene was used as a mitochondrial DNA marker. Sequencing this gene is commonly used to differentiate among cryptic species (Decaëns et al., 2013) as well as for assessing population structure and genetic diversity in metazoan aquatic species. Many factors make the mitochondrial genes ideal for such studies; firstly, Mt DNA possess high mutation rate (5 to 10 times) compared to the nuclear genome (Brown et al., 1979; Thomas et al., 1997; Denver et al., 2004). Secondly, Mt DNA lacks introns (Costanzo & Fox, 1990; Matsuzaki et al., 2004) which means that the gene is continuous without any interruption with noncoding DNA portions; i.e. it is purely made of exons. Thirdly, Mt DNA genes are inherited only through the maternal side (Shitara et al., 1998; Wan et al., 2004). The cytochrome c oxidase subunit locus I gene has been specially used by many authors (Hebert et al., 2004; Lambert et al., 2005; Costa et al., 2007; Lakra, et al., 2011; Liu et al., 2011) for species barcoding as well as showing the interlinks of populations and past demographic events such as population expansion and shrinkage (Khamnamtong et al., 2009; De Jong, et al., 2011; Jimoh et al., 2013). In addition to the characteristics of the mtDNA mentioned above, the gene has a high degree of conservation with rare insertions and deletions rates (Moritz and Cicero, 2004) with many rapidly evolving nucleotide sites, which will allow

for differentiation among even recently evolved species (Nylander et al., 1999) or newly formed populations.

Most tropical countries (with exception of Singapore and Hong Kong), are considered developing. Consequently, enhancement in genetic improvement of aquatic species including *M. rosenbergii* is still in its infancy. So to the moment, little effort has been exerted for its genetic improvement. Consequently, problems related to genetics are expected to appear in these countries.

Malaysia, a country that witnessed the first ever closure of the species life cycle in 1961, has just started its genetic improvement program in response to high local and global demand for the commodity. Recently growers of the species were complaining of low postlarvae (PL) survival, slower growth rate and consequently a decline produce. As they have only two difficult choices of seed source; either to get them from domesticated broods which were hypothesized to be repeatedly recruited from few broods thus with less genetic diversity and subjected to inbreeding depression or from broods brought from the wild with high genetic variability but undomesticated, unimproved for production traits and with high risk of bringing diseases as well. Moreover, due of over exploitation, even wild broods may be subjected to inbreeding effects as only few of a season cohort are expected to escape be catching vessels to be parents of the next generation. Even if supposed to be highly variable broods, they are not necessary genetically superior in performance (Na-Nakorn, & Jintasataporn, 2012). So, in order to deeply understand this problem, and find out applicable solutions for the industry, this study was conducted to test whether inbreeding due to unintentional recruitment of broods is behind this low PL survival, slow growth rate

and the consequent produce decline. Accordingly after many deep discussions with experts and communications with producers, the following were tackled as objectives of this study:

1. To interview GMP farmers in order to know the channels through which they get their seeds and the way broodstock are recruited as part of investigating problems behind low productivity.

2. To evaluate the performance of GMP ecotype populations collected from geographically different locations in the Malay Peninsula and their progenies produced through cyclical mating by investigating the most economically important traits.

3. To characterize genetic differences among local stocks of *Macrobrachium rosenbergii* using the cytochrome c oxidase subunit I gene for the possibility of securing genetic diversity and reducing inbreeding effect by using cyclical mating.

The structure of the following sections in this study is as follows:

Chapter two: The aim was to give an overview of the biology of GMP, history of its culture and to shed light on genetic research on it, both locally and globally. Essentially, this study was a review of work conducted on aquatic species including crustaceans and vertebrate fish with ongoing breeding programs in different parts of the world.

Chapter three: The aim was to interview GMP farmers in Malaysia in order to trace their seed sources, the manner broodstock used to produce these seeds are recruited and managed and to find out whether inbreeding effects are behind the problem stated above.

Chapter four: The aim of this chapter was to evaluate growth related traits of the four GMP ecotype populations collected from equivalent riverine of the West Coast Malaysian and their progenies produced through cyclical mating for genetic improvement.

Chapter five: The aim of this chapter was to investigate genetic structure and genetic diversity in four ecotype populations of *Macrobrachium rosenbergii* and the effect of cyclical mating on securing the latter among progeny families.

Chapter six: The aim of this chapter is to provide a general discussion for issues raised in chapter three, four and five and to draw the study conclusions and recommendations.

CHAPTER TWO: LITERATURE REVIEW

2.1 Malaysian aquaculture

FAO has defined aquaculture as the farming of aquatic organisms in inland and coastal areas, involving intervention in the rearing process to enhance production and individual or corporate ownership of the stock being cultivated. In Malaysia, the Federal Fisheries Act defines aquaculture as the propagation of fish seed or the raising of fish through husbandry during the whole or part of its life cycle.

The fisheries industry in Malaysia is considered to consist of two major components, namely capture fisheries and aquaculture. This sector provides Malaysians with an important cheap source of high quality protein. For these products, Malaysia is a net importer; nonetheless monetary wise, the balance of fish import and export is positive. The sector contribution to the national economy in the year 2013, the fisheries sector had grasped about 3276 million USD (RM11466.53 million) showing an increase of 0.23% compared to 2012. The Food fish sector which comprises of marine capture fisheries, inland fisheries and aquaculture (excluding seaweed) produced 1749314 metric tons that worth 3091 USD or RM10818.60 million, with a decrease of 1.73% in terms of quantity but an increase 2.09% in term of value compared to 2012. Fish production from the fisheries sector remained to be the major sector's contributor; it reached 1.1% of the national GDP which is equivalent to 2.26 billion USD or RM7.91 billion.

Climate change is currently an issue that affects all agricultural sectors. Although Malaysia had recently faced long drought spells, but as a part of the Pacific region, the scenario of

climate change is more towards increase sea level, floods and tropical typhoons. However, the region is very vast and it is difficult to generalize few case studies conducted by FAO. In this study it is clear that a pronounced number of fish farmers complained of the consequences of water scarcity.

2.2 History and culture status of the giant Malaysian prawn (GMP) *Macrobrachium rosenbergii*

Macrobrachium rosenbergii is one of the major species produced under freshwater aquaculture worldwide because of its fast growth, large size, and pleasant taste, omnivorous eating habit as well as its better earnings compared to tilapia aquaculture and many other types of farming (Nandlal & Pickering, 2006). It is known under several local and common English names, the giant Malaysian prawn, the giant freshwater prawn, the river prawn, scampi and udang galah, its popular name in Malaysia and parts of Indonesia, are just a few. As cited by New et al., (2009), the species was known to Western scientists since 1705.

Wowor & Ng (2007) reported that habitat wise, the GMP, *Macrobrachium rosenbergii* (de Man 1879), is native to east coast of India, Bay of Bengal, Gulf of Thailand, Malaysia and the northern Indonesian islands of Sumatra, Java and Kalimantan. So, all this area is considered as the center of diversity of the species. Nevertheless, the species has been transferred from its natural home to other different places of the world, for either research purposes or commercial culturing; hence it is the one which has been introduced to more countries. Figure 2.1 shows states in which this prawn is currently under aquaculture. The species was first introduced to Hawaii from Malaysia, where Fujimura & Okamoto, (1972)

first developed its larviculture in the footsteps of the pioneer work of Ling (1969). As a result of this it overwhelms many countries in the tropics and subtropics for commercial culturing (Iketani et al., 2011). Actually commercial GMP production under complete captivity became possible in 1965 following the discovery of the obligatory requirement of larval stages of the species for brackish water conditions, which followed the development of commercial hatching protocol just mentioned. Currently, its aquaculture has expanded in several countries within and outside it natural range of distribution such as Thailand, India, Vietnam & Malaysia for the within habitat group and China, Brazil & USA for outside its natural range one.

Figure 2.1 Main producer countries of *Macrobrachium rosenbergii*
Note: Viet Nam is also a major producer of farmed *M. rosenbergii*; this is not illustrated on this map because Viet Nam includes its production within the category 'freshwater prawns, shrimps nei' (not elsewhere included) when making data returns to FAO.
*Source: FAO Fishery Statistics, http://www.fao.org/fishery/culturedspecies/Macrobrachium_rosenbergii/en

For instance, in Brazil, which area is more than 25 times that of Malaysia and is almost totally equatorial with immense freshwater sources; GMP is cultured semi-intensively, in earthen ponds systems, mainly in Espírito Santo State, where the largest Brazilian cooperative of producers (CEAQ) is located. In almost all cases, prawn culture is a secondary farming activity (New & Kutty, 2010), but some producers believe that prawns have more stable sale prices when compared with the primary products of the farms, and thus present higher profitability. Monoculture is predominant, but in Sa˜o Paulo, Minas Gerais and Parana´, there is little polyculture with tilapia. This polyculture, although it is still limited, but it has potential for expansion as the introduction of giant Malaysian prawn in tilapia ponds was reported to increase profits, (Almeida Marques & Moraes-Valenti, 2012). Therefore, they concluded that GMP farming currently has a favorable framework due to demand curve going higher as well as great improvements in the production chain.

A second example is Thailand, which is a neighboring country to Malaysia, is second only to People's Republic of China in annual production in 2008 (FAO, 2010); even though the species represents only 6.6 % of total aquaculture produce in the country in the year 2008 (Na-Nakorn & Jintasataporn, 2012). The conventional practice of this prawn culture in Thailand is more or less similar to the practice in Malaysia with a yield is approximately 2000–3000 kg ha^{-1} over 12 months. The disadvantage of this practice is having a long production period, producing different-sized animals which affect the price of the harvest and the intensive application of drugs especially antibiotics to prevent infection that may result from crowding caused by partial harvesting operations. However, recently an improved or modern culture practice has been described by Na-Nakorn & Jintasataporn, (2012). The practice considers the growth difference between sexes. In the initial period,

the mixed sexes PL are stocked for about 3 months after which they are manually sexed; the females are sold and the males are restocked at a relatively low density.

Malaysia is one of major producers of the species, but it faces fluctuations in its production. To improve production this research addressed the problem of seed as a bottleneck for expansion. A hypothesis of inbreeding depression due to recruiting broodstock based on readiness to spawn was discussed. Results of the study are expected to give good recommendations.

2.3 Biology of the giant Malaysian prawn or *Macrobrachium rosenbergii*

2.3.1 Taxonomy

Just few years back the nomenclature of this species had been confusing, however, this matter has been recently fully resolved by Wowor & Ng, (2008) and Ng & Wowor, (2011). So, the currently accepted taxonomy for GMP could be briefly stated as follows:

Kingdom: *Animalia* - Phylum: *Arthropoda* - Subphylum: *Crustacea* - Class: *Malacostraca* - Order: Decapoda - Family: Palaemonidae, Rafinesque, 1815, (superfamily: *Palaemonoidea Rafinesque*, 1815) - Genus: *Macrobrachium Bate*, 1868 - Species: *rosenbergii, de Man 1879*.

As just designated in the classification above, the species is a member of the genus *Macrobrachium* which is the broadest genus of the family *Palaemonidae* (New *et al.* 2009). Brackish water is obligatory for most of the species under the genus *Macrobrachium* in their larval stages to which *M. rosenbergii* is not an exception. Consequently, they are found in fresh waters linked directly or indirectly to marine waters. These fresh water

sources include rivers, irrigation canals, ponds and swamps in estuarine areas. Some of them prefer rivers of clear water, while others live in extremely turbid conditions; *M. rosenbergii* is an example of the latter group. It is the largest known palaemonid in the world and its males can reach up to 33 centimeters in total body length, whilst females can grow up to 29 centimeters (New, 2002).

2.3.2 Morphology

Figure 2.2 shows the external morphology of *M. rosenbergii* as reported by New et al., (2009). The body is composed of two major parts; the cephalothorax (head) which is comprised of 14 segments and the abdomen or the tail which is made up of 6 segments. The former portion is covered by a thick protecting cover called the carapace. Body colour is usually greenish to brownish grey sometimes bluish. It possesses very long second chelipeds covered by velvet-like fur in adult male with blunt spines. The rostrum, which is a protruding anterior projection from the cephalothorax; is long with 11 to 14 dorsal teeth and 8 to 10 ventral ones and later ridge may show red colour. As the name decapoda indicates, it possesses five pairs of true legs, last three pairs are walking legs; first and second pairs are for chelae or pincers so called chelipeds. Antennae and chelipeds are often blue. The telson is a median appendage on the posterior margin of the sixth abdominal somite, serve as tail fan, which is used, in making the animal able to propel itself in a backward direction. The antennae are often blue. The chelipeds may also be blue. The body is segmented with each segment bearing a pair of appendages.

According to Nagamine & Knight, (1980), *M. rosenbergii* can be sexually distinguished with the first appearance of gonopores in juveniles, at 5.9 mm (carapace length) for males and 7.6mm for females. Male gonopores are situated at the base of the coxae of the fifth pereiopods and are covered by coverings, while female gonopores appear as oval apertures on the coxae of the third pereiopods and are enclosed by a membrane. Male *M. rosenbergii* has a central knob or point, which can be felt with the finger. This feature is absent in female. Mature females have proportionally smaller heads and claws than males (Sandifer & Smith, 1985). They exhibit a typical brood chamber, formed by the first, second and third abdominal pleurae.

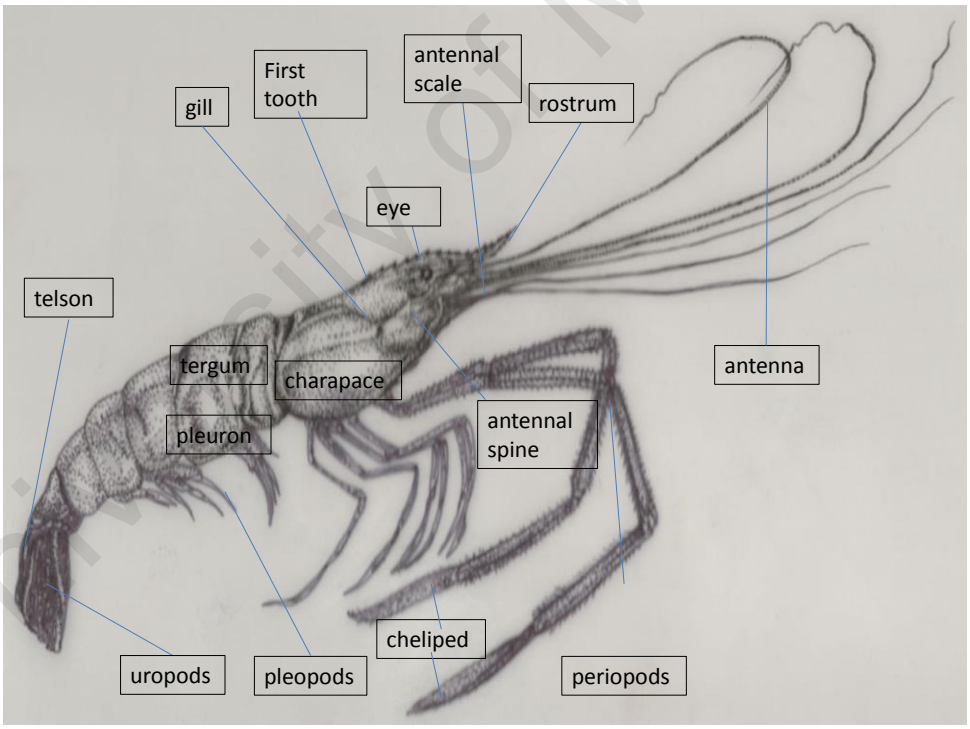

Figure 2.2 Morphology of the giant Malaysian prawn, *Macrobrachium rosenbergii* (drawn by "Designer" Mr. Ahmad Zakuan Bin Abdul Hamid (Center for Foundation Studies in Science, University of Malaya, 2016).

Macrobrachium also exhibit reproductive setae on the pleopods and thorax, which are functionally distinct: the ovipositing setae, which are mostly permanent, on the coxae of the last three pairs of pereiopods and pleopods guide the eggs during spawning, and the ovigerous setae, which only occur following a pre-spawning moult, are used to secure the eggs to the pleopods for brooding.

2.3.3 Reproductive system

The reproductive system of this species is situated in the cephalothorax. In the male it consist of a pair of testes, which are fused and lie mid-dorsally in the cephalothorax, each giving rise to a vas deferens (New, 2009). The paired vasa deferentia are made of tubes that end in terminal ampullae, which contain the spermatophores or a capsule containing spermatozoa that open at the gonopores on the coxae of the fifth pereopods. Two androgenic glands are found each on a vas deferns. This gland is responsible for the development of the male primary and secondary sexual characteristics.

A female prawn of this species possesses a pair of ovaries which are located dorsally on the sides of the proventriculus and dorsal to the digestive gland. They extend to a pair of oviducts which extend towards, and open into, the gonopores on the basal segment of the third pereopods.

Revathi et al., (2012) reported that ovarian development of *Macrobrachium rosenbergii* could be classified based on the size, colour and texture of the ovary. Stage I (spent stage) ovary is thin strand-like structure, very small in size, transparent, with no apparent ovarian tissue formation. Stage II (early previtellogenic stage) ovary is slightly larger in structure,

transparent and elongated. Stage III (late previtellogenic stage) ovary is further thickened, became dark yellow. Besides, Stage IV (early vitellogenic stage) ovary is characterized by orange colour. Stage V (late vitellogenic stage) ovary is dark green in colour and enlarged. At this stage of development the ovaries fill up the entire dorsal region of the prawn. Mature oocytes can be seen by an unaided naked eye. These variations were found to coincide with histological and biochemical changes. The protein content of the ovary in stage V shows significant increase (40 folds) compared to stage I. Hepatopancreatic lipid content also increases gradually through the previtellogenic stage and decreased at the vitellogenic stage. The variations in the vitellogenin content, an important protein as a food source for embryos, in hepatopancreas and hemolymph are significant during different ovarian stages. Cellular level changes were observed in oocytes during ovarian development. The stages I, II and III show oogonia, primary oocytes and previtellogenic oocytes, indicating that these stages correspond to early development of the ovary. The follicle cells are so distinct because immature oocytes lack yolk material. Besides, IV and V stages illustrated noticeable vitellogenic oocytes which indicate the mature stage of ovary.

In terms of hormonal control of gonadal development, the X organ- sinus gland complex located in the eye stalk plays a major role. The gonad stimulating hormone (GSH) which is present in the brain also has a role in reproduction control. When molting inhibiting hormone (MIH) and GSH levels in the haemolymph are low, and the levels of GIH and the molt hormone (MH) secreted by the Y organs are high, molting is induced. Low titres of GIH start the vitellogenic and spermatogenic processes. The ganglionic XOSG complex is the major site of neuroendocrine control in crustaceans, and is involved with several physiological processes, such as moulting, growth, sexual maturation and the regulation of

metabolism (Adiyodi 1985). Weixin et al. (1995) had induced spawning in the species by adding a juvenile hormone analogue. This can achieve synchrony which is beneficial for hatchery operation. Methyl farnesoate was also found to have a role in both reproduction (Laufer et al. (1993) and ecdysteroids levels which are essential in morphogenesis (Laufer & Biggers 2001).

2.3.4 Reproductive cycle

The reproductive cycle of this crustacean comprised of four major stages: egg, larva, postlarva and adult. Damrongphol & Jaroensastraraks, (2000) reported that mature females usually undergo pre-spawning molt which normally occurs at night resulting in animals becoming soft-shelled, however, males do not undergo through this stage. Once a female has undergone this molt, the pre-chosen male will begin to insert a sperm mass into its ventral thoracic region. As eggs extrude through the female gonophore, fertilization occurs externally. Fertilized eggs then adhere to the pleopods of the females and remain till hatching.

Nhan et al., (2009) and Habashy (2013) reported that a mature female may lay in a range of 435.2 eggs (body weight 4.71 g) and 3849.1 eggs (body weight 39.1 g). This is not much deviated from New, (2002) who reported that a fully mature female of *M. rosenbergii* can lay between 80000 and 100000 eggs in each clutch, but, their first clutches are often not more than 5000–20000 eggs. Ovaries frequently ripen again while eggs are being carried in the brood chamber. Moreover, Habashy (2013) also reported that fecundity was strongly correlated with total length and carapace length as well.

In the wild, gravid females migrate downstream, where salinity range falls between 8- 15 part per thousand (p.p.t) for spawning. At temperatures of 28-30ºC, normal egg development is characterized by a series of colour changes from bright yellow to orange to brown to grey-green. Gray-green eggs generally hatch within 24-72 hour, (D'Abramo et al., 2003). After hatching, larval development goes through eleven zoeal stages before transforming into postlarvae (PL) which is a small but resembles the adult prawn in shape and behavior. After reaching this stage, they migrate back to fresh water up stream but this migration is not immediate (Ling, 1978).

In nature larvae feed on planktons and larval stages of other crustaceans. They actively swim tail first with the ventral side facing the surface, (Nandlal & Pickering 2005). PL, like adults, swims forward with the dorsal side uppermost (Kumar, 2014), they also become benthic and can walk on substratum as well as becoming nocturnal omnivores in feeding habits (Scudder et al., 1981).

Morphologically, males of this species are classified into: blue- claw (BC), orange- claw (OC) and small males (SM). The BC males are socially dominant and have a low growth rate, while both OC and small males are subordinates with OC growing rapidly. With exception of SM, males are easily distinguished from females by their long and strong chelipeds compared to females. Apart from these features, the presence of gonopores in males may also be recognized by the appendix masculine, a spinous process adjacent to the appendix interna on the end of the second swimmeret, (Sandifer and Smith, 1985). In comparison to BC males, a female has smaller head, slender claws and the first three pairs of swimmerets are longer and broader, forming an egg incubating chamber. Females

generally become reproductively mature by 6 months of age. In more details, Nandlal & Pickering, (2005) explained that females are of three types of females; virgin females, berried females (egg carrying females) and spent females (open brood chamber). The virgin females are the young ones which had never spawned before; the berried females are the ones carrying eggs while the spent are those which spawned already.

2.4 Hatchery operation

Hatchery operation is executed to obtain seeds in order to stock grow out ponds. The first step for producing seeds is to obtain parents or broodstock that are capable of reproduction. The term broodstock normally includes only ovigourous or egg bearing females; however, under extended holding conditions such as in temperate regions or as recently practiced in hatcheries for genetic improvement programs (e.g. Pulau Sayak); adult males may also be included (New et al., 2009). In ovigerous or berried GMP females, eggs are easily visible through the translucent carapace. In equatorial tropics, such females can be obtained year round from farm ponds containing adult animals but the quantity of berried females available may vary according to the time of year. They can be obtained by cast netting but are frequently selected at times of partial or total harvest. Berried females can also be obtained from rivers, canals and lakes in areas where the species is indigenous. Some hatchery operators prefer to use berried females from natural waters based on the belief that wild females produce better quality larvae than pond–reared ones.

However, Nhan, (2010) reported that this is not necessary true, as some domesticated broodstock exceeded the wild ones in larval quality. Moreover, collecting ovigerous females from the wild often results in considerable egg loss during transport, therefore,

many hatchery managers prefer to use adjacent rearing ponds for their broodstock provisions. In addition, because of many viral diseases reported (NACA-FAO, 2011) for the species in wild stocks such as the white spot syndrome virus (WSSV), getting parents from the wild necessitates viral screening before bringing to hatchery facilities.

Most hatchery operators in area where GMP is native, do not aware of the genetic merits of their broodstock, hence they do not keep parental broods for a long time; as a consequence there is no much care about their nutrition. Rations with 40-44% protein and gross energy level of 400 kcal (100 g^{-1}) have recently been recommended, (Das et al., (1996) and Cavalli et al., (2001). Females collected for reproduction should have more than 75% of their carapace filled with eggs. For males, both BC and OC are chosen. Both sexes are kept sparsely in cement or fiberglass enclosures. When females approach egg laying stage by having ovaries filled with eggs; they will be put with males for mating. Successfully mated females will then be kept till hatching of eggs commences.

2.4.1 Artificial larval rearing

Developments in larviculture of GMP produced two main types of hatchery systems for seed production as described by New et al., (2009); which are namely: the flow-through and the recirculation systems. Flow through hatchery systems are based on regular water exchange, in order to reduce concentrations of toxic nitrogenous substances, faeces and food remains that accumulate in the larval rearing water. It can be a clear water system or a green water system. Clear water system envisages the rearing of larvae in clear water devoid of algae. The green water system makes use of algae rich waters for larval rearing. However the clear water system is generally favored due to the easier management and

higher efficiency (New et al., 2009; New, 2002). The recirculation system on another hand is based on continuous flow of larval water through closed physical and biological filters to remove food remains, faeces, dead larvae, exoskeleton debris of both cultured species larvae and artemia as well as their nitrogenous wastes. Consequently the recirculation system tends to increase larval stress and mortality, therefore, it is a recommended procedure for larviculture of *M. rosenbergii* (New, 2002). However, water requirements in flow through systems are very high and their use is recommended in hatcheries where both sea water and fresh water are available to the desired level. Luckily enough, both freshwater and sea water are abundant in all Malaysia as it is surrounded by sea and receives copious rainfall annually.

Generally hatchery operators feed newly hatched brine shrimp nauplii and freshly prepared non-live ration (D'Abramo and New, 2010). GMP larvae do not actively search for food, this is why *Artemia nauplii* that swim actively in the same part of the water column as the larvae) are such a suitable food. Feeding is recommended in the late afternoon of the first day of a hatching cycle. *Artemia nauplii* are provided five times per day in the second, third and fourth days, with the last and main feeding offered in the evening. Number of artemia nauplii meals is gradually reduced from the fifth day; by the tenth day, *Artemia nauplii* are only given at the evening feeding time. Larval survival depends upon many factors related to water quality and stocking density and feeding rate (Phuong *et al.*, (2003). Although lower stocking densities yield higher survival rates, but still medium densities result in greater numbers of postlarvae upon completion of the rearing cycle. About 3-6 *Artemia nauplii* ml^{-1} must be available in a larval rearing tank immediately after feeding, depending on the age of the prawn larvae, and at least 1 nauplii ml^{-1} should be left in the water just

before the next ration. The period required for larvae to develop into postlarvae is variable. It is practical to terminate a larval rearing cycle when 90% of larvae had metamorphosed into PL. Usually the first PL is expected to be seen between day 20 and day 26 (Ismael et al., 2001).

2.5 Management of GMP, *Macrobrachium rosenbergii* in earthen ponds

2.5.1 Grow-out systems

In order to produce GMP in captivity, growers need to mimic conditions of the river without facing the problem of low survival. Its production in complete captivity actually started after Ling's discovery of the fact that larval stages of this species require brackish water conditions in 1961 at the Marine Fisheries Research Institute in Penang, Malaysia (New et al., 2009). Following this event, Takuji Fujimura commencing his experiments in 1965 developed the first hatchery practices (Ling & Costello, 1979). After some failures applying the green water system, many growers in different countries abandoned it and changed to clear water system hatchery operation that may be followed by one or multiple stages of nursery periods. Hatchery operation and nursery systems were well described by (New et al., 2009). The main and the longest stage in breeding GMP is the grow-out stage. Different systems of grow-out were practiced in tropical and subtropical regions where prawn culture could almost be practiced all the year round. Under monoculture, prawn growing systems may be extensive, semi-intensive or intensive. These systems differ according to the pond design and the level of farmer intervention in the production process. Their definitions were standardized by New et al., 2009; accordingly they are described as below:

2.5.1.1 Extensive culture of the giant Malaysian prawn

A culture system is described as extensive when it is practiced in ponds, reservoirs, irrigation canals and rice fields with a total produce of less than 500 kg / ha / yr of giant Malaysian prawn. These waters are stocked, often from wild sources, /with PL or juveniles at 1-4 m^{-2}. Water quality is not controlled nor growth or mortality of the prawns is monitored; no additional feeding practice; and organic fertilization is rarely applied. Usually, it is impossible to drain these bodies of water or to carry out seining; hence the harvesting process is difficult and inefficient.

2.5.1.2 Semi-intensive culture of the giant Malaysian prawn

It is practiced in earthen ponds under different management approaches. After a keen selection of the site, ponds will be constructed and stocked with PL or juvenile GMP (usually from hatcheries) at 4-20m^{-2} in ponds, and result in a productivity level that range from 500 kg $ha^{-1}y^{-1}$ - 5000 kg $ha^{-1}y^{-1}$ depending upon the level of technology used. Fertilization is usually practiced and a balanced feed ration is supplied. Predators and competitors are also controlled with monitoring water quality, prawn health and growth rate. This form of culture is the most common in tropical areas.

2.5.1.3 Intensive culture of the giant Malaysian prawn

Intensive culture refers to GMP farming in small earthen or concrete ponds (up to 0.2 ha) provided with high water exchange and continuous aeration, stocked at more than 20 prawns m^{-2} and achieving an output of more than 5 000 kg $ha^{-1}y^{-1}$. Construction and maintenance costs are high and a high degree of management is required, which includes the use of a nutritionally complete feed, the elimination of predators and competitors, and

strict control over all aspects of water quality. The economic feasibility of this type of culture has not been proved yet.

Actually, farming giant Malaysian prawn differs from country to country; for example in Bangladesh there are two prawn farming systems: pond and gher. Approximately 71% of farmers are involved in gher systems which is an integrated prawn-fish-rice joint culture and the 29 remainder in pond systems. Although prawn farming practice is still traditional and extensive in nature, many farmers (20%) practice improved methods where prawns are cultivated semi-intensively. Extensive production typically use slightly modified versions of traditional methods and are described as low-density (10000-18000 post-larvae / ha / year) and low-input system. The system relies mainly on natural productivity (e.g., phytoplankton, zooplankton and benthos) of the pond, but organic and inorganic fertilizers are occasionally used to promote the growth of natural foods (Ahmed et al., 2008).

GMP in China is produced solely from aquaculture, as the animal, being cold sensitive; it is incapable of tolerating cold temperatures during winter in all Chinese provinces. The areas of the two main provinces that produce 40% of China's production are 102600 km^2 for Jiangsu and Guangdong 179,800 km2 respectively, (Hongtuo et al., 2012). The first, although the second smallest province in the whole country, it is the hottest economic spot. China as a whole is far from the equator (22 N is the nearest point to the equator), while Malaysia is totally equatorial. In India, a majority of prawn farmers own small farm areas of <4 ha followed by medium (4–8 ha), and big farmers holding more than 8 ha. Large-scale investments are rare in giant Malaysian prawn farming in India, because many of the corporate companies who had invested in coastal aquaculture during mid-1990s had

abandoned those areas in consequent to the operations becoming less profitable. Intensive giant Malaysian prawn culture systems do not exist in India. (Nair & Salin, 2012).

In Brazil, an equatorial country with immense freshwater sources but outside the natural habitat of the GMP is more than 25 times of the area of Malaysia; GMP is cultured semi-intensively, in earthen ponds systems, mainly in Espírito Santo State, where the largest Brazilian cooperative of producers (CEAQ) is located. In almost all cases, prawn culture in Brazil is a secondary farming activity (New & Kutty, 2010), but some producers believe that prawns have more stable sale prices when compared with the primary products of the farms, and thus present higher profitability. Monoculture is predominant, but in São Paulo, Minas Gerais and Parana´, there is little polyculture with tilapia. This polyculture is still limited, but it has potential for expansion as the introduction of giant Malaysian prawn in tilapia ponds can increase profits, (Zimmermann et al., 2010). Therefore, Almeida Marques & Moraes-Valenti (2012) concluded that GMP farming currently has a favourable framework due to demand curve going higher as well as great improvements in the production chain. Thailand which a neighbouring country to Malaysia, is second only to People's Republic of China in annual production in 2008 (FAO 2010); even though the species represents only 6.6% of the aquaculture produce in the country in 2008 (Na-Nakorn & Jintasataporn, 2012). The conventional practice of giant river prawn culture in Thailand is more or less similar to the practice in Malaysia with a yield is approximately 2000–3000 kg ha^{-1} over 12 months. The disadvantage of this practice is having a long production period, different-sized animals which affect the price of the harvest and the intensive application of drugs especially antibiotics to prevent infection that may result from the partial harvesting operations. However, recently an improved or modern culture practice

has been described by Na-Nakorn and Jintasataporn, (2012). The practice considers the growth difference between sexes. In the initial period, the mixed sexes PL are stocked for about 3 months after which they are manually sexed; the females are sold and the males are restocked at a relatively low density.

2.5.2 Pond preparation

Before a pond is constructed, the site should be surveyed to determine the elevation and the best layout for water intake and its discharge, access roads, elevation of dykes, pond bottom and structures such as piping. Ponds should be designed to be completely drained within a day. The size of the pond will depend on the availability of land and the farmer's choice of length and width. A length of 20-100 m and a width of 15–20 m are recommended. These dimensions will also match the size of the seine nets used for harvesting. The average depth of water in giant Malaysian prawn ponds in tropical areas should be about 0.9 m, with a minimum of 0.75 m and a maximum of 1.2 m; deeper ponds are difficult to manage. The banks of the ponds must be high enough to provide a freeboard of 30-60 cm above the highest water level expected in the pond, (New, 2002). Availability of the following equipment and facilities in any farm of the giant Malaysian prawn is crucial and vital:

1. A source of electrical power. 2. Roads and access paths. 3. Accommodation: every farm should have accommodation for some of its workers to live on site. 4. Fencing: a perimeter fence and, on larger farms, lighting, to prevent poachers. 5. Storage facilities: dry storage is needed for feeds (or ingredients), chemicals, nets, etc. and refrigerator (s). 6. Feed distribution and monitoring equipment. 7. Nets. 8. Water quality monitoring kit. 9. Predator protection. 10. Transport: larger farms will need trucks for feed distribution and prawn collection.

2.5.3 Water sources

The main water sources for aquaculture ponds are precipitation, runoff from watersheds and regulated addition of water from ground water aquifers or surface water bodies such as streams, reservoir and estuaries, (Tidwell, 2012; Swann & See, 1992). As with other aquatic species, excellent water quality should be maintained throughout the holding period. The tolerance of prawn broodstock to normally toxic compounds has not been defined yet, but based upon existing knowledge for other life stages; some general guidelines can be extracted. It can be assumed that the levels of ammonia (especially unionised), nitrite and nitrate should be minimised, with unionised ammonia and nitrite kept at negligible levels. Information concerning the effects of heavy metals on reproduction, either lethal or sub-lethal, is also not available, but extrapolations from the available literature suggest a need to be extremely cautious, (New et al., 2009).

2.5.4 Stocking

Stocking density in grow-out ponds varies according to the pond system (intensive, semi-intensive or extensive). Density has a major effect on prawn growth and a minor effect on survival. New et al.,(2009) reported that high stocking density (close to $20m^{-2}$) is usually associated with a high production of small prawns while lower stocking densities lead to the production of larger prawns, but low total weight per unit area. The stocking practices differ from country to another, in Thailand, in the conventional culture technology, postlarvae are stocked at a high density and prawns are harvested partially. The cropping period is long and the practice has led to disease outbreaks and deteriorated pond bottoms that result in frequent detection of antibiotic residues.

The improved culture technology involves manual sexing of the prawns at 3 months and restocking only the male prawns into new ponds at low density. The technique gives higher yield and reduces incidence of disease and thus the need for prophylactics, (Na-Nakorn & Jintasataporn, 2012). Whereas in China, the PL stocking density for grow-out culture is 900000–1500000 prawns ha^{-1} and the average density is 1200000 prawns ha^{-1}. Some silver carp fish are also stocked 10 days after the giant Malaysian prawn PL to eliminate blue algae. The fish density is 1125-1500 prawns ha^{-1} with a body size of 50–100 g $individual^{-1}$. All prawns are harvested before late October; the marketing size is 10–17 g (60-100 individuals kg^{-1}), (Hongtuo et al., and 2012).

2.5.5 Harvesting

FAO, (2002) stated basically two methods of harvesting: culling and draining. Cull harvesting is used to harvest market-sized animals from ponds at intervals and removes the faster growing prawns. In tropical ponds cull-harvesting usually starts 5-7 months after PL have been stocked, or sooner if juveniles have been stocked. After cull-harvesting commences, total seining of pond once per month or partial seining twice a month is necessary (i.e. seine half the pond twice per month or all of it once per month).

The method and efficiency of drain harvesting depends on the design of the pond. Efficient total harvesting can best be achieved through the addition of a catch basin within a drainable pond. As the pond is drained, prawns will accumulate in the basin. Oxygen deficiencies in the sump should be avoided.

2.6 Rice-Malaysian prawn integration

The Malaysian prawn and rice are integrated in many Southeast Asia countries, especially in the Mekong delta. In this system juveniles of this prawn are released into rice fields and are allowed to forage in an extensive system without feed provision. Canal area, which is used for prawn culture, covers about 15–20% of the total area of the rice field itself. Water levels are controlled to be 1– 1.2 m in depth in the canal and 0.2 – 0.3 m depth on the rice field (Wilder et al., 1999). Juveniles are usually stocked at a size of 5 – 10 g with a stocking density of 0.5 – 2 individuals per square meter. The major obstacle to further development of rice-prawn farming is not the system itself; it is rather due to the lack of a stable supply of seed and the inability of national hatcheries to meet farmers' demands, as described by Wilder et al., (1999).

2.7 Polyculture of the giant Malaysian prawn

Polyculture is the art and science of growing two or more compatible aquatic species together in a single pond with the objective of maximising production using fish species with different feeding habits or spatial distribution. The giant Malaysian prawn is cultured under polyculture systems in many countries in Southeast Asia and outside its natural zone. The most commonly used species are: tilapias; carps (Cohen et al., 1983; Wohlfarth et al., 1985), catfish (Miltner et al., 1983; Heinen et al.1987) and most recently the mola carplet (*Amblypharyngodon mola*) has been tried in Bangladesh (Kunda et al., 2007).

Both tilapia and prawns feed on benthic detritus in a multi-species polyculture system (Hepher & Pruginin (1981). This results in minimizing the amount of material to be decomposed by microbiota and thus reducing the biochemical oxygen demand. Prawns

haven't the ability to 'filter' free algae directly from water, however, Nile tilapia (*Oreochromis niloticus)* filter feeds on phytoplankton (Perschbacher & Lorio 1993) and, alongside, produce a second crop of marketable animals in polyculture systems (Dos Santos & Valenti, 2002). Tilapia and giant Malaysian prawn eat the faeces of grass and common carp, while common carp forage on partially digested algae from silver carp faeces. Garcia-Perez & Alston (2000) reported that under polyculture of prawns and tilapia returns increased by 21% compared to monoculture of each species. So, these species may be complementary to each other. The negative aspects of polyculture of tilapia and *Macrobrachium* prawns could be controlled by enclosing tilapia in cages. Tidwell et al. (2000) observed that total pond productivity increased by about 81% compared to prawn production in monoculture; suggesting that polyculture of tilapia may improve overall pond efficiency of pond production of the giant Malaysian prawn, even at temperate latitudes.

2.8 Genetic parameters

A phenotype (P) of an individual organism for a quantitative trait consists of two main elements, namely its genotype, (G) and the environmental components (E) which have influenced the phenotype during its lifetime. This relationship is expressed below:

$$P = G + E$$

When two groups of organisms that have similar genetic composition for a trait are raised in two environments which are not too different; it may be realistic to assume that the covariance between genotype and environment is zero. For under practical farming conditions with small or moderate environmental differences, there is often a zero or very low covariance between genotype and environment. The total genetic value, G, may be

divided into different components. The additive genetic value, (A), represent the sum of additive genetic effects from each locus and is also called the breeding value. Similarly, dominance genetic value, (D), represents the sum of all genetic effects from each locus which is caused by interactions within each locus. Epistatic genetic value, (I), represent the sum of all interactions between loci.

So, total genotypic value can be written as the sum of additive, dominance and epistatic genetic effects. (Gjedrem, 2005) as follows:

$$G = A + D + I.$$

Dominance (D) and epistatic (I) effects are not guaranteed to be passed to next generations because of their reconstruction as a result of mitotic cell division during gametogenesis. Consequently, the only guaranteed portion that can be passed to the following generation is the breeding value (A). Individual selection is usually the simplest method to operate and in many circumstances it yields the most rapid response

2.9 Procedures of selection

To pass a valuable trait to next generations, it must be selected for. Selection can be simply defined as allowing some organisms to be parents of the next generation while depriving others of this privilege. Thus castration which was practiced in animals can be considered as one of the earliest forms of selection. What selection actually does is it changes the frequency with which certain genes (or combinations) occur in a population, (Dalton, 1980).

Like domesticated plants and terrestrial animals, selection in aquatic animals could be done through different approaches. (Ponzoni et al., 2009) had described some of these procedures which include: individual selection or mass selection, within family selection, combined selection and cohort based selection.

2.9.1 Mass selection or individual selection

It is a sort of directional selection where superior animals for heritable traits are selected as broods based on their own performance in order to result in a change in gene frequency at the loci affecting the trait in the next generation. It is usually the simplest method to operate and in many circumstances it yields the most rapid response (Akvaforsk, 2005). It is successful with traits of high heritability estimates, and refers to selection based solely on individuals' phenotypes. However, in aquatic animals (with high fecund) its success is coincided with avoidance of inbreeding. Mass selection had resulted in problems in a number of fish breeding programs (Moav & Wohlfahrt, 1973; Moav & Wohlfahrt, 1976; Hulata et al., 1986; Teichert-Coddington & Smitherman, 1988). It is not successful for characters that require challenging fish with disease causing organisms or killing them to measure the trait in concern such as survival and meat quality characters. In addition this kind of selection will result in decreasing or loss of genetic diversity what so ever precautions are taken.

2.9.2 Within-family selection

This kind of directional selection requires families to be known. Such known families can be achieved by keeping fish in different enclosures. Then individuals with best performance above the family mean will be chosen to be the parents of the following generation. The

family could be of full-sibs or half-sibs. It is recommended when there is a large environmental effect common to members of the same family. Under this situation selection between families would be misleading for the confounding between genetic merits and environmental variance. So, selection within the same family, raised under the same environmental conditions could be reliable. Yet, this type of selection may increase the probability of decreasing genetic diversity, so at least care must be taken not to select siblings or closely related individuals to mate in the breeding programme.

When DNA information is incorporated in family selection it is termed walkback selection (Doyle and Herbinger, 1994). In this case families can be mixed in the same enclosure, later they will be traced back to their families through parents' identification.

2.9.3 Enhanced individual selection procedure through recurrent challenging (Prosper)

The Prosper process (PRocédure Optimisée de Sélection individuelle Par Épreuves Répétées) was designed to overcome the potential problems encountered in mass selection in order to achieve an efficient individual selection in fish. Its theoretical background relies on:

a) Maintenance of genetic variability by increasing the numbers of broods used at each generation in the selection process should be high enough (in the range of Ne = 100) to keep inbreeding to a reasonable level, b) Reduction of maternal effects variance by undertaking selection within groups of offspring from five dams with similar mean egg sizes, each group being crossed with a minimum of 10 sires. The rationale for this is that selection within group is relative efficiency better than individual based selection (Falconer

& Mackay (1996), Recurrent challenges by are distributed in 3 size classes, using the same truncation points for all groups (Chevassus et al., 2004).

2.9.4 Combined selection

This method of selection is based on individual information consolidated by information from relatives' such as offspring, full-sibs or half-sibs. It can result in improvements in growth related traits. But applying this method leads to decrease in genetic diversity. Moreover, it is costive and labor intensive.

2.9.5 Selection augmented by cyclical mating

In this method broods are divided into several arbitrary groups and mating is planned between individuals from different cohorts on a rotational basis to avoid inbreeding. An example of this method was performed by McPhee et al., (2004) in selecting crayfish. The experimenter divided the population into several cohorts or groups sampled from a previously established foundation population. Then a selection line was created, consisting of 20 cohorts as well as a control line of 8 cohorts. Offspring of each cohort were hatched and grown in a separate pen within a pond. At harvest, individuals of the heaviest weight in each cohort were chosen to be the parents of the next generation in the selection line, whereas individuals of average weight were chosen in the control line.

The giant Malaysian Freshwater prawn, *M. rosenbergii*, producers were noticed to recruit their Broodstock on the basis of readiness to spawn, which may lead to a loss of performance as the practice exerts an indirect negative effect on weight-at harvest. This can result in the smallest females being used, which may lead to a substantial reduction in mean

size across generations, (New, 1995). Conversely, (Kitcharoen et al., 2010) provided empirical evidence that selection made from early maturing female individuals of the giant Malaysian prawn *M .rosenbergii* yielded offspring that grew faster than did those from females selected from the later maturing batches. They found that the matured females were larger than immature individuals of the same sex, as shown by the significantly higher (P<.001) carapace length, body length, and total length for every maturing batch. It is likely that there was a threshold size for maturation, hence females were found to reach maturation at carapace length within (26.22–26.65 mm), body length (53.18–53.96 mm), and total length (103.88–108.30 mm) ranges.

Using another crustacean, in a program based on a founder stock from two populations of crayfish, *Cherax destructor*, as having good traits for aquaculture and involved a within-family selection protocol, coupled with a circular mating strategy, to select for faster growth rate. After two generations of selection, males and females from the selected families were 29.5% and 32.7% heavier than controls, respectively, (Jerry and Denis, 2005).

To make the picture clearer, light was shed on three shrimp species. Richardson & Humphries, (2010) collected *P. australiensis*, *C. mcculloch* and *M. australiense* from a 500 m reach of the Broken River in south-eastern Australia, they found that Although *P. australiensis* and *C. mccullochi* were similar in size (mean carapace length 5.38 and 4.58 mm, respectively) and *M. australiense* was much larger than either (mean carapace length of 12.33 mm), the two-way ANOVA indicated that all species were significantly different from each other (Tukey's tests between each pair (P<0.001). Results of this study and

literature reported confirm that growth related characters are heritable. So, they can be improved by applying genetic selection approaches.

2.10 Improvement of growth related traits of *Macrobrachium rosenbergii*

The giant Malaysian prawn, like all crustaceans, has a hard exoskeleton or casing that must be shed or moulted regularly for growth to occur. Increases in body weight and length of the prawn principally occur soon after completion of each ecdysis or moulting cycle. Growth is therefore, in a frog leap manner, which means it is incremental rather than continuous. Ra'anan et al., (1991), noticed that growth variation and the role of size in social structure of populations of the Malaysian prawn have clear discrepancy within different morphotypes. They discovered that about half of the population grows rapidly and variably, while the other half grows slowly and relatively uniformly, leading to a markedly, positively skewed size distribution.

Small immature female prawns had high growth rates, but their growth nearly ceased after maturation. This growth process produces adult females having a unimodal, symmetrical size distribution with a mean above the size threshold for maturation (about 18-26 g). In fact, the fastest growing, largest orange- claw male is the first to metamorphose to the blue -claw morphotype (at a size of 35 g). As other orange claw- males exceed this size, they transform in a sequential process so that the most recent blue- claw male is generally the largest blue- claw male in the population. Thus, growth of males is dispensatory throughout the process of morphotypic differentiation, leading to a wide size range of orange and blue -claw males. This makes genetic improvement in this species somewhat complicated (Kitcharoen et al., 2012).

Different results were reported for harvest weight of GMP in different localities. (Mohanta, 2000), declared that giant Malaysian prawn *Macrobrachium rosenbergii* was shown to attain an average weight of 92 g in males and 65 g in females in 11 months of rearing with a monthly increment of 0.70 to 20.0 g in males and 0.70 to 12.0 g in females.

While (Jain et al., 2008) found that the mean *Macrobrachium rosenbergii* weight gain in grow out at 2p.p.t was 73.13±0.06g started from 20 days old prawn larvae with a mean body weight of 0.010 g and reared for 150 days. On the other hand (Sripatrprasite & Kweilin, 2003) illustrated that the average weight of prawn caught from the reservoir that was drastically affected by the Pak Mun dam in Thailand was 236.5±102.1g, and the weight varied during hot, rainy and cool season at 288.1±94.9, 337.6±88.9 and 156.5±21.4g respectively. However, there was no significant difference ($P>0.05$) in average weight between hot, rainy and cool seasons.

Uraiwan & Panom, (2005) who studied growth comparison of three stocks of *Macrobrachium rosenbergii* after six months of rearing in grow out in concrete ponds in Thailand found that in NAGRI the length was 13.007±1.076 cm and weight 25.701±7.842 g, where as in Chantaburi the length was 12.517±1.034 cm and weight 23.363±11.588g and in Petchaburi the length was 12.463±1.343cm and weight 21.824±8.139 g. They concluded that *Macrobrachium rosenbergii* from the NAGRI stock are larger by 4% in length and 9-15% in weight than those of the Chantaburi and Petchaburi stocks.

Thanh et al., (2009) evaluated the growth performance of three strains of the giant Malaysian prawn that originated from geographically separated locations in Vietnam. At harvest (15 weeks) mean body weight, standard length and carapace length were 25.2 g, 9.67 cm and 3.30 cm (for both sexes combined) respectively and the coefficient of variation were 35.3%, 11.1 % and 14.5 % respectively. In the following year (Thanh et al., 2010) estimated additive genetic effect using a diallel cross of two local wild strains of the giant Malaysian prawn, *M. rosenbergii*, and a third domesticated Hawaiian strain raised in Vietnam . Among the three purebred strains, the Hawaiian strain performed best and the Mekong strain was the poorest performer. The Hawaiian and Mekong strains were 10.2 % heavier and 11.6 % lower the mean body weight of the pure strains (combined individuals from the three pure strains), respectively. Similarly, estimates of carapace and standard lengths for the Hawaiian strain were 4.0 and 3.6% above the means of the pure strains for these traits, while carapace and standard lengths for the Mekong strain were 4.3 and 3.9 % below the means of the pure strains, respectively.

2.10.1 Heritability of growth traits

Falconer & Mackay (1996) explained that heritability expresses the proportion of the total variance that is attributed to differences in breeding values, and this is what determines the degree of resemblance between relatives. But the most important function of heritability in the genetic study of metric characters is its predictive role, expressing the reliability of phenotypic value as a guide to the breeding value. Only the phenotypic values of individuals can be directly measured, but it is the breeding value that determines their influence on the next generation.

Different researchers in different regions have studied heritability for growth related traits in *Macrobrachium rosenbergii* and other related and less related species of aquatic animals. (Kitcharoen et al., 2012), estimated heritability for growth-related traits of GMP (*M. rosenbergii*) in Thailand prior and after morphological sexual differentiation; his estimates varied considerably with age. At two months of age, he found heritability estimates for carapace length to be (CL; 0.35 ± 0.15) and body weight (BW; 0.26 ± 0.13) which were higher than those estimated at 5 months old, based on mixed sex data. However, when data were sorted by sex, heritability calculated from data of females were higher than those of males for CL (0.26±0.16 vs. 0.10 ± 0.06), BW (0.28 ±0.17 vs. 0.12 ± 0.08), body length (0.40 ± 0.17 vs. 0.11 ± 0.07), total length (0.47 ± 0.18 vs. 0.11 ± 0.07) and claw length (0.29 ± 0.16 vs. 0.03 ± 0.04). The same trend was observed for the traits at 6 months old in both bulk and individual rearing systems.

Pillai et al., (2011) declared that when three populations of the giant Malaysian prawn *M. rosenbergii* were sampled from the states of Gujarat (West), Kerala (South West) and Orissa (East) in India to estimates additive genetic effect, the mean final body weight was 21.5 g for females and males combined and the coefficient of variation was 67.2 after 16-17 weeks post-stocking. Among the pure breeds, the growth performance of the Kerala stock was best and that of Gujarat was the poorest. Mean harvest body weight of the Kerala stock was significantly greater ($P<0.001$) than that of either Gujarat or Orissa stocks, but the harvest body weight of Orissa stock was not significantly different ($P>0.05$) from that of Gujarat stock.

In another study, heritability estimates on growth-related traits of matured GMP were found to be high only from the female side whereas those measured on the male side were not significantly different from zero (Kitcharoen et al., 2012). Therefore, these authors suggested to select for these traits through the female parent or to select before sex differentiation. However, finding a correction factor for male data may be a better approach than ignoring them. Chenette & Frahm (1981) deduced sex correction factor for birth, weaning and yearling weights in cattle. For these kinds of traits are controlled by additive genes carried on different chromosomes, no one with large effect. A male and a female animal differ in only in one chromosome that is unlikely to be responsible for this huge variation in all production traits.

On another crustacean species, Gitterle et al., (2005) conducted an experiment on the marine shrimp *Penaeus vannamei* , in a total of 448 full-sib families representing offspring from 204 sires and 442 dams, the estimated heritability for harvest body weight varied substantially and ranged between 0.24 ± 0.05 and 0.17 ± 0.04. They commented that the effect common to full-sibs is caused by the separate rearing of the families in net-cages until tagging and by possible maternal and non-additive genetic effects, which are confounded in their study. For harvest body weight this effect accounted for 7 % to 9 % of the total phenotypic variance. The degree of this effect in the study showed that body weight records should take account of this effect when estimating heritability.

In a study on *P. japonicus*, the average realized heritability for weight at six months of age was 23.4% which did not differ significantly from the estimate from the regression of offspring on mid parent of 27.7 % It was concluded that the heritability of growth in *P.*

japonicus is moderate but the rates of response to selection will be high largely due to the high levels of natural variation, (Hetzel et al., 2000).

In an experiment on *Penaeus monodon*, eighteen half-sib groups were obtained using artificial insemination of two females by each male. The total length and wet weight of the broods' were measured at 6 and 10 weeks after hatching. Heritability estimates for length and weight based on sire components were not significantly different from zero, and its mean for both ages was approximately 0.10. Heritability estimates based on the dam side were far larger than those based on the sire components and for both length and weight it decreased from 0.5-0.6 at 6 weeks to 0.3-0.4 at 10 weeks, (Benzie et al., 1997).

2.10.2 Correlations between growth parameters

An observed phenotypic correlation may be due to environmental and (or) genetic covariance. The covariance between two traits can be estimated at both the phenotypic and genetic levels, in a manner similar to estimation of phenotypic and genetic variance of one trait. Actually, genetic covariance and standard deviations are combined to estimate the genetic correlation, (Gjedrem, 2005). Final body weight of the giant Malaysian prawn, which is the ultimate resultant of growth, was found to be associated mostly with total body length, standard body length, and the carapace length. Accordingly, selection for this trait could be strengthened by measuring these parameters.

Growth performances of the giant Malaysian prawn (*Macrobrachium rosenbergii*) in different compartments of an integrated culture system were evaluated during 12 weeks culture period. Result on growth performance of *M. rosenbergii* shows the growth relative

rate, final length and final weight differed significantly ($p<0.5$). For the length-weight relationship, the growth coefficient (b) of *M. rosenbergii* was found to range between 2.37 to 3.38 at 95% confidence limit which concentration with prawns weighing 20 g recording the higher value and those weighing 40 g recording a lower slope. Regression between length and weight showed a positive relationship with R^2 values ranged of 0.89 to 0.99, (Idris et al., 2011).

Primavera et al. (1998), who studied *P. monodon* , reported that the most striking difference for both length-length and length-weight relationships in the species is by life stage/age, such that younger (and smaller) nursery juveniles have higher slopes than older (and bigger) grow-out and broodstock. They also added that body weight in proportion to length is greater in older animals.

In a study on Pacific white shrimp, (*Penaeus vannamei*), a population of the shrimp replicated in two environments was evaluated for genetic variability and co-variability of size traits. The genetic correlations for each size trait after the removal of fixed effects were lower than the phenotypic correlations, but results were found to be significant for all traits. The lowest ones were those between cephalothorax length with all other traits, and the largest ones were those between the abdominal length with total length, and abdominal weight with total weight, (Pérez-Rostro & Ibarra, 2003).

In an experiment conducted on black tiger shrimp, *Penaeus monodon,* in Taiwan it is clear that during the first three months of grow-out, different age groups tend to have different intercepts for their length-weight regressions. For individuals of the same length, those of

an older age (with slower growth in length) are heavier. Statistically, such a difference is highly significant (analysis of covariance, P<.0005 in most cases). Beyond the first three months, such a difference among age groups become less conspicuous and the significance of the difference is invalidated by heterogeneity of variances in many instances, (Cheng & Chen, 1990).

Most of the studies on length-weight and length-length relationships concentrated on drawing simple regression equations tackling the traits in pairs. In this study a multiple regression approach was performed. This study included all length measurements in one equation to estimate harvest weight using total length, carapace length and tail length as covariates or predictors.

2.11 Molecular population genetics

Genetic variation in diploid organisms is attributable to four main factors; namely; DNA mutation, gene flow, selection and random drift. These factors are behind population differentiation. Before the advent of molecular biology, in 1908 Hardy and Weinberg independently extended Mendel's principles to population level and derived a model known today as the Hardy-Weinberg equilibrium, which is the basic principle of population genetics. This principle could be stated as "In a large randomly mating population, gene and genotypic frequencies will remain the same from generation to generation in the absence of mutation, gene flow and selection. Assuming large population will eventually abolish random drift which can happen exclusively in small populations.

Current advances in molecular biology enable population geneticists to address genetic diversity and population structuring (Chauhan & Rajiv, 2010) by employing genetic markers. Actually, DNA carries genetic information in all living organisms, except for RNA viruses. Consequently, a difference in any of the molecular markers is associated with some difference in the physical structure of DNA. These structure differences could be reflected in the followings:

2.11.1 DNA sequence

A difference in nucleotide sequence is a clear way in which two homologous strands of DNA may differ. The differences may be in amino acid translated portions of protein genes (exons), portions of protein genes that are transcribed but not translated (e.g., introns, 5' or 3' untranslated regions), non-transcribed functional regions (e.g., promoters), or regions without obvious function.

2.11.2 Protein sequence

All amino acids have multiple genetic codons except for methionine and tryptophan, each of which possesses a single codon. This means that nucleotide substitutions in positions of a protein-coding locus may or may not result in proteins with a different amino acid sequence. Also some loci code for RNA strands that have an immediate function without being translated to a protein, e.g., ribosomal RNA and various small nuclear RNAs.

2.11.2.1 Secondary, tertiary, and quaternary structure

Differences in amino acid sequence may or may not lead to a different distribution of alpha-helices and beta-sheets resulting in a different protein structure.

2.11.3 Sequence organization

Particular genes may differ between organisms because of differences in the position and number of introns. At the whole genome level, there may be differences in the amount and kind of repetitive sequences, in the relative proportion of G-C relative to A-T, or even in the identity and arrangement of genes that are present. In microbial species, only a subset of genes is present in all strains. For example, in *Streptococcus pneumoniae* "the core genome" contains only 73% of the loci present in one fully sequenced reference strain (Obert et al., 2006). Similarly, a survey of 20 strains of *Escherichia coli* and one of its closest relatives, *E. fergusonii*, resulted in identification of only 2000 homologous loci in all strains compared to 18,000 orthologous loci identified (Touchon et al., 2009).

2.12 Copy number variation

Even within diploid genomes, there may be substantial differences in the number of copies of particular genes. In humans, for example, 76 copy number polymorphisms (CNPs) were identified in a sample of only 20 individuals, and individuals differed from one another by an average of 11 CNPs (Sebat et al., 2004). It is worth of mentioning that there are two main different types of genomes in nearly all eukaryotes; specifically: the nuclear genome and the mitochondrial genome. The mitochondrial genome is typically inherited only through the maternal line, although some instances of biparental inheritance are known.

2.13 Techniques of studying variations

Various techniques were used to reveal genetic dissimilarities. Of these are:

2.13.1 Immunological distance

Some molecules, especially protein molecules, induce an immune response in common laboratory mammals. The extent of cross-reactivity between antigens of two closely related species can be used as a measure of evolutionary distance.

2.13.2 DNA-DNA hybridization

Temperature degrees at which DNA of different species anneal can reveals the average percent sequence divergence between them. The percent sequence divergence can be used as a measure of evolutionary distance. Currently, the usage of both immunological distances and DNA-DNA hybridization in molecular evolution studies has drastically decreased.

2.13.3 Isozymes

Allozymes are different forms of a protein found in the same individual. They are co-dominant in nature which means that heterozygotes are also detectable beside homozygotes. So, studies applying allozymes detect genetic variation as polymorphism in proteins. Differences in a protein sequence result from the differences in the amino acids that form that protein and have a different structure. The most common proteins used in the electrophoretic studies are enzymes (Kapuscinski & Miller, 2007). But, currently studies using allozymes markers have shrunk due to its limitations that include low reproducible, less sensitive and less reliable results (Belaj et al., 2003).

Besides, patterns of variation at allozyme loci may not be representative of genetic variation that does not result from differences in protein structure such as variations that are related to variation in insoluble proteins.

2.13.4 Restriction fragment length polymorphism (RFLPs)

Restriction enzymes cleave DNA at specific 4, 5, or 6 base pair sequences. A single nucleotide change in a recognition site is usually enough to eliminate it. Thus, presence or absence of a restriction site at a particular position in a genome provides compelling evidence of an underlying difference in nucleotide sequence at that positon.

2.13.5 Random Amplified Polymorphic DNA (RAPDs)

With the introduction of the polymerase chain reaction (PCR) in the late 1980s, several related techniques for the rapid assessment of genetic variation in organisms for which little or no prior genetic information was available. These methods produce larger amounts of variation of the same taxa than allozyme and bi-allelic, dominant markers. They have the advantage, relative to allozymes, that they sample more or less randomly through the genome. They have the disadvantage that heterozygotes cannot be distinguished from dominant homozygotes, meaning that it is difficult to use them to obtain information about levels of within population inbreeding.

2.13.6 Microsatellites

Genetic variation of any nuclear DNA (nDNA) marker is characterized either at single loci or simultaneously at multiple loci. Single loci nDNA markers are either microsatellites (1–6 base-pair repetitive sequence) or single nucleotide polymorphisms (SNPs) (single nucleotide changes occur in DNA sequence). Because of high mutation rates of microsatellites at each locus, they commonly have many alleles. Moreover, they are typically co-dominant, making them more generally useful than dominant markers.

However, identifying variable microsatellite loci is more laborious than identifying AFLPs, RAPDs, or ISSRs.

2.13.7 Gene sequencing

The advent of automated sequencing has greatly increased the amount of population-level data available on nucleotide sequences. Nucleotide sequence data has an important advantage over most of other types of data such as allozymes, RFLPs, AFLPs, RAPDs, and ISSRs which may all hide variation. Nucleotide sequence differences need not be reflected in any of those markers. On the other hand, each of those markers provides information on variation at several or many, independently inherited loci. Nucleotide sequence information reveals differences at a location that rarely extends more than 2–3kb.

Electrophoresis, polymerase chain reaction and restriction enzymes (PCR-RFLP) are among molecular genetic techniques that are commonly employed to characterize genetic markers. Electrophoresis is applied to realize genetic variability at protein and DNA levels. Single locus DNA electrophoresis is determined from the pattern of the fragments that are labelled with fluorescent before undertaking gel electrophoresis. Regarding the multilocus DNA electrophoresis technique, restriction enzymes are used to cut genomic DNA at specific regions and these are then amplified with specific PCR primers.

PCR is a technique that requires very small amounts of DNA. Amplification of a specific region using specific primer produces millions of copies of DNA fragments. The DNA copies have to be processed by gel electrophoresis or subjected to further analyses such as DNA sequencing, cloning etc.

Detection of variation is based on whether or not the DNA was cut, amplified, and resulted in a different banding pattern. The size of the fragments and the presence or absence of the bands provides information on the genetic variation or similarity of the studied individual compared to other individuals.

Mitochondrial DNA markers have been successfully exploited in many research areas such as forensic medicine, phylogenetic and population genetic studies. Different regions of mitochondrial genes could be used as genetic markers. They could be either a single gene or a combination of several genes. The most popular mitochondrial markers are the control region and cytochrome oxidase I.

2.14 Analysis of sequence data

2.14.1 Neutrality tests

2.14.1.1 Tajima's D

This test distinguishes between a DNA sequence evolving randomly ("neutrally") and one evolving under a non-random process, including directional selection or balancing selection, demographic expansion or contraction or genetic hitchhiking. So, if the nucleotide sequence variation among certain haplotypes is neutral and the population from which they were sampled is in equilibrium with respect to drift and mutation, i.e Tajima's D will not be statistically different from zero. If it is either significantly negative or positive, we can infer that there's some departure from the assumptions of neutrality and/or equilibrium. When Tajima's D is positive the population is either under over dominance selection or population bottleneck. On the other hand if it is negative it indicates the population is under expansion or purifying selection.

2.14.1.2 Fu's Fs test

Fu (1997) proposes a different statistic based on the infinite sites model of mutation. He Has suggested estimating the probability of observing a random sample with a number of alleles equal to or smaller than the observed value under given the observed level of diversity and the assumption that all of the alleles are selectively neutral.

A negative value of Fs is an evidence for an excess number of alleles, as would be expected from a recent population expansion or from genetic hitchhiking. A positive value of Fs is an evidence for a deficiency of alleles, as would be expected from a recent population bottleneck or from over-dominant selection. Fu's simulations suggest that Fs is a more sensitive indicator of population expansion and genetic hitchhiking than Tajima's D. Those simulations also suggest that the conventional P-value of 0.05 corresponds to a P-value from the coalescent simulation of 0.02. In other words, Fs should be regarded as significant if $P < .02$ or less.

2.14.1.3 Mismatch distribution

It is the distribution of observed number of differences between pairs of haplotypes. This distribution is multimodal when tested samples are drawn from populations at mutation-drift equilibrium, but unimodal in populations that passed a recent expansion (Rogers and Harpending 1992) or through a range of expansion with high levels of gene flow between two neighbouring demes (Ray et al,2003, Excoffier 2004). The real data are compared with sets of simulated data under the assumption of demographic population expansion. To check their accordance, parametric bootstrap tests are used. The first test is sum of square deviation (SSD) between expected and observed mismatch of real population data and

simulated data and the seconded is raggedness index statistic. Raggedness index is calculated based on maximum number of differences and frequency of the allelic classes. Populations that have undergone demographic expansion are expected to have small SSD and smaller raggedness index in their mismatch distributions than non-expanded populations.

2.15. Cytochrome C Oxidase subunit I

Metazoan mitochondrial DNA markers are commonly used to assess genetic diversity and population structure in aquatic animals as well as other taxa. So, in this study the cytochrome C oxidase subunit 1 mitochondrial gene (COI) was used to study specified ecotype populations of the giant Malaysian prawn. Many factors make the mitochondrial genes ideal for such studies, including their high mutation rate compared to the nuclear genome (Brown et al., 1979; Thomas et al., 1997; Denver et al., 2004) their lack of introns (Costanzo & Fox, 1990; Matsuzaki et al., 2004) and their mono parental mode of inheritance (Shitara et al., 1998; Wan et al., 2004). Among the mitochondrial genes, the cytochrome c oxidase subunit locus I gene has been used by many researchers (Hebert et al. 2004; Lambert et al. 2005; Costa et al., 2007; Lakra, et al., 2011; Liu et al., 2011) for species barcoding as well as showing the interlinks of populations and past demographic events such as population expansion and shrinkage (Khamnamtong et al., 2009; De Jong, et al., 2011; Jimoh, et al., 2013). The reason behind this is that it has high degree of conservation with rare insertions and deletions rates (Moritz & Cicero, 2004) with many rapidly evolving nucleotide sites, which will allow for differentiation between even recently evolved species (Nylander et al., 1999).

Actually only few studies had extensively carried out on *Macrobrachium*. *rosenbergii* specimens collected from Malaysia. Bhassu et al., (2007) reported no genetic structuring retrieved from 18 populations in Malaysia using the LP-RADP marker. A second study on the same populations using another molecular marker, RADP, found that three major clusters exist in the populations studied (See et al., 2008) but no significant genetic differentiation was detected among populations belonging to Peninsular and east of Malaysia. A third unpublished study showed that populations exhibited high genetic differentiation (FST: .62503) mainly due to the sample from Sabah. However, an adjacent population to it, ,Sarawak ,was similar to those in Peninsular Malaysia, even though Northern Sarawak showed sub-population differentiation from the main cluster indicating that genetic diversity of Northern Sarawak was more restricted. The present study utilized a fast-evolving molecular genetic marker, mtDNA of the cytochrome c oxidase I (COI) gene using a sequencing technique to characterize the genetic structure of wild populations of *M. rosenbergii*.

CHAPTER THREE: THE REALITY AND CHALLENGES IN FARMING THE GIANT MALAYSIAN PRAWN, *MACROBRACHIUM ROSENBERGII*: INVESTIGATIONS IN LEVERAGING THE MALAYSIAN INDUSTRY OF THE MALAYSIAN GIANT PRAWN IN NURTURING AN INDIGENOUS NICHE

3.1 Introduction

The enhancement of agricultural sectors, including aquaculture, has been shown to have a strong and positive association with poverty alleviation and economic development in developing countries (Anríquez & Stamoulis 2007; Christiaensen et al. 2011; Delgado et al. 2003; Muhanji et al. 2011; Ndanga et al. 2013). The development of aquaculture in particular can create new job opportunities that may contribute to economic momentum (Anríquez & Stamoulis 2007; Cai et al.; 2009; Mapfumo 2011). Currently, aquaculture is the fastest growing agricultural sector in the world (FAO 2012; WWF 2015). As a relatively new sector, it is expected both to boost the existing supply chain of fish in some countries and create totally new sectors in others, such as small food stalls in pasar malam (literally night markets) in Malaysia or bukas (a type of local restaurants) in Nigeria (Cai et al. 2009; Hishamunda and Ridler 2006; Simopoulos & Bhat 2000; Miller and Atanda 2011; FAO 2014,), and thus contribute to poverty alleviation and sustainable agriculture in accordance with the commitments of organizations such as United Nations Environment programme (UNEP), Food and Agriculture Organisation of The United Nations (FAO), International Maritime Organisation (IMO), United Nations Development Programme (UNDP), The International Union for Conservation of Nature (IUCN) and World Fish Centre .

In Malaysia, as in other developing countries, investment in aquaculture is expected to substantially increase incomes in rural and coastal societies, reducing unemployment and poverty in these communities as well as imported food bills. All this is in line with the Malaysian national plan to place the nation among high income nations by 2020 (Mahathir Mohammad, (1991), Tenth Malaysian Plan, 2010). At the same time, even in developed countries such as Canada, the development of aquaculture has played an important part in revitalizing rural, coastal and aboriginal communities by providing meaningful year-round employment and a reason for youth to remain in their communities (Chopin, 2015).

Worldwide, aquaculture provides close to half (47 %) of all fish supplies destined for direct human food consumption, albeit fish is estimated to account for only 6.4% of global protein consumption and about 19% of total animal protein supply (FAO, 2012). In Malaysia, the fisheries sector as a whole currently provides direct jobs for more than 111,000 citizens (Malaysian Department of Fisheries or DOF 2013), with aquaculture representing 10 % of the sector. Within the latter, cultivation of the Malaysian giant prawn (GMP), *Macrobrachium rosenbergii*, which is a free-swimming crustacean , is gaining popularity because of its large size, fast growth, pleasant taste and omnivorous eating habits-all of which mean it can yield better income than tilapia or many other agricultural activities. Indeed, this prawn is one of the major freshwater commercial aquaculture species worldwide.

The species is known by several names: the Malaysian prawn, the Malaysian giant prawn, the giant river prawn, the giant Malaysian prawn, udang galah and scampi being just a few. The animal is indigenous to Malaysia (Wowor & Ng, 2007), thus its Farming is both

convenient and environmentally sustainable (Tidwell, 2003), as its territorial nature limits its density in grow out ponds (Karplus et al. 1992; Shivananda et al. 2012). To cultivate it successfully, suitable sites with warm temperatures, satisfactory quantities of cheap fresh water, enough sunshine and good wind exposure are needed (Nandlal & Pickelings 2005). All these conditions are abundant in many parts of Malaysia, making virtually all parts of the country suitable for such activity with exception of areas prone to flooding, landslips or storms. Furthermore, this species has something of a special relationship with Malaysia, as the country that witnessed the first ever closure of its life cycle in 1961 when Shao-Wen Ling, an ex- FAO fish biology expert, discovered that its larvae require brackish water conditions for survival above five days and completion of their developmental stages until metamorphosis into PL (New et al., 2009).

For all the above reasons, farming of *Macrobrachium rosenbergii* is important, and is anticipated to have a high future potential, in Malaysia. Currently, the total area using the fresh water aquaculture pond system is estimated to be around 5175.08 ha. This has resulted in the GMP being on the top list of exported commodities (Department of Fisheries 2013). With its near-ideal natural environment, well-trained fish biology experts and excellent infrastructure, Malaysia should be capable of developing an even more flourishing industry of this omnivore. Water, the vital input, is very cheap if not free, while land prices and rental are reasonable. Moreover, there is significant governmental aid available in the sector, through the provision of assets such as paddle wheels and seeds as well as extension services.

Despite all this, agribusiness companies still seem hesitant to invest in the sector, even though this does not involve large risks or uncertainties. There were several reports of incidences of viral diseases in Malaysia since 1997. But GMP producers surveyed in this study conveyed having experienced losses only once, that is – when a viral plaque of white spot syndrome virus or (WSSV) hit the country (NACA-FAO, 2011). Nevertheless, worries about disease and other constraints have led to production being lower than might be expected, as well as production fluctuations in the amount of total crop for the country (Figure 3.1).

The current study was conducted through in-depth interviews with investors in the entrepreneurial pond culture system of the GMP in Negeri Sembilan, in order to investigate the reasons behind these declines and fluctuations in productions.

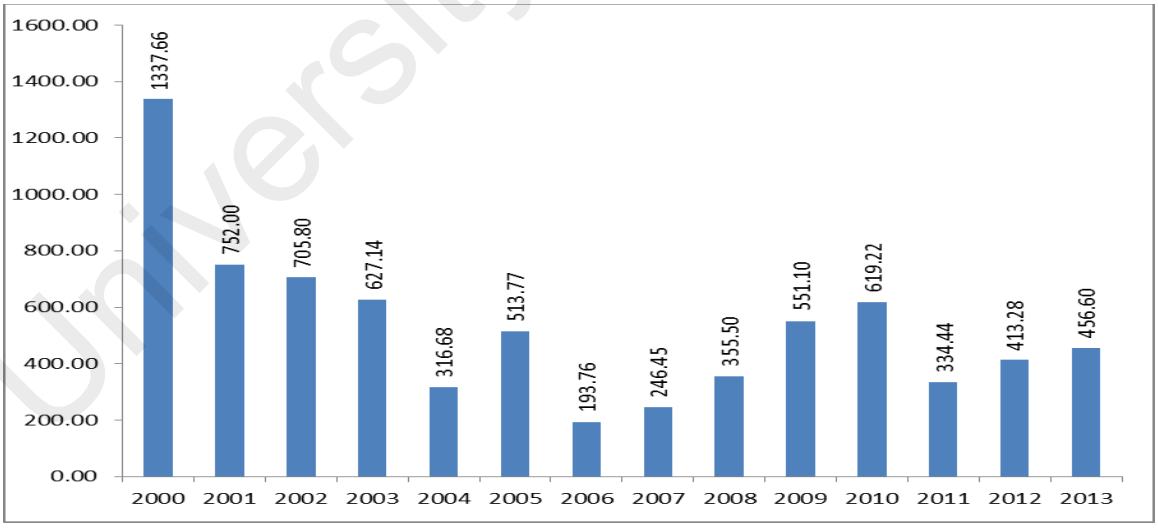

Figure 3.1 Fluctuations of the Production of *Macrobrachium rosenbergii* (metric tons) in Malaysia (2000 – 2013).
*Source: Annual Fisheries Statistics, Department of Fisheries, Malaysia.

3.2 Methodology

The data and information used in this paper were collected from available literature, unpublished data on the website of the Department of Fisheries (DOF), augmented with in-depth interviews with main stakeholders in the entrepreneurial pond culture-based GMP industry in the Malaysian state of Negeri Sembilan. The goal of the interviews was to explore in depth the respondents' views, feelings and perspectives concerning all aspects of their GMP farming agribusiness. For this purpose, intermediary persons were contacted in order to select suitable interviewees, as the address sheet available on the website of the DOF was not up to date. The interviewees were selected through different intermediaries based on purposive sampling. None of the informants was previously known to any of the research group or the interviewers. Earthen ponds are used by almost all of those involved in the entrepreneurial pond culture system (Figure 3.2). In order to reduce and focus further the number of interviewees, only those breeding in at least four ponds, with a minimum area of two hectares, were included in this research.

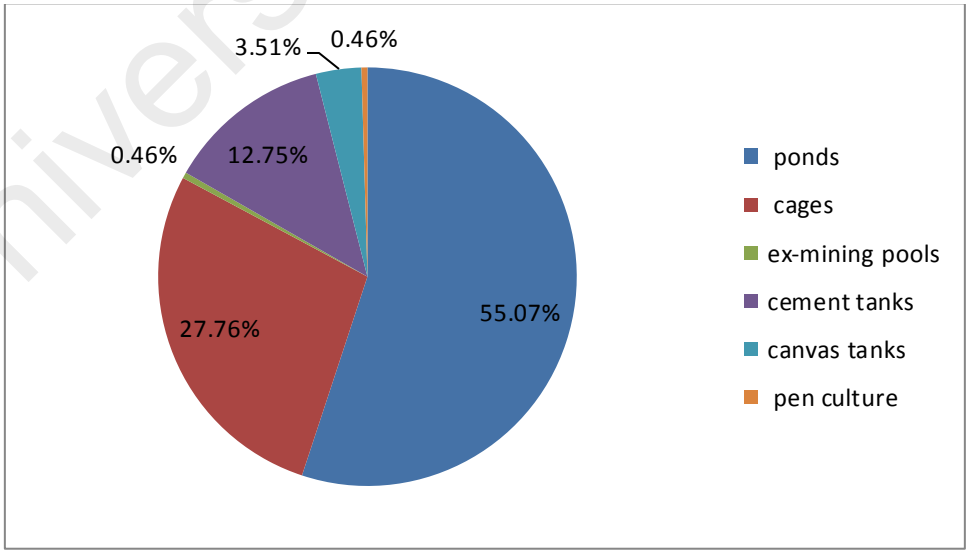

Figure 3.2 Methods of Freshwater aquaculture production in Malaysia (2013).
* Source: Annual Fisheries Statistics, Department of Fisheries, Malaysia

The interviewees were concentrated in the district of Kuala Pilah, because aquaculture itself is unevenly distributed within the state and, additionally, the major water source of springs are also uneven in their geographical distribution.

Interviewers made appointments with respondents at least a week before conducting actual interviews with them. All discussions were held on site, in order to allow the interviewers to conduct transect walk (Appendix A) to see at first hand the relevant farming conditions as well as to minimize disturbing the respondents' work. The questionnaire consisted of three parts: first, the personal data of the GMP farmers in the state; second, their occupations and the organization of their businesses; and third, their current pond management data, including any problems facing them. Negeri Sembilan was chosen for this study because it is a major production area of GMP as well as for its relative geographical proximity to Singapore, to where especially significant quantities of live prawns of the GMP species are exported (DOF 2013). Moreover, Negri Sembilan is also quite close to Kuala Lumpur, in which the bulk of Malaysian middle class income earners live. This class, as well as the rich, spends substantial amounts on recreational fishing, especially during weekends. These fishing ponds allow more people to fish while sustaining wild stocks.

The results presented in the sections that follow are first-wave (initial) results. The intention is that further waves will follow in due course, based on an updated and revised questionnaire and with wider coverage of all the major producing states in the country in order to produce a more comprehensive survey.

3.3 Results

3.3.1 Farmers and land tenure

The group of GMP producers in the entrepreneurial pond culture system who agreed to participate in the study were generally of middle age when they got into the business, and had a reasonable level of education (secondary school or above). They were all married, responsible people; some are even community leaders. Most of the respondents had no direct background in fish breeding, agriculture or biology. However, a few families had purposely sent their sons to study animal agriculture-related disciplines to help expand their business and sustain it in the long run.

Most of the respondents were practicing GMP farming under the entrepreneurial pond culture system as a full-time business. Most had also taken the step of registering their business, with few exceptions, to get bank loans and government assistance. A small proportion of them had registered their business names in order to be able to bring in foreign workers.

The majority of respondents cited better returns as the reason why they had opted for GMP production under the entrepreneurial pond culture system rather than other types of agricultural activities such as tilapia aquaculture or coconut or banana farming. The majority also said they were unlikely to go for longer-term plans that might require high input expenditure, mainly because they operate their business on rental land rather than owned land. Most respondents were reluctant to adopt recommended sustainable practices, simply because they feel that these practices had no tangible benefits for them and no direct effect on their sales.

3.3.2 Water sources, quantity and quality; and extreme weather events

In general, water sources for aquaculture are springs, wells, rivers, streams, lakes, surface runoff, ground water and municipal supplies (Valenti et al., 2009). But all the GMP farms under the entrepreneurial pond culture system included in this study depend solely on spring water from creeks on which small dykes are built (Figure 3.3).

Figure 3.3 A dyke built on a creek flowing from a spring at a farm of *Macrobrachium rosenbergii* in Negeri Sembilan State, Malaysia.

These springs are ideal sources of water because they are free of agricultural pollutants such as fertilizers and pesticides. Farmers use a continuous flow water system mainly to dilute metabolites produced by their cultured species, to aerate their ponds and to replace water loss by evaporation and percolation.

However, recently the area has faced water scarcity problem, mainly because of the prevalence of adverse weather conditions as Figure 3.4A and 3.4B show photographs of ponds that receive sufficient and deficient water respectively.

Figure 3.4A A pond receiving water from a sufficient water source at a farm of *Macrobrachium rosenbergii* in Negeri Sembilan, Malaysia

This resulted in some farmers to encounter problems with water or soil chemical properties. However, some growers who had never checked the quality of their soils and water unluckily faced soil pH and/or salinity problems that rendered their ponds unsuitable for GMP culture. Most producers feel that water quality was not an issue, and so only carried out rough checks on water quality based on the colour of the water.

Figure 3.4B A pond receiving water from a deficient water source at a farm of *Macrobrachium rosenbergii* in Negeri Sembilan, Malaysia

3.3.3 Entrepreneurial pond management practices

The entrepreneurial pond culture system is a sort of a balancing act between anabolic (building) and catabolic (degrading) processes resulting in growth when there is a positive difference between the former and the latter. Every aspect of management should therefore be tailored to achieve this goal. More specifically, the aquatic environment of a pond is an ecosystem that provides food, space, shelter and oxygen for all its components, including the species being farmed, phytoplankton, zooplankton, and benthic and microbial communities; and in turn receives metabolites (faeces, ammonia gill excretion, CO_2 etc.), from these components. Excessive concentrations of either released ammonia and nitrites on the one hand, or faeces on the other hand, are environmentally costly, since the former are potential toxins, while the latter consume oxygen as part of the process of their decomposition. To achieve the best crop, farmers therefore have to make compromises

between lower stocking densities – which means lower production – and replenishing the system with oxygen through aeration or water exchange (Brune & Drapcho 1991).

The entrepreneurial pond culture system chosen for this study inevitably involves significant capital investment – unlike the homestead system, which is semi-subsistent in nature and merely makes opportunistic use of existing ponds at a low opportunity cost. In terms of economics, Belton et al. (2012) described entrepreneurial farming as 'quasi-capitalist', shading into capitalist at the larger end of the scale, where paid labour completely takes over from family labour in routine farm operations. A summary of key data on the earthen ponds used by entrepreneurial pond management stakeholders respondents together with a flowchart of the activities carried are presented below in Table 3.1 and Figure 3.5.

3.3.4 Pond stocking

Under the GMP entrepreneurial pond culture system, grow-out ponds are filled with water about 10 days prior to stocking with juveniles (PL45- PL60). Even though the majority of farmers interviewed acknowledged that stocking with juveniles at 10 – 12 prawns m^{-2} is best, 16 prawns m^{-2} is reasonable, while above 20 prawns m^{-2} can lead to slow growth, their general practice is to stock more than 15 juveniles per square meter.

Table: 3.1 Criteria of Entrepreneurial pond culture system of GMP, *Macrobrachium rosenbergii,* in Negeri Sembilan

Feature	**Criterion**
Number of ponds	4 – 25, with most having less than 20
Pond length (m)	1500 –2100 m
Pond width (m)	12 – 45m, with most between 20 – 30m
Pond depth (m)	0.9 – 1.8m, with most around 1.5m
Pond construction cost (USD)	3000
Water depth (m)	1.2 – 1.5m
Electricity and fuel consumption (USD) month /	50 – 420
Water maintenance	Pump and ventilation
Stock population density (PL45) / m^2	Mostly 10 –15; a few in the wider range of 16 – 25
Feed	Starter feed (38% protein), grower feed (30% protein)
Length of cycle (months)	5 – 6 months
No of prawns / Kg	30 – 50
Type of harvesting	Continuous, beginning at 4 months of stocking up to 8 months (every two to three weeks)
Survival rate (%)	60 –70%
Production kg^{-1} $pond^{-1}$$cycle^{-1}$	2500 – 3000
Pond repair interval (years)	1 – 3 years
Time required to empty pond (hours)	4 – 48 hours, with most less than 6.
Facilities (offices, residential area, fridge, stores, vehicles etc).	Most possess these. A residential area is provided when workers do not live in a nearby village.
Nursery operation	Seldom practice
Nursery period (days)	45 – 60 days
Record keeping	Seldom
Source of fry	Hatcheries in Perak and Kedah states

In addition, a few of the respondents practice nursery operation, stocking newly produced PL in smaller ponds for a period of eight weeks and then grading these juveniles before final stocking in the main pond (Table 3.1).

1. Pond construction or cleaning of previously existing ponds by removing mud from pond bottom using pressurized water.

2. Drying ponds for about 10 days, until cracks appear on the surface of the bottom soil.

3. Application of lime at 1.6 tons / ha.

3a. Soil tillage after the soil dries (optional).

4. Filling the ponds with water to a depth of 1 – 1.5 m.

5. Fertilization: by urea and cow dung.

6. Seeding: PL 45, density 55 – 60 seeds / m^2 only good seed used in the pond at a density of 15 – 25 seeds / m^2

7. Seeds grading and restocking: diverting 45-60, density 16 – 20 seeds / m

8. Grading process: Grading is done twice: at 120 days and 135 days

9. Raising process: this process takes about 150 – 160 days

10. Harvesting: 35 – 40 prawns / kg (28 – 35 gram / prawns)

Figure 3.5 Flowchart for entrepreneurial GMP grow-out system

Moreover, grading and restocking at a lower density reduce aggressive behaviour between different prawn morphotypes. So, some farmers use recycled car tiers as substrates and place of hiding (Figure 3.6). The results of this study confirmed that stocking at a rate of 12 prawns m^{-2} is optimum and limiting the breeding cycle to 150 days being more beneficial.

Figure 3.6 The use of Recycled tyres as substrates and hiding places

3.3.5 Supplementary feeding

Apart from the natural food produced in the pond water by phytoplanktons and zoo planktons, the GMP farmers interviewed provide two additional types of concentrated feed: starter (mash form) and grower feed (pelleted), with crude protein contents of 38% and 30% respectively (Table 3.2). Feed represents some 40 – 60% of running costs, and so is rationed according to the age and mass of the prawns (Mitra, et al. 2005).

Table 3.2 Feed compositions of starter and grower types for *Macrobrachium rosenbergii*, grow-out in Negeri Sembilan, Malaysia

Ingredient	Starter (%)	Grower (%)
Crude protein	Min. 39.0	Min. 30.0
Crude fat	5-7	Min. 4.0
Crude fiber	Max. 4.0	Max. 4.0
Ash	15.0	Max. 13.0
Moisture	Max.12.0	max 11%

3.3.6 Pond preparation, chemical manipulation and predator control

A pond used for grow-out of the GMP is either new or old. Newly constructed ponds may need limestone to be added to adjust their total alkalinity 40 - 50 mg / L (New et al., 2009). Other than this, pond management practices are generally the same for both types. Respondents in this study disclosed that they repair their ponds at approximately three-year intervals. Repairs are usually carried out by hired labourers on contract based work. Pond drying should normally be carried out between every two stockings; all our respondents unveiled to do this. However, some farmers mentioned that they rarely see the bottoms of their ponds, indicating that they do not dry these ponds completely. As a rule of thumb, pond bottoms must be dried properly at least once per year (Boyd 1995).Respondents also mentioned that they control predators and unwanted fish by poisoning them at the end of each production cycle, using poisons like saponin extracted from local poisonous plants such as the *Madhuca* spp. (Kanuajia et al., 1996). Most of the respondents' feel that they do not need to fertilize their ponds, either by chemically manufactured fertilizers or by organic

by-products such as chicken manure or cow dung. Also most of them used to keep records of their pond management at their stating up their business; but after few years many became reluctant to do this.

3.3.7 Input supply

Contacted growers generally buy feed, fertilizers and other chemicals from company venues. But sometimes they may have inevitably to buy from local stores, at higher prices. All the producers complained about increases in the price of feed. There are many feed suppliers; however, there is only one company that produces feed.

Another vital input in this business is seed. The vast majority of respondents revealed that they faced problems in getting PL to stock their ponds, sometimes having to wait as long as three months. Some interviewees also stated that PL from foreign sources were all males, larger in size as a result, and with higher survival rates; on the contrary, others mentioned that, even although foreign-sourced PL are cheap, they are also delicate and so more likely to suffer illness, injuries or death.

3.3.8 Workers

Quite a few of the labourers working in the GMP farming are non-Malaysians. However, as most of these jobs are unskilled, this is not a sensitive issue. This kind of work can be taught to new labourers in a short period of time while on the job. Furthermore, overseas workers are cheap and, as they generally live in the farm, they are indirectly always on call. In any case, these immigrant workers still do not represent a significant proportion of

workers in the industry; moreover, only a very small number of them have become farmers (Table 3.3).

Table 3.3 Culturists and workers in Malaysian *Macrobrachium rosenbergii* industry according to race and gender (2013)

Farmers	**Total**	**Male**	**Female**
	18004	16924	1080
Bumiputeras		14437 (566)	1069
Chinese		2,319 (233)	7
Indian		61 (10)	1
Others		107	3
Workers			
	12114	10832	1282
Local Malay		8954	1225
Local Chinese		991	51
Local Indian		79	3
Indonesian		539	0
Thais		29	3
Bangladeshi		115	0
Others		125	0

* Data in brackets show the number of farmers in Negeri Sembilan. All farmers are local.

* Source: Annual Fisheries Statistics, Department of Fisheries, Malaysia.

3.3.9 Harvesting and production economics

Entrepreneurial GMP farmers in Negeri Sembilan harvest their prawns by reducing the amount of water in the pond and then gently scooping them up in a net. Prawns are then transferred to a cement tank filled with clean spring water, where they remain until being shipped to the company that ordered them. Most of the crop is sold to companies running recreational fishing ponds or is exported to Singapore. The mean selling price, for both types of customers, depends on the grade (size) of prawn such as the one shown in Figure 3.7 below. Prawns weighing 30-35 pieces per kilogram sell for approximately 16 USD. However, prices as high as 23 USD / kg can be earned for a grade with 6 to 7 or fewer pieces per kg.

Figure 3.7 A farmer showing a healthy male prawn *Macrobrachium rosenbergii* ready to harvest in his farm (Negeri Sembilan state, Malaysia)

The above prices are high because most customers either use the prawns for recreational fishing or are exporters. By comparison, in Thailand the mean price for similar prawns was 4.0–9.3 USD per kg in 2010. However, ninety per cent of Thai product is consumed locally (Na-Nakorn & Jintasataporn, 2012). In countries outside the region, such as Brazil, GMP are also sold according to weight, being generally classified as small (15–25 g), medium (25 – 35 g) and large (35 – 45 g), and with size preferences varying among regions (Almeida Marques & Moraes-Valenti 2012).

Table 3.4 The GMP, *Macrobrachium rosenbergii*, grow- out costing details according to age and different stocking densities for two types of cycle lengths.

Feed			**Stocking**		
Age (day)	feed (g)/prawn	Cost USD/prawn	Number of postlarvae	180 days (USD)	150 days (USD)
1 – 15	0.01	0.0215	1	12.27	12.25
15 – 30	0.019	0.0308	1000	67.74	57.4
31 – 45	0.108	0.0462	11000	623.073	509.14
46 – 60	0.238	0.0769	12000	678.60	554.36
61 – 75	0.24	0.1077	13000	734.12	599.50
76 – 90	0.255	0.1231	14000	809.58	664.48
91 – 105	0.266	0.1538	15000	866.00	709.88
106 – 120	0.286	0.1848	16000	920.62	754.97
121 – 135	0.312	0.2000	17000	976.14	800.06
136 – 150	0.319	0.2154	18000	1031.67	845.16
151 – 165	0.34	0.2308	19000	1087.18	890.56
166 – 180	0.342	0.2462	20000	1142.71	935.70

Table (3.4.) presents some up-to-date information on feed costs for operating GMP entrepreneurial ponds on two different cycle lengths (the conventional 180 days and a modified 150 days), which we believe are of importance.

3.4 Discussion

The data in this study are based on only twenty farmers due to the unwillingness of some farmers to share cost and profit information for commercial proprietary reasons, as well as to the inherent constraints of using in-depth questionnaires to study a single or small number of units (in the case of GMP farmers using the entrepreneurial pond culture system in Negeri Sembilan). A similar unwillingness to share information was observed in a value chain analysis of aquaculture in Kenya, reported by Ndanga et al. (2013). In the case of the present study, we were also constrained by our concern to treat the respondent farmers in an ethical manner and hence not to pressurise them.

The level of education of the respondent farmers was relatively high (secondary school or higher) – which may have been a factor in their decisions to become pioneers by adopting a relatively new technology such as GMP farming. This dovetails with what was reported in Mozambique (Uaiene et al. 2009) on farmers' adopting new technologies. In the present study, the respondents made the decision to go into GMP farming despite not having any previous experience of fish-related agribusiness or any related education, albeit some respondents made good use of their engineering backgrounds to address pond design and topography problems.

Turning to how respondents developed their GMP businesses, it is clear that most took a rational and prudent approach: starting as part-timers using only a small number of ponds initially and then gradually expanding, so as to avoid the risks of investing a lot of money in a business with which they were not yet familiar. With time, most farmers gradually gained experience, and with it the confidence to expand their businesses as well as to involve themselves totally (full-time) in these.

The entrepreneurial pond culture farmers interviewed were generally reluctant to keep records after the first few cycles of production, mainly because they run a family business in which detailed accounting is not really required. Costs such as electricity, other utilities, wages and salaries, and inputs like feed are relatively easy to memorize or to be estimated accurately. This is in contrast to cooperative farms, with their many and heterogeneous stakeholders, where good record-keeping and professional, transparent management are necessary. As a general rule, as farmers gain experiences their desire for expansion increases. However, small growers like our respondents tend to require all their land to be in one place to make its management easier, so when planning to expand it may be difficult for them to find land for sale or rent in the same vicinity.

Another constraint on expansion is age – and the lack of succession planning. As the farmers we interviewed approach senior citizenship, they become less willing to expand their agribusinesses because, unfortunately, most of their sons and daughters, as university graduates, desert the business in favour of a job in a big city. The younger generation do this; it seems, not so much for the money (the city job may actually pay less than working in the family agribusiness) as for the lifestyle. This is a cause of bitterness and regret

among some of our older GMP farmers. However, a few families with clear longer-term business plans send their sons to study fishery-related sciences so that they can take over the business, expand it further, and keep the family name alive. On the other hand, younger medium-income entrepreneur farmers who do GMP farming part-time in the evenings and on weekends often have a desire to expand; but they are worried about the risks and uncertainties associated with the agribusiness, so they keep their day jobs as a guaranteed income source and continue to do farming as a source of secondary income.

In sum, what can be anticipated in the future is that while the individuals involved may change, GMP farming will continue to expand until it exhausts all available suitable land. The market for GMP is likely to remain buoyant, because as the middle class grows, so will demand for recreational fishing, as well as the consumption needs of local rich people and tourists. In addition, the export market, especially for live prawns, with markets such as Japan and Hong Kong remaining open.

As stated earlier, most of our respondents run their prawn farms on rental land. Land tenure in the study area is a complicated issue, as most of the land is inherited and thus has multiple owners. Interestingly, those renting the land are mostly men, while the land owners are mostly women; this is because the area is dominated by the Minangkabau clan, which is a matrilineal society that passes communal land ownership entirely through the female lineage (Stark 2013). Women comprise the majority of land owners but they mostly hire their lands to men who run farms.

Water quality is another key issue. The majority of the ponds in our survey receive most or all of their water directly from springs, which means that they are largely free from agricultural residues such as fertilizers and pesticides. Most of our respondents feel that there is no problems with water quality, thus they only look at water colour when monitoring its quality. Virtually none of them consider buying water quality measuring devices as a priority even though these instruments, while expensive, are crucial tools that every pond-based commercial fish farmer should possess.

Water supply, i.e. availability, is another potential problem for the future. The continuous water flow system applied by the farmers in this study is water intensive. However, there is no guarantee that such huge quantities of water will be continually and reliably available for the rest of this century, given global climate change and particularly the prevalence of extreme weather events in South–East Asia within the last twenty years (Trenberth & Hoar 1997). More specifically, Manton et al., (2001) reported a 0.1 - 0.3ºC increase in temperature per decade in the last half of the twentieth century, as well as a decreasing trend in the number of rainy days throughout the area. This was reported to negatively affect groundwater levels, resulting in diminishing water volumes and ultimately a reduction in the refilling potential of groundwater and aquifers in large pond areas (Bosma, & Verdegem, 2011).

In Malaysia, nobody will forget the dry spell of 2014 that even affected municipal water supplies in big cities such as Kuala Lumpur, forcing the authorities to introduce water rationing affecting about 90% of the residents in the metropolitan area (Tan, 2014). By the same token, many of our respondents confirmed that the dry spells of 2014 had drastically

affected their business. A number of farmers were forced to keep lower quality pond water, as their ponds might otherwise have dried up and they would then have lost their whole GMP crop. This led to ponds containing dark green water due to algal bloom, which in turn causes oxygen deficiency, especially in the early morning. The photographs in Figures 3.4A and 3.4B show the contrast between a pond suffering from water scarcity and another with no such problem. Some farmers disclosed that they had even observed their prawns trying to jump out of the water when it rained after a long dry spell due to the sudden change in the chemical properties of the water (when it rains, run-off water can carry eroded soils with different chemical properties in sufficient quantities to affect pond water characteristics).

Ultimately, tackling major environmental problems such as soil erosion and climate change impacts will require a strategic approach at a national and international level. However, the farmers themselves could take actions to mitigate potential water scarcity problems during dry spells. For example, they could harvest rainwater. Or they could invest in increasing the capacity of their dikes and pump excess water during times of abundance into elevated emergency tanks, in order to guarantee a smooth flow when water is in short supply.

Energy to pump water for the latter purposes could be generated by renewable energy sources such as solar cells, windmills, or a mix of the two. In addition, these energy sources could be harnessed to provide increased aeration in ponds, providing scope for increased stocking density and hence greater total production. These kinds of energy sources could be financed through loans provided by banks or by the Ministry of Agriculture and Agro-based industry, with farmers paying these back in small instalments. The main risk

involved is that the renewable energy equipment might be stolen, but this would be unlikely if farms were guarded or at least permanently occupied. Furthermore, using renewable energy is cost-effective in the long term because it is environmentally clean and costs very little to maintain and repair. The authorities could encourage the use of such renewable energy sources by disseminating information on them to farmers. Even better, installing a hybrid renewable energy source on the premises of the farmers' association as a demonstration for growers, and organizing visits for interested farmers by the Department of Fisheries, would be an effective way of encouraging renewable energy usage by example.

The respondents differed in various pond management practices such as stocking density, juvenile grading, nursery operation, fertilization, feeding, liming, pond repair and drying. Although the vast majority of respondents knew the recommended stocking density (number of PL45 to be stocked) and the consequences of overstocking, most did not adhere to this – perhaps in an attempt to compensate for their high PL mortality rates (more than 30 %). Overcrowding leads to slower growth and hence delayed production cycles. However, luckily the effects of such delays on farmers are not so serious, because the environment is suitable all year round and so there is no rush to complete cycles before the onset of winter, as is the case in temperate regions. Furthermore, farmers can still sell delayed produce at decent prices, because the latter are mainly dictated by prawn size.

Quality and quantity of seed are further vital elements for the GMP business to flourish. Unfortunately, the industry in Malaysia has faced unstable production (Figure 3.1), similar to other countries in the region such as India (Nair & Salin 2012). One of the reasons for

this low productivity are high PL mortality rates, which may in part be caused by the practice of recruiting small numbers of broods on the basis of their readiness to spawn rather than their genetic merits (Mather and De Bruyn 2003; New et al. 2009; Kitcharoen et al. 2010; Nair and Salin 2012), leading to cumulative inbreeding effects. High PL mortality may in turn have affected PL prices, which are generally expensive in Malaysia at around 20 USD for 1000 PL (this study), compared to other countries in the region. For instance, PL prices in Thailand were estimated to range between 1.9 – 2.2 USD for 1000 PL (Na-Nakorn & Jintasataporn, 2012).

The results of this study showed that detrimental inbreeding effects are likely to be among the major causes of production fluctuations in Malaysia, because hatchery operators often collect broodstock from the same farms they previously supplied with seeds. Similar results were reported in India (Nair & Salin, 2012). Stunted growth as a result of diseases in ponds attributable to poor water quality cannot be ruled out either as causes (Pillai *et al.*, 2005) of such fluctuations. However, although the Thai GMP industry faces similar problems to Malaysia and India, loss of genetic diversity and inbreeding effects have had a much smaller impact on their production levels (Charoentawee et al., 2007). This may be because, according to Charoentawee et al. (2007), Thai hatchery operators (unlike their Indian and Malaysian counterparts) tend to pool broodstock during the collection process without regard to their source or origin, which may have the unintended effect of broadening their genetic variation resulting in heterozygosity ranges comparable to those of wild populations (.69 – .70).

Information on the genetic heterozygosity of GMP populations in Malaysia is available only for wild stocks, with the range being 0.53 – 0.83 (Abdul Razak, 2011). Respondents in this study gave contradictory information on the quality of foreign-sourced GMP seeds, primarily because most of them had not tried such sources. However, the Malaysian Ministry of Agriculture and Agro-based Industry informed the researcher that, as of December 2014, no company had applied to import seeds of the GMP species from anywhere abroad. We suspect that the lack of reporting by our farmers on procuring seeds from abroad may due to the fact that most foreign seeds available in Malaysia are actually smuggled from neighbouring countries. This means that they have not passed through the quality tests that ought to be routinely performed by the Department of Fisheries.

A permanent strategic solution for this seed problem would be to keep the whole production chain under complete captivity, keeping in mind that the genetic diversity of broodstock is of paramount importance in the breeding and management of this species. Broodstock should be collected from a variety of different ponds to prevent an accumulation of detrimental inbreeding effects and to guarantee genetic diversity. Furthermore, this would minimize, if not completely eliminate, the risk of bringing disease in from the wild. Using molecular biology for regular checks of genetic diversity could also be practical and affordable as such tests are becoming cheaper.

The failure of some GMP hatcheries in Negeri Sembilan may have been caused by pollution from the oil-spill dispersant Corexit 9527 reported in Malaysian estuarine waters (Law, 1995). However, hatcheries that rely on deep sea water sources are generally successful (own observation). Actually, the number of hatcheries in the area is quite

limited, making bringing seeds from distant states the only choice. A small proportion of our respondents possessed specialised nursery ponds, in which they keep PL_{45} bought from hatcheries for about eight weeks, and practice regular juvenile grading throughout the production cycle, enabling them to substantially reduce the interval of their production cycles and eventually produce five cycles over two years instead of the four cycles obtained by most other farmers. Moreover, their produce is more homogenous in terms of size.

Pond fertilization may not be necessary if a complete diet is offered. But with feed prices escalating, feeding complete diets may become increasingly expensive. When prawns are given only supplementary feeding, fertilization indeed has a positive effect on production (Tidwell et al. 2013).

Feed availability and quality is a major obstacle for the aquaculture industry as a whole (FAO, 2005), and that was the main area of complaint from most of our respondents. Regarding availability, the paradox is that good quality imported feed is expensive, while some locally produced pellets are of low quality. That may explain why the trade in good quality feed is governed by an invisible cartel, if not by outright monopolism. As a result, farmers have no choice over where to buy from. It is high time for the government of Malaysia to protect farmers, who are part of the food security chain. Turning to quality, protein and crude fat content tend to be within recommended ranges, but the percentage of carbohydrate – an essential component of diet for any animal – is frequently not given, nor is the energy level. In addition, minerals are just put under ash without specifying the Ca and P, which are macro elements. Vitamin C, another important requirement, is also often not given. Some respondents seek to improve their feed by adding corn, which they believe

makes the prawn meat firmer. Moreover, crushed corn is large in size, so unwanted fish cannot compete with prawns for it. Further study on this issue may need to be conducted.

Pond repair is another problematic issue, as the overwhelming majority of respondents are renters, which makes it is difficult for them in terms of cost benefit analysis to take decisions to repair enclosures that they might have to leave in due course. One possible solution to this problem might be to include in rental contracts a clause providing for compensation to be paid to renters for any repairs they have carried out on ponds within a specific period (say a maximum of three years) prior to the termination of the lease. This could be fair to both parties, as landowners can run ponds themselves or lease them at a better rental if they are in improved condition.

Complete pond drying between consecutive cycles is important in order to break the life cycle of some predators as well as diseases. Failing to do this can lead to an increase in predator populations in ponds and adversely affect the following season's production. Predators and other unwanted fish species are commonly controlled by treating dried out ponds with a poison from local plants that contain saponin, such as Madhuca spp. (Kanuajia et al. 1996). As a rule of thumb, pond bottoms must be dried adequately at least once per year (Boyd, 1995).

Currently, a lot of technical information is available on the DOF Malaysia website. However, detailed information on cost analysis is available only in commercial project proposals intended for sale, not online. Making this kind of information available could

help to bring millions of dollars to the country that hundred times overweigh the costs of selling books containing it. We therefore suggest that the Ministry of Agriculture and Agro-based Industry buy the right to publish such proposals and put them free on its website. As a contribution to this process, the current study (Table 3.4) provides up-to-date information on feed cost, which accounts for more than 60% of running costs. However, many factors other than the costs of inputs and outputs can affect prawn prices, such as social and religious events like Chinese New Year, Christmas and Aidulfitri (a religious festival for Muslims), plus competition from wild catch.

As GMP is produced for commercial purposes, we should take a hard look at the market for the product. Statistics on GMP production and trade in Malaysia used to be included with shrimps, which are significant, representing 50% of fishery sector exports in 2009 as an example. But we are confident that the market for GMP produced in Malaysia is quite open and expanding. Supply still lags behind growing demand. That demand is driven by, on one hand, the increasing number of local recreational fishing ponds; and on the other hand, by the growing middle class and tourists. High demand for live prawns in Singapore is a further factor. Malaysia is best suited to respond to this demand due to its proximity. It has a substantial advantage over other, more distant major producers of GMP such as Bangladesh, China and India (New & Nair 2012; Hongtuo & Yiwei, 2012), which have to ship live prawns by air, dramatically increasing costs and reduce competitiveness. In addition, China and India have a large enough and growing middle class to consume the bulk of their own production. Neighbouring Indonesia, for its part, does not have any significant production of prawns on the mainly touristic islands near to Singapore. Hence, among the major producers, the main potential competitors are Thailand and Myanmar

(New & Nair 2012; Na-Nakorn & Jintasataporn, 2012). Thai exporters, however, need to cross the entire Malay Peninsula from north to nouth, which requires at least one resting station for the prawns in water for two or three days, plus feeding them and accommodating for the travelling crew, and the risk of higher mortality. All these factors combined reduce Thailand's competitiveness. For Myanmar the situation is even more difficult.

Finally, larger-scale investment is greatly needed to improve and expand the GMP sector in Malaysia. This task should be pursued vigorously by the Department of Fisheries, as well as by investment and commercial attachés in Malaysian embassies that could help to persuade larger companies to bring international capital to GMP aquaculture.

CHAPTER FOUR: EFFECTIVELY IMPROVING GROWTH RELATED TRAITS, MEASURING THEIR HERITABILITY ESTIMATES, AND PREDICTING BODY WEIGHT IN MACROBRACHIUM ROSENBERGII

4.1 Introduction

The Malaysian Giant Prawn (MPG), *Macrobrachium rosenbergii* (de Man, 1879), is a catadromous crustacean under the genus *Macrobrachium*. It is known under several common English names as well as local names. These names include: The giant river prawn, the giant Malaysian prawn, the Malaysian prawn, scampi in India, galda in Bangladesh, udang galah in Malaysia and parts of Indonesia (Java and Borneo) is universally important for aquaculture in the tropics and subtropics. Its natural distribution includes east coast of India, Bay of Bengal, Gulf of Thailand, Malaysia and the northern Indonesian islands of Sumatra, Java and Kalimantan, (Wowor & Ng, 2007).

Genetic improvement of farmed fish by selection has a paramount advantage over other types of genetic manipulations such as polyploidy and sex reversal by being permanent, i.e. it is fixed in the line or strain once it is achieved (Akvaforsk, 2005). In general, the response to selection for growth rate in aquatic species is exceptionally high compared to that of terrestrial farm animals. Particularly, genetic response for growth rate in these species with begun selective breeding programs ranged between ten and fifteen per cent per generation (Gjedrem, 2000; Quinton et al., 2005; Ponzoni et al., 2005; Eknath et al., 2007). In addition, these species have high fecundity that allows applying narrower selection differentials in improvement programs, thus reducing time required to achieve a certain level of improvement. Particularly, economically important quantitative traits for *M.*

rosenbergii were reported to show significant differences between ecotypes or strains (Thanh et al., 2009; Thanh et al., 2010; Pillai et al., 2011). These traits were described to express heritability estimates that range from moderate to high (Nugroho et al., 2005; Kitcharoen et al., 2012; Dinh et al., 2013; Dinh & Nguyen, 2014). Therefore, geneticists can use such information to achieve progress in improving these important traits through selective breeding as long as there is variation.

Aquaculture of the GMP as an important species under culture in Malaysia has recently experienced fluctuations in production (DOF, 2013). These fluctuations in production may have been caused by high postlarvae mortalities as a result of high inbreeding levels attributed to procurement of broods directly from grow-out ponds based on their readiness to spawn that includes early maturing small females rather than their breeding values (Mather and Bruyn, 2003; Bhassu et al., 2009; Thanh et al., 2010). This latter practice may have caused an indirect negative response on weight at harvest. These reasons lead the researcher to study variation of harvest weight (WT), total body length (TL), carapace length (CL) and tail length (TA) in four ecotype populations of this prawn originally collected from different geographical locations in the Malay Peninsula and produce cohort progenies in order to estimate heritability values of the traits in question.

On another hand, yield amount combined with some other economic factors such as scarcity and season; ultimately dictate the decision of starting time of harvesting in grow-out ponds of the Malaysian prawn, *M. rosenbergii*. More precisely, harvest weight (WT) as a whole and tail weight as the most valued and greatly consumed part, are of paramount economic importance. It is easier to measure any length measurement of live prawns than

weighing them, so regression models that can be applied to estimate WT as response variable using morphometric measurements as predictor variables (Andem Andem et al., 2013**;** Hung & Nguyen 2014) are crucial. These measurements are proved to be less variable and more easily taken under field conditions (Primavera et al. 1998; Lalrinsanga et al. 2012), helpful in describing growth in wild populations (Abohweyere & Williams 2008; Deekae & Abowei 2010) demarcating the stocks and comparative growth studies (Primavera et al. 1998; Sampaio and Valenti 1996; Peixoto et al., 2004), management of cultured species without putting them under unnecessary stress, as well as having high association with fecundity and total yield (Habashy, 2013).

Most of the research conducted on morphometric measurements of prawns in general concentrated on obtaining mathematical relationships between two variables at a time (Abohweyere & Williams 2008; Deekae & Abowei 2010; Andem Andem et al., 2013) for various purposes. In this research the researcher shepherded a stepwise multiple linear regression model to estimate harvest weight (WT) as a response variable and using total body length (TL), tail length (TA) and carapace length (CL) as predictors. The researcher thinks that multiple regression- approach seems to be more logical, more robust and more realistic for estimating WT than performing several simple regression relations between WT and a single predictor at a time and choose the one with highest R^2 as the best predictor. There may be high collinearities among predictors, as in this study case, and hence they overlap in explaining the same variance. In addition, researchers should not forget that simple regression is special version of multiple- regression. Moreover, from aquaculture point of view, estimating WT more accurately has a paramount economic

importance. After all, reports applying multiple regression models of any type for estimating harvest weight in the Malaysian prawn are still subtle, if any.

However, most researchers who studied weight length relationships used drawing simple regression equations between WT and the predictor variables at a time and concluding that the predictor variable with the highest coefficient of determination (R^2) is the best estimator. Unluckily, much information may be hidden behind excluded predictors just because of their relatively lower (R^2) and hence completely ignored and considered unimportant. Consequently, it looks more logical and practical that all predictors simultaneously affect WT, hence stepwise multiple linear- regression seems more robust and broader than the simple model conventionally used especially under aquaculture conditions. Accordingly the researcher decided to apply multiple linear -regression model as statistical model to estimate body weight using all or most known morphometric body measurements that were reported to be associated with growth. Currently, to the researcher knowledge there is no any report of a multiple- approach, either linear or polynomial on WT length relationships in this species under aquaculture conditions. This approach was expected to show a clearer picture of the species growth under aquaculture. It would also allow involved companies' personnel to be able to estimate the yields of their ponds more robustly and hence be able to take the decision of starting harvest optimally.

4.2 Materials and methods

4.2.1 Collection and conditioning of broodstock ecotype populations

Four GMP broodstock ecotype populations were collected from geographically different natural drainage basins along the west coast of the Malay Peninsula (Figure 4.1), namely;

Kedah from Kota Kuala Muda River, Perak from Perak River, Negeri Sembilan from Timon River and Johor from Muar River. The ecotype populations were named according to state of origin in Malaysia, namely Kedah, Johor, Perak and Negeri Sembilan. Samples of these ecotype populations were conveyed to a hatchery belonging to the Marine Aquaculture Institute, National Prawn Fry Production and Research Centre (NAPFRE) (i.e FRI) of the department of fisheries (DOF), Malaysia, located at Pulau Sayak village, (5.6667 N and 100.3333 E) and kept separately in 5000 L cement tanks at initial stocking density of 7 individuals / m^2.

Figure 4.1 the locations of the studied *Macrobrachium rosenbergii* ecotype populations in Malaysian states of Kedah, Perak, Negeri Sembilan, and Johor

Air supply systems were previously set up for each tank and operated continuously to maintain adequate (5 – 7parts per million or p.p.m) dissolved oxygen concentrations. The animals were kept with least possible disturbance for conditioning. Thus water changing was practiced only on weekly basis, if its quality did not deteriorate so badly.

Broodstocks were fed on blood cockle and squid once a day. However, females with eggs at grey stage which just require 2 – 3 days to hatch would be fasted as their eggs do not benefit from their mother's nutrition, so balancing this out with water quality deterioration that may occur by feeding them as well as the disturbance that may be caused beside. Prawns will be quite fine with skipping feeding for 3 – 4 days.

4.2.2 Broodstock data collection

Broods of the four ecotype populations were collected. Four body traits were measured on each individual in all samples; they included: harvest weight (BW) in grams, total length (TL) in centimetres (cm) from the tip of the rostrum to the tip of the telson, carapace length (CL) in cm from the eye to the first abdominal segment and tail length (TA) in cm, from the first abdominal segment to the tip of the telson. Estimation of tail weight required scarifying the animal and because the animals may be used for breeding purposes this trait was excluded. In addition to body and carcass weight traits, records of sex were also documented.

4.2.3 Mating design

Newly brought broods would be quarantined for two weeks besides checking for the most common viral diseases prior entering the hatchery. This was followed by choosing them for production characteristics and morphotypic states for both sexes. Females chosen were adult, virgin with more than 90 % of their ovaries filled with eggs. The eggs could be seen through their translucent carapaces. For the reproduction period was relatively short, all chosen males were of BC morphotype with persisting pairs of chelipeds.

A cyclical mating design as shown diagrammatically in Figure 4.2 was adopted for this study. This design is ideally based on arbitrary dividing of broods intended for breeding into groups such as putting four groups of a certain ecotype population or genetic group (as an example for J ecotype population J1, J2, J3 and J4) each in a separate tank and rotates the males to produce the first generation. This kind of mating will hamper development of deleterious inbreeding effects. The principle is that male progeny of one ecotype population are mated with females of a different ecotype population or family. The transfer follows the pattern indicated in the diagram. Males are transferred in the directions indicated by the arrows, whereas females stay in the group where they were born. The pattern of transfer varies with the generation number, (Nomura & Yonezawa, 1996).

For the current data, each of the four parental ecotype populations was considered as a separate foundation population, as well as being selected for growth traits, thus suitable for cyclical mating design. Accordingly, each progeny family was obtained from a maximum of four sub-groups, if all were successful, or a minimum of one group depending upon the success of obtaining PL. Eight progeny families were attained in the first generation.

Successfully produced progenies would not be full sibs or half-sibs with exactly determined or known parents but an amalgamation of individuals which at least share being from a common population. However, if any two individuals were taken at random they would share a common ancestor in the few back generations with a higher probability than with individuals from other populations. The data were statistically analysed to investigate differences among the four parental groups and their progenies.

The sex ratio of the breeding stock was 1:2 female to male for each of the four sub-populations made from each of the four groups. They were then held in a separate fiberglass tank for conditioning. The ratio of males was deliberately made so high in order to guarantee that every female that had gone through a premating moult would be fertilized, to guard against many females becoming berried at the same time and also to guard against some females' preference to certain males in couple choosing because the social hierarchy in this species is very complex (New et al., 2010). All mating tanks were checked daily to pick out elite berried females to remove them and put them into a larger (100 L) tank at a stocking density of 40 individual per m^2 to avoid unnecessary disturbance from checking activities. Berried females were checked 10 days after being restocked into a purposely darken which would be checked every two days after the first check

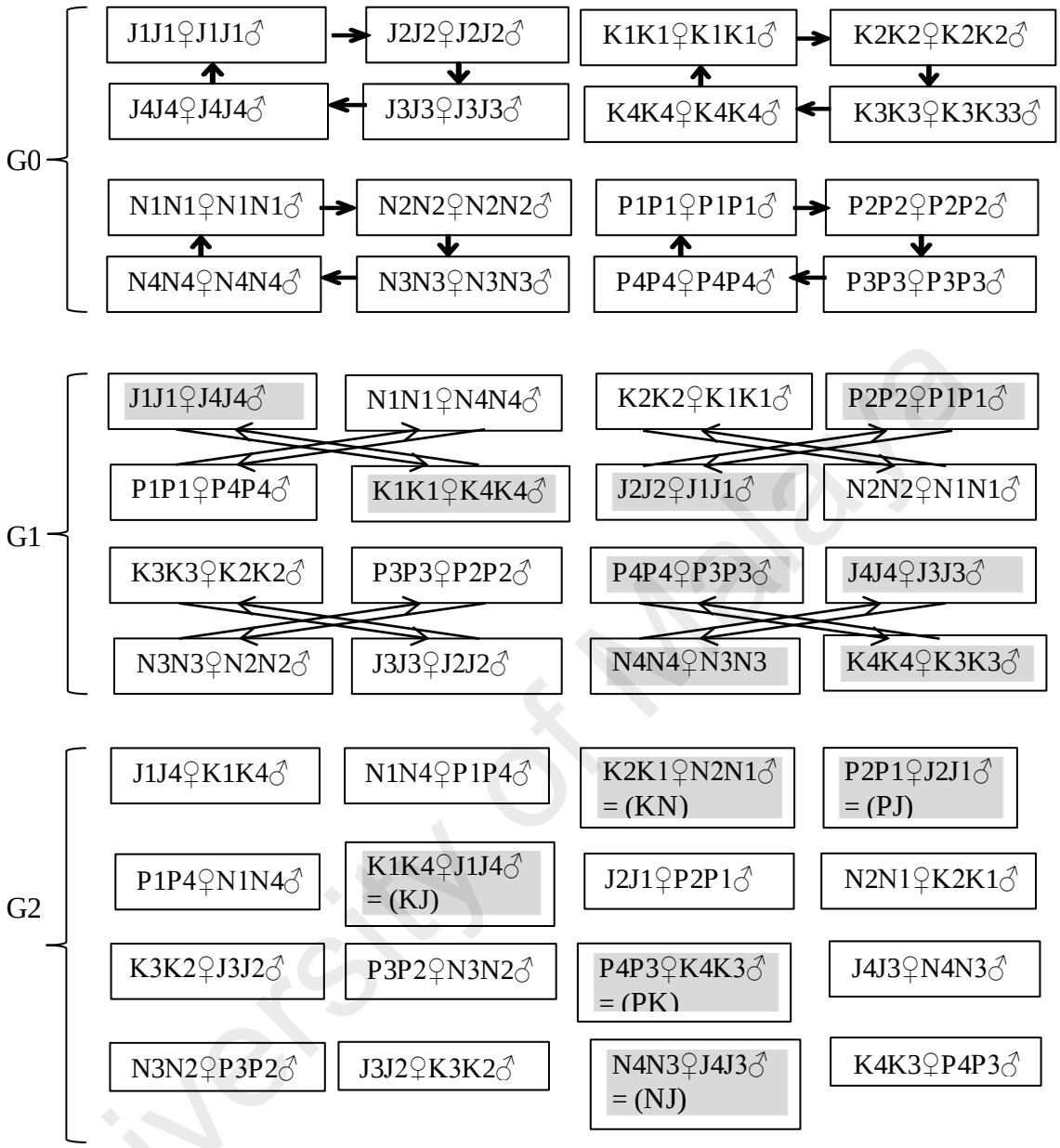

Figure 4.2 Diagrammatic illustration of cyclical mating system for (J) Johor, (K) Kedah, (N) Negeri Sembilan and (P) Perak ecotype populations. The five successfully produced cohorts were mated to pure parental ecotypes which had not been used as parents for any of the coming predecessor haplotype.

*Successful cohort families are highlighted.

E.g: when J1J1 subpopulation was mated to J4J4 subpopulation all offspring produced were of J1J4 composition and when K1subpopulation was mated to K4subpopulation all offspring produced were of K1K4 composition.

In the following generation these offspring were mated together K1K4♀×J1J4♂ to produce (KJ) irrespective to designation.

When eggs colour changed from orange to dark grey, then berried females would be considered ready to spawn. Ripe berried females were then transferred to a 500 L fiberglass tank for hatching larvae with a water column of 50 cm. They had to be disinfected by immersing them in 30 to 50 p.p.m of formalin for an hour. Temperature was always kept in the range of 28º – 31ºC which is reported by Cavalli et al. (2001) to be optimum for the species by installing immersed automatic heaters as the optimum temperature for the GMP. However in Malaysia where equatorial climate is prevalent, temperatures dropping below tolerable levels rarely occur as temperature fluctuations may be expected on rainy days.

Salinity on another hand was increased gradually starting with 6 p.p.t (part per thousand) for berried females before hatching, then increased to 8 p.p.t for the first four days after free larvae emerged from eggs, then it would be kept at 12 p.p.t till the appearance of the first few postlarvae (PL), finally decreased gradually to fresh water conditions. Aeration is set up prior to starting a hatching cycle. Oxygen level was kept in the range of 5 – 7 p.p.m. spawning usually commences during night hours (between 10:00pm and 2:00 am); thus newly hatched larvae from each family were collected the next morning. Larvae from each progeny family were disinfected with 5 p.p.m formalin for 1 minute and families placed separately into larval rearing tanks.

4.2.4 Larvae rearing

Larvae from each family were reared separately in 100 L tank with stocking density of 40 individuals per liter. Open clear water larval culture system was employed (Phuong et al., 2006). This is currently the most popular larval rearing system used in the Malaysia.

Larvae were fed only on newly hatched brine shrimp nauplii (3 times per day) plus egg

custard as a supplement from the tenth day of larval stocking onwards. For the first ten days, no water exchanges were conducted, following which, 10% water change was practiced daily plus 50% weekly one. Aeration system was based on a central aeration system. Thus adequate oxygen supply was always assured.

Water quality parameters such as pH and dissolved oxygen were monitored daily and were confident to be within the normal ranges. Water temperature was maintained as 28°C using pre-installed immersed heaters with little errors. Few early metamorphed PL individuals were seen on day 25 – 30 after larval stocking with complete metamorphosis of almost all larvae occurred on $32^{nd} – 45^{th}$ day.

4.2.5 Juvenile nursing and grow-out ponds

Produced PL beings of each progeny family were raised up to early juvenile stage in separate 5000 L capacity nursery tanks at a stocking density of 1000 PLs per m^3. They were fed on commercial prawn pellet at starting feed size of 32% crude protein. Continuous aeration was set and waste matter was removed everyday via a siphon. Polyvinyl chloride or PVC pipes and mesh nets were placed in the nursery tanks to allow PL to hide, in order to reduce cannibalism. After 60 days of raise in nursery, each family was comprised of 1 – 2 g fries were transferred separately to grow-out ponds at Tapah village (4.183° N and 101. 267° E). Each family was replicated three times in hapas constructed in ponds at a stocking density of 14 individuals per m^2. Hapas look like inverted mosquito nets enclosure (2 m x 1.5 m x 1.0 m) stitched out of square-meshed mosquito netting cloth and tied on to bamboo poles fixed in ponds or tanks so that about 0.3 m is above the water level while its bottom is 0.3 m above the pond bottom (Appendix B), were supplied with air from 9:00pm

to 6:00am and juveniles were fed on a 40% crude protein commercial prawn pellet. Hapas were cleaned every two weeks to ensure good water flow and water was exchanged at least twice a month via gravity flow or by pumping if it was urgent. After 150 days in grow out, samples of 50 individuals from the three replicates of each of the successful progeny families were collected and their morphometric data were recorded. Individuals with the highest weight and healthy were chosen as brood stock for the next generation.

4.2.6 Data collection of progeny families

Random samples of 50 individuals of each of the three replicates of each of the successful cohort families were collected. Then measurements of the same morphometric traits were taken in a similar manner as those of the parental groups described previously.

Morphometric data were again collected for the progeny families produced in the second round of cyclical mating between the giant Malaysian prawns ecotype populations described above. This later, data was used to study the relationships between harvest weight (WT), total length (TL), tail length (TA) and carapace length (CL) as growth indicators and as measuring lengths under field conditions is more easier than weighing live animals, multiple regression model was applied to estimate it as a response variables for the morphometric variables as predictors.

4.2.7 Statistical analyses

Data were analysed using version 9.3 of the Statistical Analysis System software (SAS Institute Inc., 2012). The SAS procedures applied were the General Linear Model (GLM), mixed model, ANOVA and the regression. MANOVA procedure was applied to test for the

existence of systematic effects of ecotype populations and their progeny families considered that causes mean differences in the traits examined. Then Duncan's multiple-range test was applied to identify which ecotype populations are significantly different for each measured trait. The mixed model procedure was implemented to detect fixed effects of family, sex, replicate and their interactions in the eight progeny families according to the following equation:

$$Yijk = \mu + C_i + S_j + V_k + (C*S)_{ij} + (C*V)_{ik} + (S*V)_{jk} + e_{ijk}.$$

Where Yijk= the measurement of a morphometric body trait, μ = a constant, Ci= fixed effect of family (i = 1,2,3 …….8), Sj is the sex (j= 1,2), Vk is the replicate effect (k=1,2,3), (C*S)ij is the family by sex interaction, (C*V)ik is the family by replicate interaction, (S*V)jk is sex by replicate interaction and eijk is a random error which is distributed normally.

To estimate heritability of traits in consideration; parents were identified as ecotype populations in the mating design. Data of the progenies were then used to evaluate their parental groups using ANOVA under GLM procedure of the software. Similar to penaeid prawns, as heritability through sire side was reported not to differ from zero (Kitcharoen et al., 2012; Benzie et al., 1997).

This may be attributed to the absence of an agreed on correction factor for sex in the considered species as the case in cattle for instance (Chenette & Frahm, 1981). Therefore,

in this study, it is only estimated through maternal linage according to Pirchner, (1983) equation substituting sire by dam as shown below:

$$h^2 = 4\,\sigma^2D / (\sigma^2D + \sigma^2w)$$

Where: h^2 = heritability; σ^2D = Variance due to dams and σ^2w = Variance within progenies produce by the same dam.

Moreover, separate stepwise multiple linear regression analyses were performed on morphometric data of the parental ecotype populations and their haplotype progeny families produced in the second round of cyclical mating. Prior to analysis, these data were logarithmically transformed for the nature of their relationships with WT is exponential (Hayes et al., 1995), while the stepwise multiple linear- regression under the regression procedure of SAS software version 9.3 (SAS Institute Inc. 2012) procedures assumes linearity.

In these equations harvest weight WT was treated as a response variable, while TL, CL and TA were predictors. The two sexes of each parental ecotype population were treated separately in one time and mixed in another round, while their haplotypes produced under the mating system designated were combined without sexing.

Correlation strength between WT and its predictors were utilized to decide priority of adjustment in generating the multiple linear regression equations (Jensen & Sørensen,

2007). The general formula applied for these linear multiple- regression equations is as shown below:

$$WT = \mu + \beta_0 TL + \beta_1 CL + \beta_2 TA + e_{ijk}$$

Where μ is intercept (constant), eijk is a random error which is independent and normally distributed, $\beta 0$ is the expected difference between two experimental units for which the variable TL differs by one unit, with all other explanatory variables kept the same, β_1 and β_2 are Similar to β_0 with changing the explanatory variable to CL and TA respectively.

4.3 Results

4.3.1 Comparisons among quantitative traits of the parental ecotype populations

Naturally growth in the GMP, *Macrobrachium rosenbergii* (de Man, 1879), is highly skewed with multimodal curves in males and a mono-modal normal distribution in females (Kuris et al., 1987; Nguyen et al., 2007). The GMP males are categorized into three major distinct morphotypes; blue- claw, orange- claw and small males (Karplus & Sagi, 2009) and are mostly preferred by females for mating (New et al., 2010). This encourage the researcher to use only blue claw- males, because the breeding period applied was short.

Figure 4.3 is a boxplot that shows variations of studied growth related traits in the ecotype populations considered. Table 4.1 shows basic descriptive statistical data on the four parental ecotype populations, explicitly: least square means (LSM), standard errors for males, females and combined sexes as well as Duncan's grouping which is displayed for the groups of combined sexes as an example. As shown in the table, means of morphometric

measurements, TL, CL, TA and WT of the GMP were found to differ significantly (P<.05) according to ecotype population source. Kedah progeny family recorded the highest results for all the traits investigated.

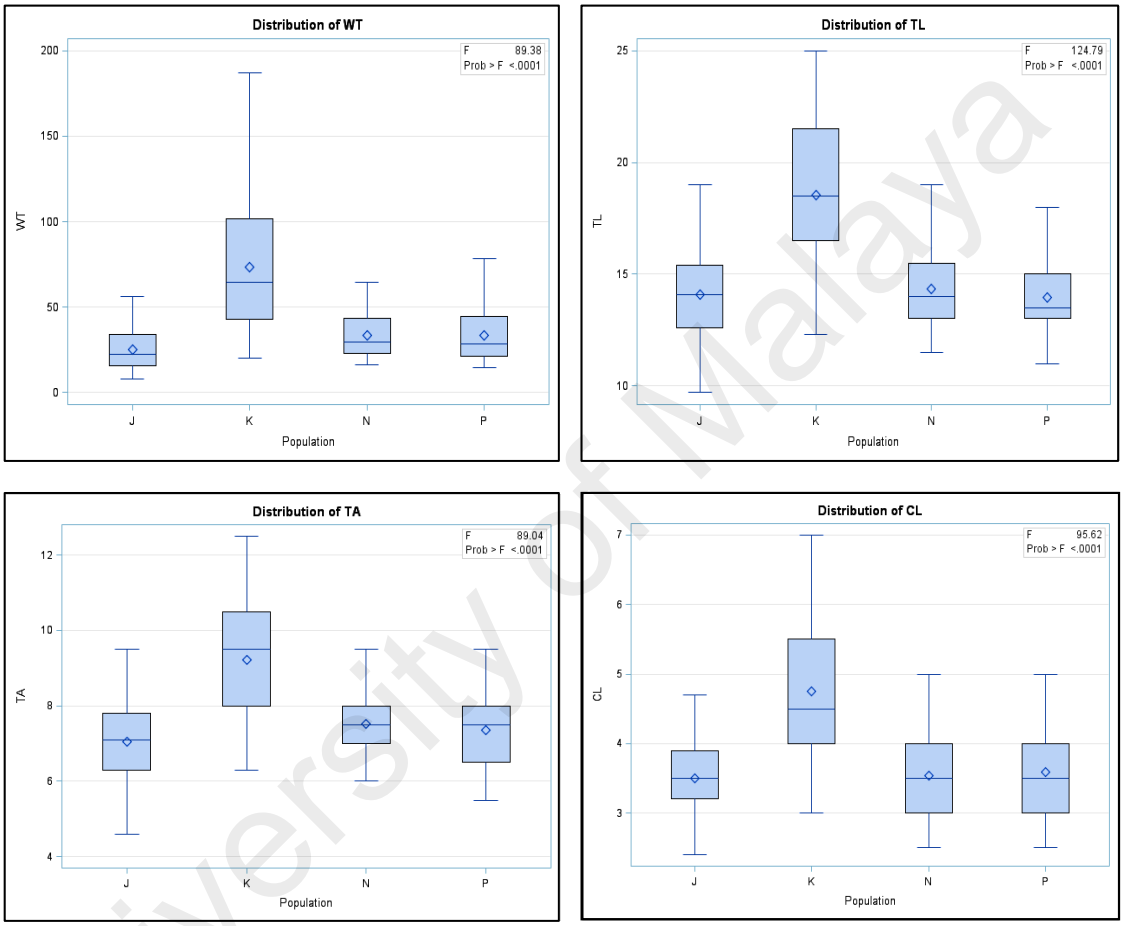

Figure 4.3 Boxplot of WT (g), TL (cm), TA (cm) and CL (cm) of *Macrobrachium rosenbergii* ecotype populations of Johor (J), Kedah (K), Negeri Sembilan (N) and Perak (P)

*Left upper= WT, right upper= TL, left lower= TA, and right lower= CL

Table 4.1 Least square means (LSM), standard error and Duncan's grouping (mixed sexes) of body weight (WT), total length (TL), tail length (TA) and carapace length (CL) for female, male and mixed sexes of Kedah (K), Perak (P), Negeri Sembilan (N) and Johor (J) ecotype populations of *Macrobrachium rosenbergii*

Population	No.	LSM	WT (g)	TL (cm)	TA (cm)	CL (cm)
K	100	Female	60.762±3.951	17.996±0.275	9.070±0.138	4.404±0.082
	100	Male	106.810±4.161	19.964±0.289	9.709±0.146	5.402±0.086
	200	Mixed	82.592±3.139**a**	18.929±0.213**a**	9.373±0.105**a**	4.877±0.069**a**
P	100	Female	25.740±4.489	13.327±0.312	7.009±0.157	3.318±0.093
	100	Male	50.946±5.473	15.541±0.380	8.189±0.192	4.176±0.114
	200	Mixed	35.877±3.803**b**	14.217±0.258**b**	7.484±0.127**b**	3.663±0.084**b**
N	100	Female	30.292±3.051	14.043±0.211	7.427±0.107	3.377±0.063
	100	Male	53.036±4.805	15.917±0.334	8.121±0.168	4.292±0.100
	200	Mixed	36.829±2.823**b**	14.578±0.191**b**	7.625±0.094**b**	3.638±0.062**b**
J	100	Female	25.308±6.529	14.250±0.399	7.326±0.200	3.538±0.119
	100	Male	25.874±5.709	13.823±0.454	6.846±0.229	3.408±0.136
	200	Mixed	25.549±4.749**c**	14.076±0.322**b**	7.134±0.159**c**	3.498±0.105**b**

*P= Perak, J=Johor, K=Kedah and N=Negeri Sembilan. *a, b, and c refer to Duncan's grouping between the four ecotype populations for a traits in a column at $p<.05$.
*different letters indicate statistically significant differences between designated ecotype populations.

4.3.2. Comparisons among quantitative traits of the progeny families

Table 4.2 displays LSM and standard errors of the these traits in the eight progeny families that were successfully produced in this study and Figure 4.4 represents a histogram illustrating sexual dimorphism in WT means in the progeny families as an example trait. Appendix C shows the histograms of the rest of the traits.

Table 4.2 Least square means (LSM), standard errors and Duncan's grouping of body weight (WT), total length (TL), tail length (TA) and carapace length (CL) for the eight *Macrobrachium rosenbergii* progeny families

Family	No.	WT(g)	TL(cm)	TA(cm)	CL(cm)
P2P2×P1P1	150	14.267±0.642**c**	11.059±0.121**c**	5.435±0.061**e**	2.831±0.040**c**
J1J1×J4J4	150	19.186±0.642**ab**	11.754±0.121**b**	5.888±0.060**c**	3.139±0.039**ab**
J2J2×J1J1	150	13.056±0.609**c**	10.590±0.115**d**	5.450±0.057**e**	2.623±0.038**d**
J4J4×J3J3	150	13.165±0.636**c**	10.854±0.120**cd**	5.926±0.059**bc**	2.664±0.039**d**
P4P4×P3P3	150	13.527±0.648**c**	11.089±0.123**c**	5.693±0.061**d**	2.826±0.040**c**
K4K4×K3K3	150	20.884±0.648**a**	12.500±0.123**a**	6.427±0.061**a**	3.240±0.040**a**
K1K1×K4K4	150	18.407±0.642**b**	11.962±0.121**b**	5.969±0.060**bc**	2.880±0.039**c**
N4N4×N3N3	150	20.749±0.604**a**	12.074±0.114**b**	6.083±0.056**b**	3.084±0.037**b**

*P= Perak, J=Johor, K=Kedah and N=Negeri Sembilan. *a, b, and c refer to Duncan's grouping between the four ecotype populations for a traits in a column at p<.05.
*different letters indicate statistically significant differences between designated ecotype populations.

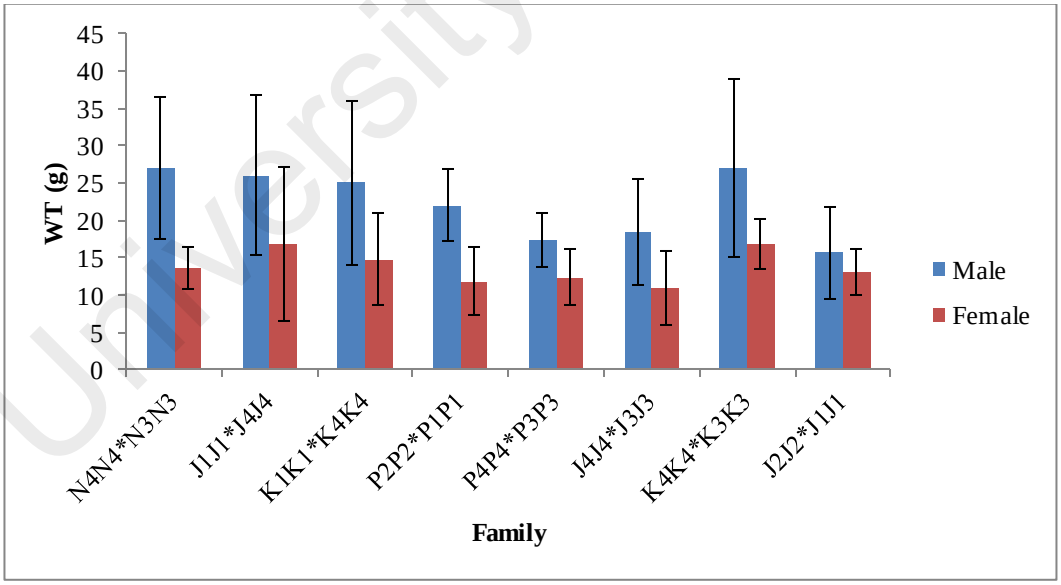

Figure 4.4 A histogram showing weight (WT, g) means of the males and females of the eight progeny families of *Macrobrachium rosenbergii*

Moreover, fixed effects of family, Sex and their interactions were found to be significant ($p<.001$) for all the traits, while replicate was significant ($p<.05$) only for WT. But its interactions with family and sex were significant ($p<.001$) except for WT (Table 4.3).

Table 4.3 Analysis of variance (ANOVA) using mixed procedure showing the effect of family, sex, replicate and their interactions on body weight (WT), total length (TL), tail length (TA) and carapace length (CL) of *Macrobrachium rosenbergii*

Trait	Effect	d.f	F value	P
WT	Family	7	51.63	<.0001
	Replicate	2	3.04	0.0483
	Sex	1	1227.82	<.0001
	Family × sex	7	23.64	<.0001
	Replicate × sex	2	86.24	<.0001
	Family × Replicate	14	1.22	0.2525
TL	Family	7	46.14	<.0001
	Replicate	2	0.38	0.6835
	Sex	1	1152.03	<.0001
	Family × sex	7	13.18	<.0001
	Replicate × sex	2	116.86	<.0001
	Family × Replicate	14	2.20	0.0066
TA	Family	7	40.75	<.0001
	Replicate	2	0.70	0.4968
	Sex	1	1023.91	<.0001
	Family × sex	7	9.09	<.0001
	Replicate × sex	2	134.36	<.0001
	Family × Replicate	14	2.66	0.0008
CL	Family	7	53.80	<.0001
	Replicate	2	0.81	0.4451
	Sex	1	1247.53	<.0001
	Family × sex	7	15.33	<.0001
	Replicate × sex	2	115.55	<.0001
	Family × Replicate	14	2.71	0.0006

*Multiplication sign (×) shows the interaction, d.f = degrees of freedom, F = F-statistics, P = probability.

4.3.3 Heritability estimation

Heritability (h^2) of a trait is precisely defined as the amount of the superiority of the parents above their contemporaries which on average is passed on to the offspring (Dalton, 1980). It describes the inheritance strength of the trait in question. However, it is a specific characteristic that is true for a defined group of organisms raised in specific environment including feeding and shelter. Practically, the most important aspect behind estimating heritability for any trait is its predictive value, (Pirchner, 1983) as after the occurrence of fertilization; it would be too late to change the genetic composition of the coming individual, therefore breeders can only select parents of tomorrow from the present generation. Table 4.4 illustrates the analysis of variance (ANOVA) of the quantitative traits of the successfully produced family groups (progenies) and their estimated heritability values. Each estimated value for h^2 for each trait falls in the range of moderate to high.

Table 4.4 Analysis of variance for estimating heritability values of body weight (WT), total length (TL), tail length (TA) and carapace length (CL) of *Macrobrachium rosenbergii* in Malaysia

Trait	Source	DF	Mean Square	Heritability Estimates
WT	Between families	7	643.54067	0.5788±0.2989
	Within families	392	68.02839	
	Corrected Total	399		
TL	Between families	7	20.374425	0.5156±0.2753
	Within families	392	2.425844	
	Corrected Total	399		
TA	Between families	7	3.9087286	0.4277±0.2407
	Within families	392	0.5595153	
	Corrected Total	399		
CL	Between families	7	2.1350250	0.5120±0.2739
	Within families	392	0.2560015	
	Corrected Total	399		

4.3.4 Length-weight relationship using multiple regression equations

A correlation coefficient between two traits expresses the strength or the intensity of association, while a regression equation measures the form of this relationship. Correlation coefficients between WT, TL, CL and TA were found to be .902, .900 and .846 respectively, while within predictor variables the coefficients were .889, .950 and .849 for TL with CL, TL with AT and CL with AT consecutively.

Tables 4.5A and 4.5B display the components of stepwise multiple-linear regression equations obtained for logarithmically transformed data of the parental ecotype populations of the Malaysian prawn with mixed and separated sexes correspondingly, while table 4.5C shows the same components for haplotypes of progeny families of the second round of breeding between the ecotype populations under the cyclical mating design described above.

The model used was tuned, as the alpha level used is one third of the default value used in the regression procedure under SAS software, version 9.3. Yet, in spite of that, results obtained are unlikely to occur by chance for the maxima of this probability (Pr>[t]) for each considered trait, were as low as $\leq .0007$, .0243 and .0005 in mixed parents, separated parents and mixed progeny families respectively. Further, the model coefficients of determination (R^2), are high, ranging from 72 – 94 %, with the first predictor explaining a minimum of 68% of that total variance; with a contribution, as explained in the tables by standard estimate (standard partial regression coefficient), reaching up to .86. But when simple relationships were developed, the equation would be similar to the one obtained for the female group of Johor shown in Table 4.5B; with more than 70 % R^2 value for each

predictive variable with body weight. This indicates overlapping in explaining the total variance. Equations obtained for the four ecotypes populations of the Malaysian prawn and for their progeny families haplotypes produced under the mating design mentioned earlier were found to be different.

Table 4.5A Components of the equations drawn by stepwise multiple -regression for estimating WT in response of TL, TA and CL for parental Malaysian prawn ecotypes *Macrobrachium rosenbergii* (mixed sexes)

Constants	Value	Partial R^2	Model R^2	SE	Standardized Estimate	Pr>[t]
Kedah						
Intercept	- 4.0209			0.2120	0	<.0001
β_0	1.7276	0.0405	0.9353	0.2252	0.47573	<.0001
B_2	—	—	—	—	—	—
B_1	1.4663	0.8949	0.8949	0.1766	0.51488	<.0001
Perak						
Intercept	— 2.8111			0.2640	0	<.0001
β_0	0.3159	0.0544	0.8887	0.0675	0.26615	<.0001
B_2	0.7199	0.0142	0.9029	0.2016	0.20157	0.0006
B_1	1.5041	0.8343	0.8343	0.1504	0.56090	<.0001
Negeri Sembilan						
Intercept	— 3.82881			0.1646	0	<.0001
β_0	0.35691	0.1080	0.9112	0.0392	0.31123	<.0001
B_2	2.06019	0.8032	0.8032	0.1252	0.55019	<.0001
B_1	0.50317	0.0117	0.9229	0.1011	0.20752	<.0001
Johor						
Intercept	— 3.44132			0.3648	0	<.0001
β_0	—	—	—	—	—	—
B_2	1.14120	0.0498	0.7295	0.3201	0.36603	0.0007
B_1	1.75245	0.6797	0.6797	0.3367	0.53435	<.0001

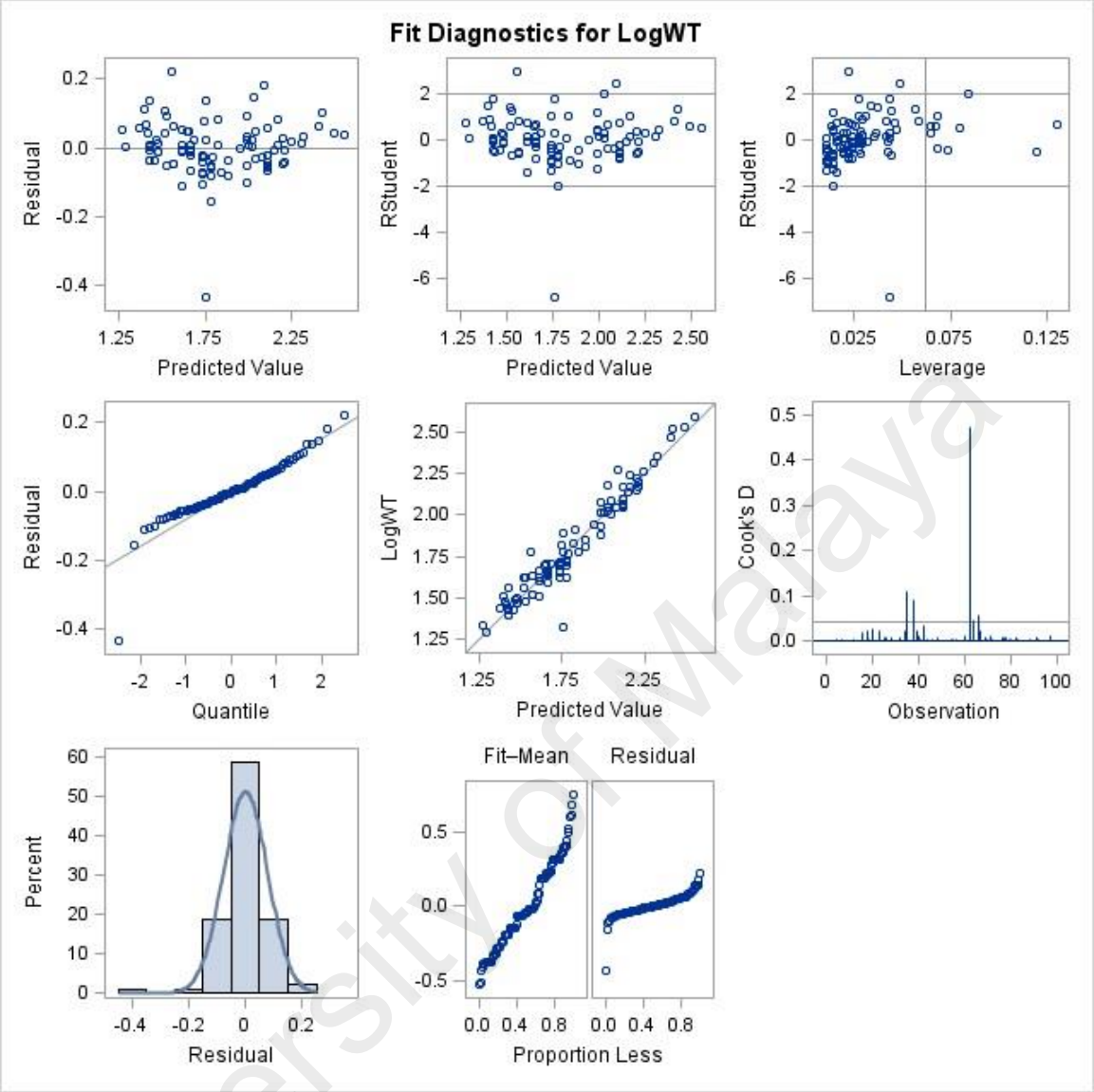

Figure 4.5A Fit diagnostic of residuals against predicted values of WT for Kedah ecotype (mixed sexes).

Furtherly, Figure 4.5B, Figure 4.5C and Figure 4.5D consecutively show plots of fit diagnostics for log WT for one example for each of the three groups.

Table 4.5B Components of the equations drawn by stepwise multiple -regression for estimating WT in response to TL, TA and CL for separate sexes of parental Malaysian prawn ecotypes.

Constants	Value	Partial R^2	Model R^2	SE	Standardized Estimate	Pr>[t]
Kedah Male						
Intercept	-4.8089			0.3298	0	<.0001
β_0 TL	1.0482	0.8950	0.8950	0.4535	0.2968	.0243
B_2 TA	1.2865	0.0254	0.9204	0.4237	0.3496	.0035
B_1CL	1.0366	0.0106	0.9310	0.2456	0.3471	<.0001
Kedah Female						
Intercept	-4.0222			0.3062	0	<.0001
β_0 TL	0.8244	0.0335	0.8691	0.3025	0.3370	.0095
B_1CL	1.5688	0.8356	0.8356	0.2977	0.6311	<.0001
Perak Male						
Intercept	-3.1176			0.4270	0	<.0001
β_0 TL	0.9665	0.7656	0.7656	0.3973	0.3800	.0204
B_2 TA	1.4092	0.0348	0.8004	0.4048	0.5438	.0014
Perak Female						
Intercept	-2.6757			0.3461	0	<.0001
β_0 TL	0.8267	0.0493	0.8173	0.2228	0.2643	.0005
B_1CL	1.6689	0.7679	0.7679	0.1621	0.7331	<.0001
Negeri Sembilan Male						
Intercept	-4.06354			0.4328	0	<.0001
β_0 TL	1.39323	0.8350	0.8350	0.4019	0.4335	.0012
B_2 TA	0.88155	0.0327	0.8677	0.3683	0.2815	.0210
B_1CL	0.62696	0.0152	0.8829	0.2479	0.2690	.0151
Negeri Sembilan Female						
Intercept	-3.95486			0.1882	0	<.0001
β_0 TL	2.53042	0.8644	0.8644	0.1392	0.8058	<.0001
B_1CL	0.44737	0.0168	0.8812	0.1106	0.1792	<.0001
Johor Male						
Intercept	-6.11865			0.2878	0	
β_0 TL	4.28833	0.9209	0.9209	0.2908	1.2745	<.0001
B_1CL	-1.11848	0.0193	0.9403	0.2809	-0.3442	0.0002
Johor Female						
Intercept	-3.50722			0.6749	0	<.0001
β_0 TL	2.63774	0.7458	0.7458	0.3629	0.8636	<.0001

* R^2= coefficient of determination, SE= standard error, Pr = probability for t test of the corresponding component of the equation.

Where WT= weight, response variable, μ= isometric constant, β_0, β_1, and β_2 = parameters associated with the predictors' i.e TL (total length), TA (tail length) and CL (carapace length).

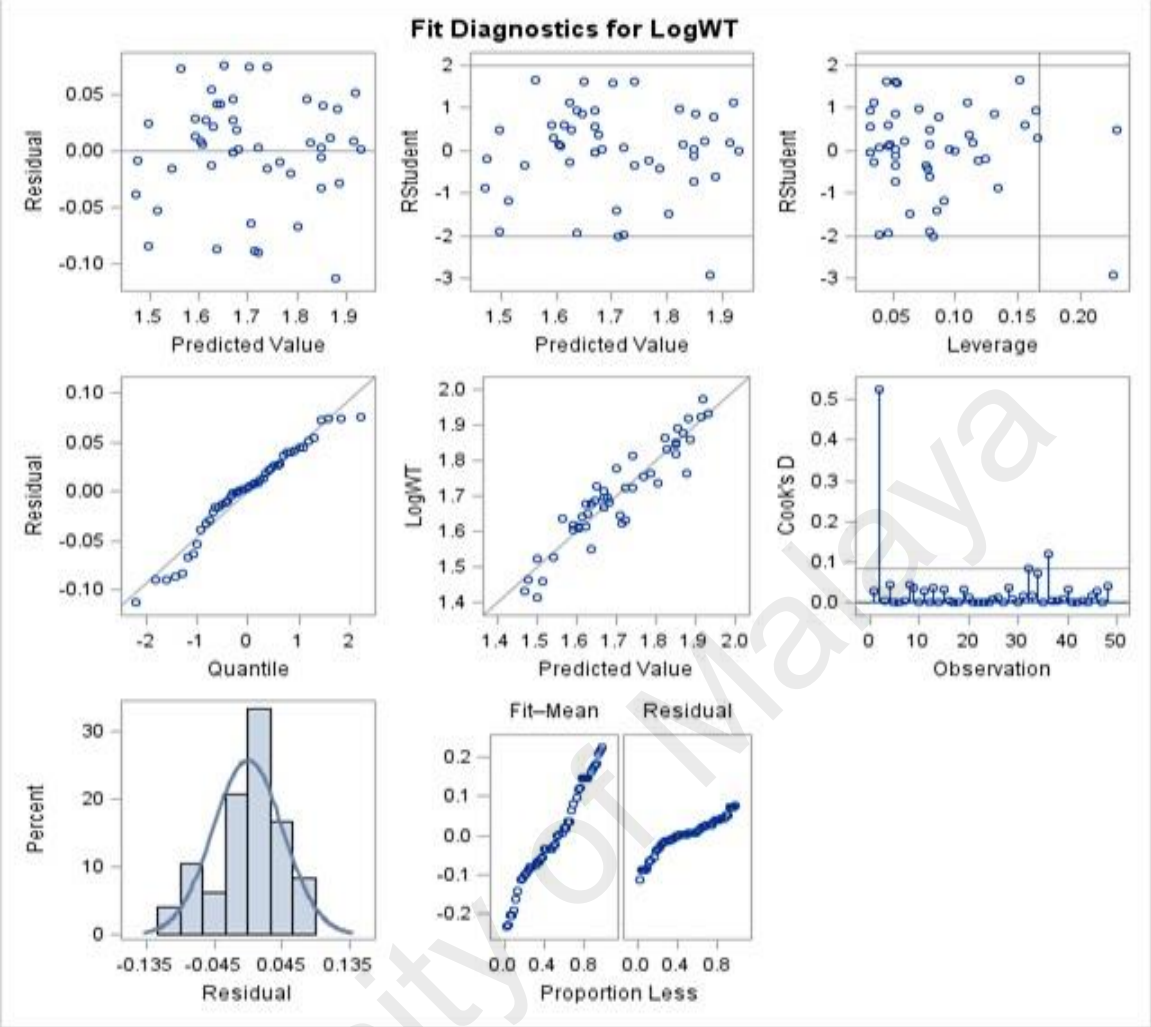

Figure 4.5B Fit diagnostic of residuals against predicted values of WT (log transformed) for Negeri Sembilan Males

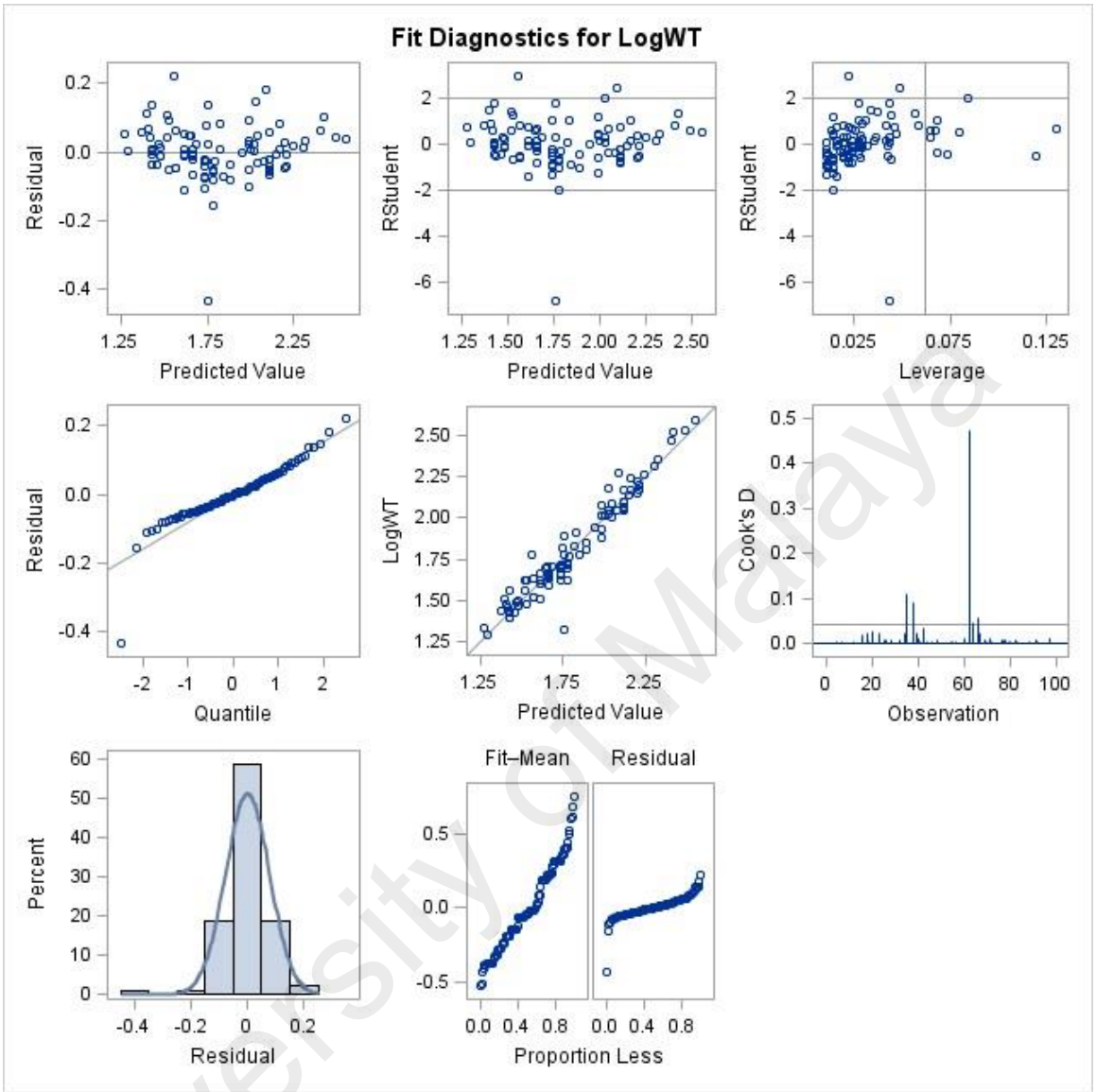

Figure 4.5C Fit diagnostic of residuals against predicted values of WT (log transformed) for Kedah females

The rest of similar plots are presented in appendix D. These plots indicate fairly random distributions of residuals, with a few observations falling out of the threshold limits, for example of the Cook's D statistic meaning that these readings are outliers. Still, for prawns are known to have skewed growth, so such data are reasonable.

Table 4.5C The components of stepwise multiple- regression equations of WT estimation for unsexed progeny groups.

Constants	Value	Partial R^2	Model R^2	SE	Standardized Estimate	**Pr>[t]**
PP×KJ						
Intercept	-5.04386			0.37201	0	<.0001
β_0 TL	2.05612	0.8295	0.8295	0.30709	0.62671	<.0001
B_1TA	1.12735	0.0429	0.8723	0.30020	0.35149	.0005
KP×NJ						
Intercept	-4.39223			0.40006	0	<.0001
β_0 TL	2.18260	0.7903	0.7903	0.25600	0.67700	<.0001
β_2 CL	0.73534	0.0559	0.8462	0.18388	0.31755	0.0002
KK×PJ						
Intercept	-5.90934			0.60435	0	<.0001
β_0 TL	3.44949	0.8177	0.8177	0.29250	0.90429	<.0001
NJ×PP						
Intercept	-4.27239			0.43769	0	<.0001
β_0 TL	2.28870	0.7889	0.7889	0.26185	0.74984	<.0001
β_2 CL	0.50120	0.0368	0.8257	0.18178	0.23653	0.0091
PP×KN						
Intercept	-4.30191			0.38637	0	<.0001
β_0 TL	2.29108	0.8843	0.8843	0.31178	0.75255	<.0001
β_2 CL	0.51889	0.0127	0.8971	0.24249	0.21914	0.0390
PJ×KK						
Intercept	-5.14127			0.46667	0	<.0001
β_0 TL	2.70878	0.8918	0.8918	0.33733	0.76640	<.0001
β_2 CL	0.50594	0.0144	0.9062	0.22505	0.21456	0.0314
KN×JJ						
Intercept	-5.33628			0.26267	0	<.0001
β_0 TL	2.52682	0.9225	0.9225	0.24601	0.78224	<.0001
β_2TA	0.73820	0.0114	0.9339	0.27047	0.20787	0.0092
JJ×KP						
Intercept	-4.42795			0.38059	0	<.0001
β_0 TL	1.16095	0.0348	0.8635	0.39452	0.41375	0.0058
β_2TA	1.81996	0.8287	0.8287	0.47300	0.54099	0.0005

* R^2= coefficient of determination, SE= standard error, Pr = probability for t test of the corresponding component of the equation.

Where WT= weight, response variable, μ= isometric constant, β_0, β_1, and β_2 = parameters associated with the predictors' i.e TL (total length), TA (tail length) and CL (carapace length).

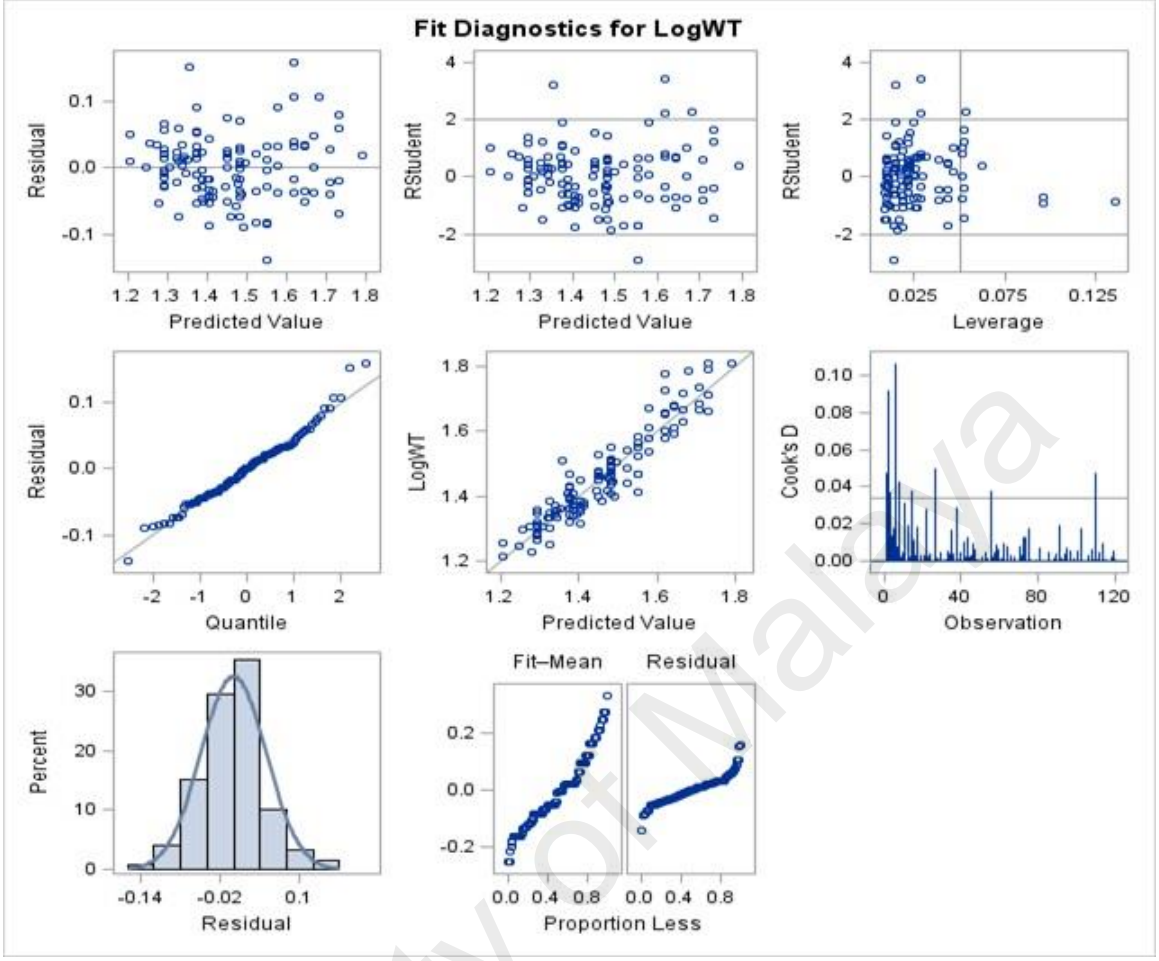

Figure 4.5D Fit diagnostic of residuals against predicted values of WT (log transformed) for Progeny family PP×KJ

4.4 Discussion

4.4.1 Comparing quantitative traits of the parental ecotype populations

Growth in the GMP, *Macrobrachium rosenbergii* (de Man, 1879), is highly skewed with multimodal curves in males (Kuris et al., 1987; Nguyen et al., 2007). Means of morphometric measurements, TL, CL, TA and WT of the GMP differ significantly (P<.05) according to ecotype population source (Table 4.1.). Consistently, similar results were reported by (Thanh et al., 2009; Munasinghe & Thushari, 2010; Kitcharoen et al., 2010; Pillai et al., 2011).

4.4.2 Comparing quantitative traits of progeny families

One family of Kedah progenies had significantly ($p < .05$) excelled all progeny families except one from Negeri Sembilan progenies for all the traits investigated (Table 4.2). On another hand, one group of Johor progenies was statistically undistinguishable ($P > .05$) from the progeny family of Kedah for both WT and CL. Results of the fixed effects, namely that of family, sex and their interactions for all the traits indicated significant ($p < .001$) genetic difference between the families. Although results of replicates were not significant ($p > .05$), yet its interactions with family and sex were significant ($p < .001$), (Table 4.3), mainly as a result of being dragged by association with sex and family that have very high effects. Results of the progeny families confirmed similar results obtained in comparing the parental ecotype populations, thus illustrating the existence of genetic differences between studied ecotype populations as well as the existence of sexual dimorphism in *Macrobrachium rosenbergii,* for these traits. Similar results were reported by Thanh et al., (2009), Pillai et al., (2011) and Sethi et al., (2013).

4.4.3 Heritability estimation

Heritability describes the inheritance strength of the trait in question. The most important aspect behind estimating heritability for any trait is its predictive value, (Pirchner, 1983) as after the occurrence of fertilization; it would be too late to change the genetic composition of the coming individual, therefore breeders can only select parents of tomorrow from the present generation. Accordingly, the ranges of heritability values obtained for the four traits in this study (WT, TL, CL and TL) are considered intermediate to high. They are in line with results obtained by Thanh et al., (2010); Kitcharoen et al., (2012); Dinh et al., (2013) and Dinh & Nguyen, (2014) for the same species. However, higher results were evidenced

by Nugroho et al., (2005) showing h2 estimates as high as .84 for body weight, and lower estimates as compared to this study were also reported by Luan et al., (2012) and Sun et al., (2013).

Results in this study show significant differences among parental ecotype populations and highly significant fixed effects of family source as well as moderate to high heritability estimates of the economically important traits that were studied which consequently encourage the author to un-hesitantly recommend selection methods for these quantitative traits for genetic improvement of this species. But care should be taken not improve characters at the expense of variability. Thus the best way is to practice selection simultaneously with adopting one of the cyclical mating methods to keep variability.

4.4.4 Length-weight relationships

Naturally, growth in the giant Malaysian prawn (GMP) is in a frog leap manner with almost complete cessation before the upcoming moult and its pace decreases with age, expressed as a drop in the slope of the curve (Guest, 1979; Kurup et al., 2000). Growth is reflected in proportional increments of weight and length measurements. Hence, in this study, results of length-weight relationships in *Macrobrachium rosenbergii* applying stepwise multiple-linear regression were presented and discussed.

According to the researcher's knowledge, this is the first report applying a multiple approach on length-weight relationship data of shrimps. For available reports are entirely simple relationships; so only first predictors in equations obtained here could be compared with those results.

The study proved that the model used better fits the data and hence considered more robust for estimating WT using all the predictive variables in comparison to performing several separate paired simple relationships at a time.

Interestingly, the study publicized that mixed sexes presented equations with high coefficients of determination R^2, this is not in contrast to the documented (Kuris et al., 1987; Nguyen et al., 2007) skewed growth of the species, because, the aim of the experiment was based on planning for genetic improvement of the species, in this case only blue -claw males were used for breeding. Actually the small male morphotype is the one that causes most of skewness in grow outs of the Malaysian prawn. Moreover, it is not advisable to use such males for breeding purposes, although they are equally fertile, but being socially submissive, they are unlikely to get a chance to mate with females in the presence of the dominant blue- claw (BC) and orange- claw (OC) males. So, adding females, with fairly normal weight distribution that reached weights equal or even higher than some BC males to such males and dealing with them as one group did not distort the data.

The study showed that eliminating a predictor/s variable can result in losing explanation of the total variance. Although applying the model in this study resulted in missing only 2 % of the total variance explained. This does not indicate that eliminated predicting variables had no contribution to the response variable, but it only means that there is no extra variance for them to explain after including their/its predecessor (s) predictive variables (Der, G., & Everitt 2008 and McDonald 2009). It also declared that predictor variables overlap in explaining the total variance. Actually removing predictor on the basis of having

low t-test probability is statistically inappropriate, for the predictors may be so correlated, as it is in the case of weight- length relationships, hence, this will result in altering the estimated regression coefficients and standard errors (Der, G., & Everitt 2008 and McDonald 2009). But, just applying simple regression approach may exaggerate some predictive variables and over simplify the form of relationship resulting in immature conclusions, by one finding an impressive coefficient of determination (R^2) and hurry to compare the slopes of the lines. So multiple regression approach seemed to be more robust, as actually, simple linear regression is a special case of multiple linear- regression model in which there is only one predictor.

The study also revealed different regression equations for the different ecotype populations, consolidated by similar results for the different haplotypes of their progenies, that are most likely belong the same cohort, indicates the strong role of the genetic composition in growth pattern in *M. rosenbergii* as well as the positive effect of stage. This coincides with the findings obtained by Lalrinsanga et al., (2012) who studied nine different crosses of the same species in India.

In conclusion, the model is proved to fit for analyses of weight length relationships and is very informative for initiating genetic improvement programs.

CHAPTER FIVE: COI GENE SEQUENCE ANALYSIS TO TEST CYCLICAL MATING AS MEANS OF SECURING GENETIC DIVERSITY OF *MACROBRACHIUM ROSENBERGII*

5.1 Introduction

Globally, aquaculture production has increased in volume and diversified into new species. Indeed, FAO reported in 2014 that about 600 aquatic species are under culture. Few years back this number was only 200 species. The giant Malaysian prawn in general is an important candidate for aquaculture, especially in Southeast Asia. They are decapod crustaceans that naturally inhabit tropical freshwater environments in large rivers and streams that are influenced by adjacent brackish water zones, as most species of their genus need brackish water conditions for the development of their larval stages (Ling & Merican, 1961; New, 2002; Wowor et al., 2009). Currently, total global production of these prawns has risen to almost 444,000 tons. Of this, more than half (51.7 %) is accounted for by the Giant Malaysian Prawn (GMP), *Macrobrachium rosenbergii* (New & Nair, 2012). GMP production in Malaysia from both aquaculture and fishing in the wild has recently experienced wide fluctuations (Figure 3.1), albeit the crop has remained substantial, with even the worst year's production exceeding 300 tons (New & Nair, 2012). This may be attributed to loss of genetic diversity.

Therefore, maintaining the genetic diversity of domesticated aquatic species like the GMP is an important issue for both researchers and producers, as this can help to stabilize production levels. The species considered here, like its sister *Macrobrachium wallacei*, is highly fecund (Habashy, 2013), meaning that only a limited number of broods are enough for a farmer to produce his coming crop. This can lead to the rapid development of inbreeding and loss of diversity (Knibb et al., 2014).

Wild aquatic stocks are an ideal source of diversity that may be used whenever there is a need to replenish genetic variability in cultured stocks. However, genetic improvement programs have only recently been developed in the various institutions belonging to the Malaysian Marine Aquaculture Institute (FRI).

For studying genetic diversity of *M. rosenbergii* the cytochrome c oxidase subunit locus I gene has been chosen. This gene is one of the mitochondrial DNA (mtDNA) markers that commonly used to assess genetic diversity and population structure in metazoan including aquatic animals. Many factors make these markers ideal for such studies, including their high mutation rate compared to the nuclear genome (Brown et al., 1979; Thomas et al., 1997 and Denver et al, 2004), their lack of introns (Costanzo and Fox, 1990; Matsuzaki et al., 2004) and their mono-parental mode of inheritance (Shitara et al., 1998; Wan et al., 2004). The cytochrome c oxidase subunit locus I gene particularly has been used by many researchers (Hebert et al. 2004, Lambert et al. 2005, Costa et al., 2007; Lakra, et al., 2011; 2011; Barman et al., 2014) for species barcoding as well as to identify linkages between populations and past demographic events such as population expansion and shrinkage (Khamnamtong et al., 2009; De Jong, et al., 2011; Jimoh, et al., 2013; Li et al., 2014 ; Han et al., 2015). In addition to the above mentioned characteristics of the mtDNA, the gene has a high degree of conservation, with rare insertion and deletion rates (Moritz and Cicero, 2004) and many rapidly evolving nucleotide sites, all of which facilitates differentiation between even recently evolved species (Nylander et al., 1999).

Accordingly, this gene was used to examine the genetic diversity and structure of geographically separate samples of GMP, *M. rosenbergii* populations and their progenies.

The aims behind were, first of all, to cast light on the current state of the species in Malaysia in terms of its genetic diversity, and to help point out problems before these deteriorate to a point where they might become difficult to correct; and secondly, to test how far adopting cyclical mating could help to maintain diversity in aquaculture stocks. The study was carried out through comparing the results of a gene sequence analysis of progeny families with those of the parental ecotype populations. Samples were collected exclusively from the western coast of peninsular Malaysia.

5.2 Materials and methods

5.2.1 Sampling

About 4,000 individuals of the GMP, *Macrobrachium rosenbergii,* were collected from four states, namely Perak, Negeri Sembilan, Kedah and Johor. These GMP ecotype populations were designated according to the initials of their state of origin, i.e P, N, K and J respectively. Initially these parental ecotype populations were checked for known diseases before introducing them to the hatchery for breeding in the premises of the Marine Aquaculture Institute (FRI) Malaysia.

Samples of sharply cut pairs of swimmerets or pleopods were taken from 20-30 individuals of each ecotype population and each progeny family (Appendix E) produced by the cyclical mating which is described in section 4.2.3 and whole animals were returned back. Swimmerets are naturally autotomizable meaning that they can regenerate again, hence causing least stress for prawns. Then the swimmeret samples were preserved in vials containing absolute ethanol. This is followed by transferring the collected and preserved

samples to the laboratory of genomic and evolutionary biology at the University of Malaya, wherein they were kept at -20 C prior to commencing DNA extractions.

5.2.2 DNA extraction

Approximately 50 mg of swimmeret tissue was used to isolate the genomic DNA from each muscle sample following a protocol described by Li et al., (2011) and Avin et al., (2013) with slight modifications. Lysis period was extended to overnight due to toughness of swimmeret. Moreover, dithiothreitol and ammonium acetate were used in this protocol to break-up disulfide protein bonds by the former on one hand and to precipitate impurities including astaxathin by the later on another hand. Extracted genomic DNA was kept at –20ºC until use. Samples of genomic DNA extracted from swimmeret tissues were checked for the quality as well as for the quantity of the product as described in the following section.

5.2.3 DNA quantification

The quality and quantity of the purified DNA samples were checked using NanoDrop 2000/2000c spectrophotometer. The spectrophotometer checks for the purity of the DNA and RNA in the samples from the ratio of the absorbance at 260 nm and 280 nm indicates the presence of contaminants absorbed at 230 nm.

5.2.4 Target gene fragment amplification

A fragment of the mitochondrial DNA of COI was then successfully amplified using LCO1490 (Forward): 5GGTCAACAAATCATAAAGATATTGG3 and HC02198 (Reverse): 5TAAACTTCAGGGTGACCAAAAAATCA3 primers developed by (Vrijenhoek et al., 1994) based on a complete mitochondrial sequence of the crustacean *Artemia fransiscana*

(Valverde et al., 1994). The length of the gene fragment obtained was 683 base pairs without any insertion or deletion. During optimization of the PCR reaction, some parameters were put into account such as the amount of the DNA template, the annealing temperature and the concentration of $MgCl_2$. Gradient PCR using Eppendorf thermal cycler was run to find the optimum annealing temperature for the species. An annealing temperature of 50 Cº was selected for the gene and DNA template of (100 ng) was used in its amplification. Using the optimized parameters, double stranded DNA amplifications were performed using Eppendorf thermal cycler in 50 µl volumes, containing 1.25 U of Taq polymerase, 10 µl of 5X reaction buffer (green GoTaq® reaction buffer, Promega), 0.2 mM of each dNTP , 0.08 µM (COI) and 0.25 µM of each of primer, 2.5 mM $MgCI_2$ (Promega) and approximately 100 ng of DNA. PCR amplification cycles were as follows: 95ºC for 5 minutes to denature double-stranded DNA, followed by strand denaturation at 95ºC for 1 minute and annealing at 50ºC for 1 minute. Primer extension was then preceded at 72ºC for 1 minute and 30 seconds. The denaturation, annealing and extension steps were repeated for 35 cycles. Lastly, the reactions underwent final primer extension for 5 minutes at 72ºC and the product was then held at 10ºC.

5.2.5 Visualization of PCR products

PCR products obtained were loaded onto a 1% agarose gel after 5 µl of 6X Orange loading dye and run in 1.5% Tris borate EDTA (TBE) buffer, with 2 µl of 10 ng/ml ethidium bromide added. 5 µl of 100 bp DNA marker was used as a size standard. The PCR products were visualized and photographed using an ultraviolet (UV) transluminator system with an attached camera, driven by the Quantity One software (Bio-Rad Inc.). All the samples were extracted with satisfactory results where high concentration of genomic DNA was obtained

and were successfully amplified. These steps are crucial to determine whether the expected fragment is amplified and also to confirm the specificity of the primer to obtain only the desired fragment.

5.2.6 Sequencing of PCR products

Prior sending for sequencing, all PCR products were purified using PCR clean-up Machery-Nagel Rev. 02, 2012 company protocol developed by Vogelstein and Gillespie (1979). Dilution of each sample was based on the concentration of the PCR product visualized by agarose gel electrophoresis and the NanoDrop 2000/2000c Spectrophotometer. The final volume of each sample was 30 µl (15 µl for each sequencing reaction). The concentration of the primer was 3.2 µM/ µl for each PCR reaction. The primer was diluted from a 100 µM stock by adding ultra- purified distilled water. For instance, if a batch of 10 samples sent for sequencing, at least 15 µl of primer will be needed. This larger amount of primer (15 µl for 10 samples where only 1 µl is needed to sequence one sample) was sent to cater for any repetitions of sequencing if required. The optimum concentration of both the PCR product and primer are critical as they are of the main keys for sequencing analyses success in. Sequencing was carried out on the both strands of the PCR product (LCO1490 used to sequence one stand and HC02198 to sequence the other strand) using the Sanger method for cycle sequencing, which labels the four didoxynucleotides with different fluorescent dyes so that the peak intensities for each position in the sequence can be detected by a laser in an automated sequencer. Sequencing both strands provides two copies of DNA strand for each samples to increase the confidence to identify the nucleotide when dealing with an ambiguous peak in the case of poor quality sequencing results. Sequencing was performed on an ABI PRISM 3730xl

Genetic Analyzer of Applied Biosystems, USA, with a Big Dye® Terminator v3.1 Cycle Sequencing Kit. The sequencing service was provided by the Centre for biotechnology Research for Agriculture Research (CEBAR) at University of Malaya.

5.3 Bio-computations

Obtained sequences were first checked using Chromas elite V4.2. Then they were edited manually to attain consensus sequence for all samples. This was followed by aligning them using the alignment option available in Mega v.6 software (Tamura et al., 2013). The same software was used to calculate the number of conserved and variable sites.

In addition, the nucleotide composition was also calculated using the same software. The relationships between ecotype populations were visualized by a maximum likelihood tree (Felsenstein, 1981) generated also by Mega v.6 software (Tamura et al., 2013) using the best fitting model suggested by Model selection and calculated with 1000 bootstrap replicates (Felsenstein, 1985). The same software was also utilized to calculate the genetic divergence rate using the best test model, Kimura 2-parameter (Kimura, 1980) and gamma distribution model with 5 categories. This model was chosen based on the lowest number of Bayesian Informative Criterion (BIC) scores. The phylogenetic tree was visualized and manipulated using the phylogeny option in Mega v.6 software (Tamura et al., 2013).

Gene diversities (haplotype and nucleotide diversity) were calculated using DNASP v5.10.01 using the aligned nucleotide sequences created using Mega v.6 software (Tamura et al., 2013). Statistical tests originally developed to assess the selective neutrality of mutations have been increasingly used in recent years to test for demographic expansion

(Holsinger, 2012). These tests are designed to distinguish between neutrally evolving sequences under mutation drift equilibrium, sequences evolving in non-neutral ways including directional or balancing selection, and demographic expansion or contraction. This is because historical biogeographical changes can have an impact on both within and among variations in populations: for example, demographic bottlenecks can be caused by past habitat deterioration resulting in the failure of a population to respond to selection because it is in gene fixation traps. In order to test for such past events, we used statistical tests similar to those commonly used to analyse demographic events. Tajima's D (Tajima 1993) uses the frequency of segregating nucleotide sites; Fu's Fs (Fu, 1997) uses the distribution of alleles or haplotypes; while the mismatch test is a frequency graph of pairwise differences between alleles. All three tests are based on the principle that a sudden population expansion associated with a non-neutral process will produce a shift in the allele frequency range when compared to a neutral Wright-Fisher model consistent with population expansion under neutral evolution. Analyses were implemented in Arlequin software v. 3.5 (Excoffier and Lischer, 2010), with p-values generated using 1,000 simulations under a model of selective neutrality. Aligned DNA sequences were assigned into the coding region of mitochondrial DNA, and the reading frame was started at the second base pair to carry out the synonymous and non-synonymous test.

Haplotype data were generated by DNASP v5.10.01 (Librado and Rozas, 2009) using an aligned nucleotide sequences data in a Fasta formatted file created using Mega v.6 software (Tamura et al., 2013). Based on the haplotype data created using DNASP v5.10.01, the sequence for each haplotype was manually copied from one of the sequences of the sample that belonged to that haplotype for further analysis using Arlequin v. 3.5 (Excoffier and

Lischer, 2010). Assessment of relative genetic diversity within and between ecotype populations was computed using Analyses of Molecular Variances (AMOVA), as implemented in Arlequin v. 3.5 (Excoffier & Lischer, 2010). Fisher's exact P-value was calculated using Arlequin v. 3.5 (Excoffier &Lischer, 2010) with 1000 number of Markov chain length. The exact P-value is to test the differentiation between all pairs of ecotype populations. Haplotype diversity represents the probability that two randomly sampled alleles are different, while nucleotide diversity is defined as the average number of nucleotide differences per site in pairwise comparisons between DNA sequences (Tamura et al., 2013). Finally, median-joining network analyses were performed using the software package Network version 4.1 (Bandelt et al., 1999).

5.4 Results

5.4.1 Indices of diversity

Quantities and qualities of obtained DNA are presented in appendix F. Before analysing the sequences obtained, some of them were submitted to the BLAST of the National Centre for biotechnology Information to confirm that the sequences obtained are for the COI gene of the species (Appendix G). Chromas elite software checking for peaks sequences chromatography is shown in (appendix H). Analysis of these partial successfully amplified sequences (683 base-pairs) of the COI gene fragment for 117 individuals from the parental ecotype populations resulted in identifying 43 haplotypes (Appendix I). No insertion or deletion was observed in any of the obtained sequences. The number of variable sites varied considerably between the ecotype populations tested (Table 5.1A). The mean nucleotide composition was 28.1%, 26.3%, 26.9% and 19.6% of the T, C, A and G bases; with a clear bias against purines.

Only one haplotype was found to be shared between all the ecotype populations (36.0% of all obtained sequences); with another one shared between three of the ecotype populations, with a frequency of only 9.0% of the total sequences. As shown in Table 5.1A.

Table 5.1A Gene and nucleotide diversity of four Malaysian *Macrobrachium rosenbergii* ecotype populations based on COI gene sequences

Ecotype population	N	Z	S	HD	π (%)
J	30	7	7	.6575±0.0867	00.2511±0.1677
K	28	16	32	.9206±0.0361	01.1942±0.6358
N	30	14	28	.8092±0.0708	00.5577±0.3213
P	29	16	33	.9138±0.0349	01.1251±0.6011

Where: N = sample size, Z = number of haplotypes, S = polymorphic sites, π = Nucleotide diversity (%), HD = haplotype diversity.

The distribution of haplotypes between ecotype populations was not uniform, with 5, 11, 9 and 11 unique haplotypes for J, K, N and P ecotype populations respectively. The representation of these haplotypes was quite variable, with some represented by multiple sequences but others by just a single sequence.

As illustrated in Table 5.1A, the level of haplotype diversity of the parental ecotype populations was moderate to high (.6575 - .9206), while their nucleotide diversity was low (0.2511 - 1.1942 %). The J ecotype population possessed only approximately half as many haplotypes as the rest of the ecotype populations. This difference was even more pronounced with regard to the number of polymorphic sites.

The mean nucleotide composition for the progeny families was 28.1%, 25.4%, 27.0% and 19.5% of the T, C, A and G bases respectively. As indicated in Table 5.1B, gene diversity among the seven progeny families ranged from .7251 to .9412, while nucleotide diversity was high at 0.5273 % to 1.8237 %.

Haplotype distribution in the progeny families was almost uniform, albeit with a lower mean than in the parental groups. The total of 53 haplotypes for the progeny families indicates the genetic richness of these groups. Haplotypes generated for both parental and progeny families were submitted to the National Centre for Biotechnology Information or NCBI under accession numbers KM234130 to KM234211.

Table 5.1B Gene and nucleotide diversity in progeny families of *Macrobrachium rosenbergii* based on COI gene sequences

Progeny	N	Z	S	HD	π (%)
PP×KJ	19	10	35	.7836± .0976	0.9864±0.5437
JJ×KP	21	8	53	.7381± .0844	2.0045±1.0470
KK×PJ	17	12	37	.9412±.0432	1.8237±0.9694
NJ×PP	20	12	15	.8737±.0653	0.4654±0.2805
PP×KN	19	6	20	.7251±.0828	0.4521±0.2745
PJ×KK	18	8	18	.7974±.0739	0.5273±0.3137
KN×JJ	20	12	33	.8789±.0654	1.1266±0.6122

Where: n = sample size, Z = number of haplotypes, S = polymorphic sites, π = Nucleotide diversity (%), HD = haplotype diversity.

Tables 5.2A and 5.2B show the average pairwise population differences of the COI gene haplotypes for the parental ecotype populations and progeny families respectively; with the former ranging between 0.7544 and 0.9532, while the latter varied from 0.0137 to 0.9532. Both the average pairwise population differences and corrected average pairwise differences were significant for the same comparisons in the parental ecotype populations, while in the progeny families a group emerged as insignificant when corrected. Consequently, about 67% of the comparisons were significant in the parental ecotype populations; while this increased to 86% in the uncorrected and 81% in the corrected comparisons of the progeny families.

Table 5.2A Average pairwise population differences for COI haplotypes of four Malaysian ecotype populations of *Macrobrachium rosenbergii*

Population	J	K	N	P
J		0.8583**	0.7544	0.8701**
K	0.0693**		0.8786	0.9532*
N	0.0211	0.0137		0.9207**
P	0.0845**	0.0360*	0.0592**	

Where : above diagonal: average number of pairwise differences between populations (PiXY), below diagonal: corrected average pairwise difference (PiXY-(PiX+PiY)/2) and ** p <. 001, * p < .05.

Table 5.2B Group average pairwise differences for COI haplotypes between Malaysian *Macrobrachium rosenbergii* progeny families

Progeny	PP×KJ	JJ×KP	KK×PJ	NJ×PP	PP×KN	PJ×KK	KN×JJ
PP×KJ		0.9875***	0.9938**	0.9816**	0.9751***	1.0000***	0.9579**
JJ×KP	0.2266***		0.9160*	0.8929*	0.7619	1.0000***	0.86905*
KK×PJ	0.1314**	0.0763*		0.9530*	0.8947*	1.0000***	0.9471*
NJ×PP	0.1529***	0.0870*	0.0455*		0.81316	1.0000***	0.8725
PP×KN	0.2207***	0.0303	0.0616*	0.0137		1.0000***	0.8079
PJ×KK	0.2095**	0.2323***	0.1307***	0.1645***	0.2387***		0.9583**
KN×JJ	0.1266**	0.0605*	0.0370*	-0.0038	0.0059	0.1202**	

Where: above diagonal: Average number of pairwise differences between populations (PiXY), below diagonal: Corrected average pairwise difference (PiXY-(PiX+PiY)/2)
- * = p < .05, ** = p < .001, *** p < .0001

Tables 5.3A and 5.3B show FST pairwise comparisons between parental groups and progeny families respectively. For the parental ecotype populations, about 67% of the comparisons were statistically significant, with the minimum significant genetic differences being between the K and P ecotype populations (0.0378) and the maximum ones (0.0975) between the P and J ecotype populations. This result is consistent with their relative geographical distances from each other.

Table 5.3A Pairwise F_{ST} COI haplotypes comparisons of four Malaysian ecotype populations of *Macrobrachium rosenbergii*

Population	J	K	N	P
J	0.0000			
K	.0815**	0.0000		
N	.0280	.0157	0.0000	
P	.0975**	.0378*	.0644**	0.0000

- * = p <. 05, ** = p < .001

Table 5.3B Pairwise FST comparison of progeny families of *Macrobrachium rosenbergii* based on COI haplotypes

progeny	PP×KJ	JJ×KP	KK×PJ	NJ×PP	PP×KN	PJ×KK	KN×JJ
PP×KJ	0.0000						
JJ×KP	.2299***	0.0000					
KK×PJ	.1333***	.0856**	0.0000				
NJ×PP	.1555***	.0978**	.0482*	0.0000			
PP×KN	.2263***	.0397	.0700*	.0166	0.0000		
PJ×KK	.2096***	.2330***	.1312***	.1639***	.2391***	0.0000	
KN×JJ	.1319**	.0700*	.0395*	-.0044	.0070	.1249***	0.000

- *** = $p < .0001$, ** = $p < .001$, * = $p < .05$.

AMOVA from the analysis of the COI gene sequences of the four parental ecotype populations of *M. rosenbergii* revealed significant ($p < .0001$) differences between them. A noteworthy percentage of the variations was attributable to differences between ecotype populations (Table 5.4A) and population subdivisions (FST = .0545), suggesting that the presence of significant structuring of the studied ecotype populations. On the other hand, the AMOVA between the progeny families, as demonstrated in Table 5.4B, pointed to a statistically significant amount of the genetic variation being attributable to differences between families.

Table 5.4A Analysis of molecular variance for COI gene sequences between four Malaysian ecotype populations of *Macrobrachium rosenbergii*

Source of variation	d.f	Sum of squares	Variance components	% variation
Between Populations	3	3.315	0.0237	5.45
Within populations	113	46.488	0.4114	94.55
Total	116	49.803	0.4351	

- Fixation index FST = .05451, $p < .00006$, d.f = degree of freedom.

Table 5.4B Analysis of molecular variance for COI gene sequences between progeny families of Malaysian of *Macrobrachium rosenbergii*

Source of variation	d.f	Sum of squares	Variance components	% variation
Between families	6	8.859	0.0558	12.01
Within families	127	51.917	0.4088	87.99
Total	133	60.776	0.4646	

- Fixation index FST = .12013, p < .000001, d.f = degree of freedom.

The Tajima's D, Fu's Fs neutrality tests and mismatch tests for number of differences between pairs of haplotypes used to describe the demographic history of the studied ecotype populations are shown in (Table 5.5).

Table 5.5 COI haplotypes neutrality and demographic expansion tests for four Malaysian ecotype populations of *Macrobrachium rosenbergii*

Test	Statistic	Population				Mean	SD
		J	K	N	P		
Tajima's D	Tajima's D	–0.0870	–0.0299	–1.6570	–0.3129	–0.5217	0.7667
	P- value	.5410	.5690	.0270	.4590	.3990	.2524
Fu's Fs	Fs	–0.9323	–2.1057	–3.9208	–2.1784	–2.2843	1.2314
	P- value	.3040	.2140	.0450	.2020	.1913	.1076
	Theta π	1.7149	8.1561	3.8092	7.6847	5.3412	3.1044
Mismatch	SSD	0.0192	0.0137	0.0114	0.0180	0.0156	0.0036
	P- value	.5500	.5300	.6600	.5000	.5600	.0698
	Raggedness index	0.0676	0.0326	0.0275	0.0256	0.0383	0.0198
	P- value	.7500	.3300	.9100	.5900	.6450	.2473
	Theta K	2.5582	14.6796	9.6142	13.8810	10.1833	5.5484
	Theta S	1.7669	8.2231	7.0678	8.4030	6.3652	3.1221

Tajima's D test results were negative for all the parental ecotype populations; however the test showed only N ecotype population to have a significant ($p < .05$) departure from neutrality. The pairwise mismatch graphs for the haplotypes did not show clear monomodal, but sum of squared deviation and raggedness statistics are both small and not significantly distinguishable from simulated data under the assumption of demographic expansion (Figure 5.1 and table 5.5). However, Fu's Fs being not significant, although negative, does not support SDD and raggedness results.

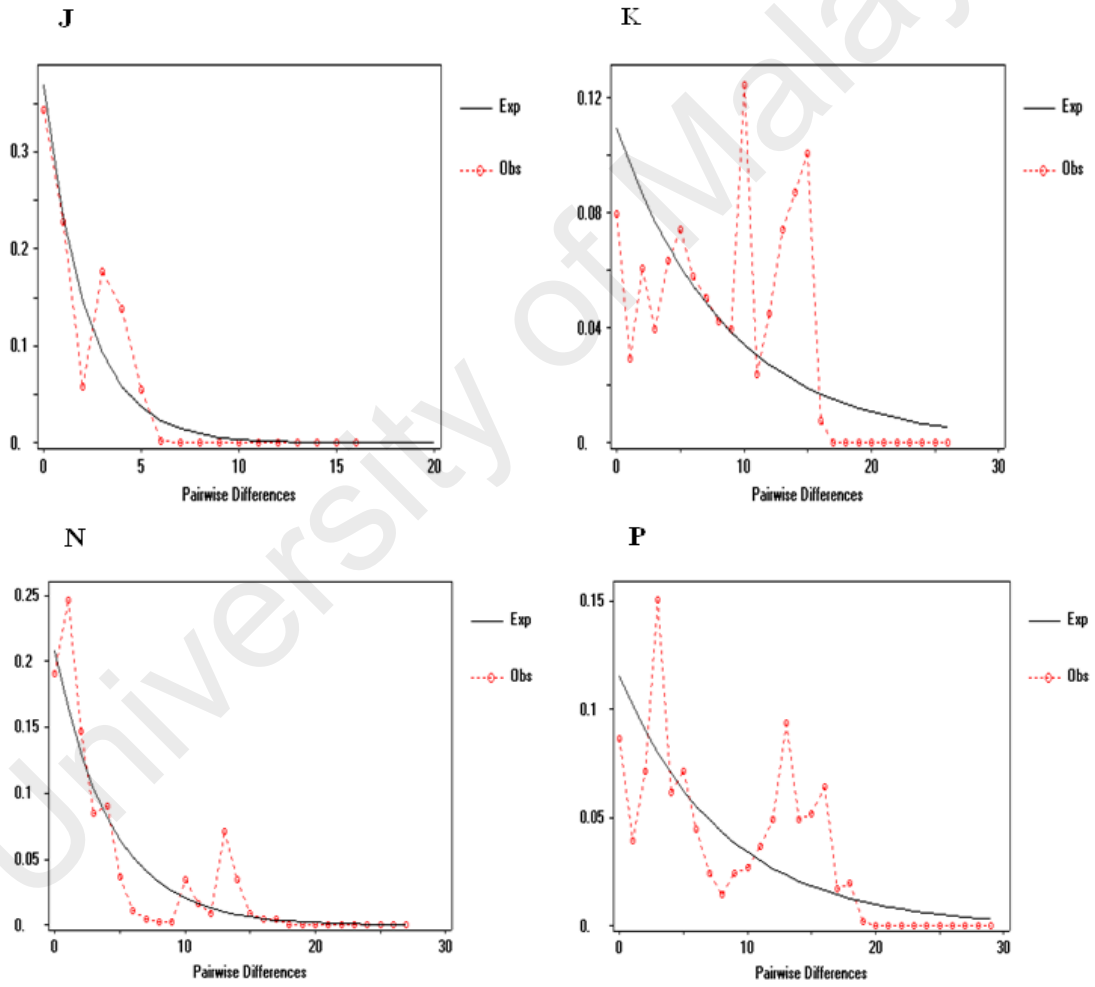

Figure 5.1 COI haplotypes Mismatch distributions of four Malaysian ecotype populations of *Macrobrachium rosenbergii*

- Ecotype populations are designated by their initials. Johor (J), Kedah (K), Negeri Sembilan (N), and Perak (P)

5.4.2: Phylogeny analysis

The phylogenetic tree presented in Figure 5.2 was drawn using only unique haplotypes of the parental ecotype populations based on maximum likelihood interpretation. It produced a division of these haplotypes into two major groups: a first group (I) representing three of the four ecotype populations, the exception being K; and a second group (II) composed mostly of haplotypes from the K and P ecotype populations, with no J haplotypes.

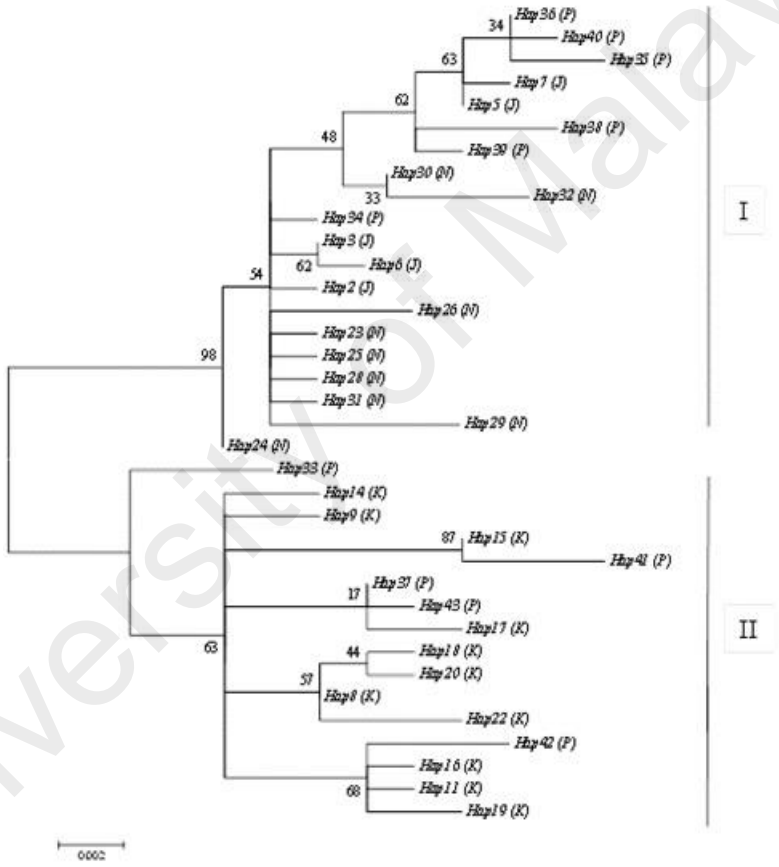

Figure 5.2 Maximum likelihood phylogenetic tree constructed for unique haplotypes of the COI gene of four Malaysian ecotype populations of *Macrobrachium rosenbergii*. Cluster I includes haplotypes from the Negeri Sembilan (N), Johor (J) and Perak (P) ecotype populations, and cluster II contains haplotypes from Kedah (K) and Perak (P) ecotype populations. The numbers at the branches represent bootstrap values of 1,000 replications

5.4.3: Networking of haplotypes

Networking all the haplotypes by median joining resulted in two clear clusters. The first included haplotypes from the K and P ecotype populations, but a complete absence of J ones.

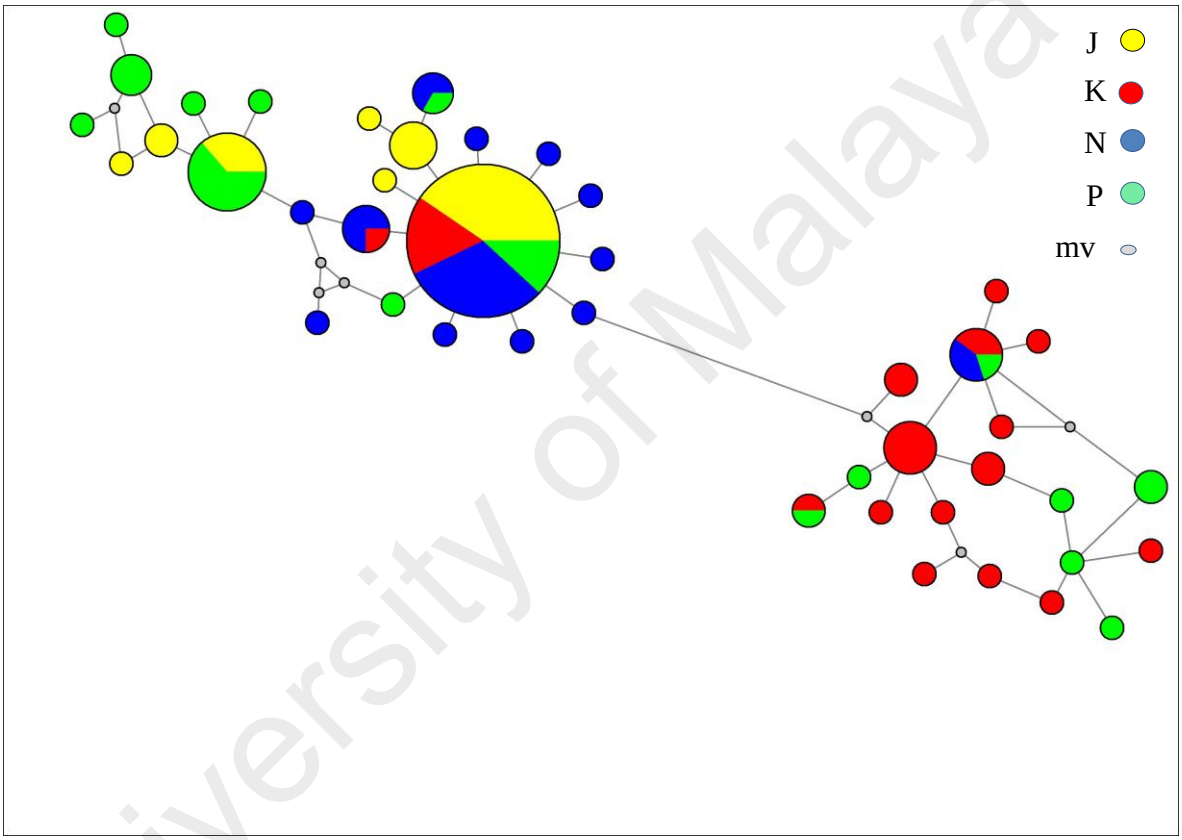

Figure 5.3 Median-joining network of COI haplotypes constructed for four Malaysian *Macrobrachium rosenbergii* ecotype populations. Circle size represents relative number of haplotype copies present in dataset. J = Johor population, K = Kedah population, N = Negeri Sembilan population, P = Perak population, mv = median vector

The second cluster was a mosaic with shared haplotypes in the form of a large circle surrounded by haplotypes differing from those at the core by a few site mutations (De Jong et al., 2011), with N haplotypes constituting the overall majority. It also had a sub-branch consisting of haplotypes from the geographically separate areas J and P.

5.5 Discussion

This study provides an analysis of the genetic structure, diversity and phylogeography of four ecotype populations of the giant Malaysian prawn (GMP) through investigating the cytochrome c subunit I locus, a mtDNA gene extensively employed by many researchers (Liu et al., 2011; De Jong, 2011; Hsu and Zeng , 2013; Xue et al., 2014). It also casts light on ways to ensure genetic diversity in cultured groups of this species. Given the GMP's popularity internationally for use in aquaculture, information on its genetic structure, genetic diversity and on the relationships between indigenous populations is of high interest-particularly for Malaysia, which is one of the top ten producers of the giant Malaysian prawn (New & Nair, 2012) and has also just started genetic improvement programs for this species. Moreover, Malaysia is the country that witnessed the first ever closure of the life cycle of this animal, as well as being the source of all the currently well-known cultured strains around the globe.

Table 5.1A shows high haplotype diversity associated with low nucleotide diversity in the parental ecotype populations studied. This pattern is similar to the results reported in marine mussels from isolated lakes (Goto & Hanzawa, 2011), as well as in the *Penaeus monodon* (Khamnamtong et al., 2009; Khedkar et al., 2013) and *Mugil cephalus* fish in the China Sea (Sun et al., 2012). In line with De Jong et al. (2011), differences between haplotypes could be explained as subtle and attributable to a few mutations. Nevertheless, some genetic structuring was found for the studied ecotype populations.

This structuring could be ascribed to geographical isolation; however, its degree was unexpectedly low. This is indicated by AMOVA showing a significant portion of variations

to be due to differences between ecotype populations, but with a low FST fixation index ($p < .05$; FST = .05451). In addition, this was corroborated by the significant pairwise comparisons of the ecotype populations (Table 5.2. and Table 5.3.) and the findings of both the phylogenetic tree and median joining networking (Figure 5.3. and Figure 5.4.), all of which showed the haplotypes clustering into two major groups, with those of P ecotype population appearing in both clusters, while the haplotypes of the J and K ecotype populations interchangeably exchange presence absence in either of the two clusters.

One explanation could be that one GMP ecotype population was artificially introduced into other rivers from Perak state, where hatcheries for prawn production were first based. Initially, therefore, all the farms in other states would have obtained their fries from hatcheries in Perak. Perhaps some feral prawns escaped into other rivers and mixed up with indigenous ecotype populations. This would be in line with the findings of a number of authors who have reported on the genetic impact of the introduction of some freshwater animals by humans such as the *Macrobrachium asperulum* (Liu et al., 2011), freshwater crab (Shih et al., 2004), freshwater fishes (Wang et al., 2004) and bamboo viper (Creer et al., 2001) in Taiwan, and *P. brisbanensiss* (Stevens et al., 2002) in New Zealand. In an agreement with Khamnamtong et al., 2009; Maneeruttanarungroj et al., 2006) suggestion for tiger prawn, we recommend using of the high genetic polymorphism of COI, with the unique haplotypes for developing DNA barcodes for broodstock used in breeding programmes.

Genetic drift is another important issue. Although the mismatch graphs of number of pairwise differences among haplotypes of GMP ecotype populations are not clear

monomodal, which is typical for populations under demographic expansion, their small SSD and raggedness index (P≥.05) may show a signature of demographic expansion. In addition, Tajima's neutrality test showed that only N ecotype population had significant ($p < .05$) signs of departure from mutation–drift equilibrium (Table 5.5. and Figure 5.2.). But still Fu's Fs test did significantly show much evidence for departure from neutrality. Tajima's D (Tajima, 1993) is more powerful when gene frequencies are within moderate levels, while Fu's Fs test is more sensitive in detecting bottlenecks, genetic hitchhiking and the over-dominance of selection which can occur when gene frequencies are extreme (Holsinger, 2012).

Results obtained here may therefore point towards a purification of selection at loci with moderate frequencies and decreasing variability of haplotypes for N and J ecotype populations. If this is the case, the situation could be serious: a warning sign of decreasing diversity that may necessitate strong decisions to combat habitat deterioration due to negative human interventions. Overfishing, illegal fishing, logging and pollution were reported by Bhassu et al. (2007) as endangering wild stocks of *M. rosenbergii* in Malaysia.

This requires a robust response, including temporary bans on fishing in some rivers. Specifically, we recommend a ban on fishing south latitude 2.9663 N to the southern borders of Malaysia for at least three months a year, to allow the GMP species to replenish. The states of Negeri Sembilan and Johor, from which the N and J ecotype populations were brought, are geographically quite close to Singapore, which offers a salutary example in this context, with the recent extinction of the *M. rosenbergii* species due to pollution (Ng 1997) and high demand, leading to over-fishing that in turn may have caused decreased

population sizes and reduced body weight resulting from high inbreeding. This is consistent with Sardà (1998), who reported a significant reduction in the mean size of *N. norvegicus*, a burrowing marine crustacean species, over a period of 20 years that could be a sign of overexploitation of the stock.

In contrast with mass selection, which can substantially reduce genetic diversity over generations (Knibb et al., 2014), cyclical mating can help to maintain such diversity as AMOVA revealed diversity between populations increased to more than double (12.01 vs 5.45) in only two cycles. Moreover, pairwise FST comparisons showed about 81 % of the groups to be significantly different, compared to 67 % in the parental ones. But, as aquatic species are highly fecund, hence it can happen by chance that a whole subgroup to be simply siblings. So, close monitoring and regular checking of aquaculture groups is important, for a sudden genetic drift may occur at any time. In conclusion, we do recommend applying this method of mating. However, for a better insight into these differences between and among the ecotype populations, further investigation using nuclear markers is required. We also recommend the utilization of unique haplotypes in evaluating restocking activities carried out by the Fisheries Department.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

The popularity of the giant Malaysian prawn (GMP), *Macrobrachium rosenbergii*, locally known as udang galah, for aquaculture is escalating, as it is currently cultured in more than 40 countries around the world. Malaysia is part of the natural credible of this species (Wowor & Ng (2007); therefore, the practice of impounding it in captivity in enclosures such as ponds and tide water has been performed since time immemorial (Wickins, 1976).

However, its modern culture comes to existence via the great breakthrough made in its biology and life cycle by Ling, a former-FAO expert in fish biology, who discovered that its larval stages require brackish water conditions to develop and metamorphose into a pre-juvenile stage or a tiny adult called post-larval stage (New et al., 2010) while working at the Marine Fisheries Research Institute in Penang, Malaysia. So, in this country that witnessed this great event, its modern culture started by the establishment of the first hatchery in 1984, but the GMP genetic improvement has just started by a program initiated in 2009. Unfortunately GMP producers in Malaysia and other countries in the region such as Thailand and India face fluctuations/or declines in its produce. Figure 3.1 presents these fluctuations in Malaysia during the period of 2000- 2013. This pattern of instability in the country produce has been hypothesized in this study to be an outcome of the unintentional practice of choosing brood stock by hatchery operators relying upon readiness to spawn rather than according to genetic weight, which in turn could consequently result in selection of small size, early maturing brood stock prawns as well as ignoring some important steps in management of the species such as juvenile grading throughout the production cycle that

ultimately reduces aggressiveness among prawns of similar sizes in comparison to non-graded prawns, overcrowding, water quality aspects etc.

Therefore, the study was planned to begin with conducting an in-depth questionnaire addressed to the adopters of the GMP entrepreneurial system of pond management in Negeri Sembilan, Malaysia. This was then, followed by an evaluation of four ecotype populations of the species as part of the genetic improvement program launched in 2009. These four ecotype populations which were previously collected from geographically apart regions in Peninsula Malaysia were compared regarding growth related trait, namely harvest weight (WT), total body length (TL), carapace length (CL) and tail length (TA). Moreover, fixed effects and heritability values for these economically important traits were evaluated. In addition multiple linear regression approach was used to estimate WT as a respondent variable and TL, CL and TA as predictor variables or covariates for two different age groups.

Finally, the study was culminated by conducting a study on genetic structure, genetic diversity and the effect of cyclical mating on this genetic diversity using these same four groups and their progenies.

Results of analysis of the in depth study questionnaire that addressed entrepreneurial pond management system adopters mentioned above showed that the major causes of produce instabilities are lack of good quality seeds and high juvenile mortality; both of which are likely to be caused by detrimental inbreeding effects. Better seeds could be obtained by keeping the whole production cycle in captivity and taking care of genetic diversity of

brood stock. Furthermore, juvenile grading throughout the production cycle substantially reduces the production cycle and result in more homogeneous produce. Adherence to the recommended stocking density is also important. The study recommends more care on water quality issues. On cost view, feed price stability needs the intervention of the Malaysian government by opening the way for other competitors and /or establishing government owned feed factories.

Results on comparing the GMP ecotype populations described earlier in Chapter 4 showed significant differences among them for all the four traits studied (P<.0001).;with Duncan's multiple range-test revealing that Kedah ecotype population is superior over the rest of the ecotype populations for all the growth related traits tested at p value of .05.

Kedah progeny family was also revealed to be superior over all progeny families for all traits tested in progenies that were successfully produced mating cohorts in a cyclical manner (Figure 4.2). In addition, estimated heritability values for the same growth related traits were moderate to high; that is to say: 0.5788±0.2989, 0.5156±0.2753, 0.4277±0.2407 and 0.5120±0.2739 for WT, TL, TA and CL respectively. These results consolidate each other and led to recommending the application of selection methods under cyclical mating design to improve the GMP in Malaysia and anywhere else in the developing world. Moreover, estimation of body weight through multiple linear regression model using body length measurements as predictors was found to be more effective and accurate in this species that encouraged the researcher to propose its adoption as model in other crustaceans and other aquatic metazoan as well. Principally, at least two of the three predictors were found to be significant (p<.05) estimators of harvest weight in more than 90% of the cases

for both age groups; explaining 73-94% of the total variance. Different equations were obtained when the two sexes were separated indicating the existence of sexual dimorphism for growth traits. In conclusion, prediction of weight through utilizing all predictor morphometric measurements at a time seemed to be more accurate and precise.

In the molecular population genetics study, sequences of the cytochrome c subunit I locus mitochondrial gene or COI were analyzed in order to explore genetic structure, diversity and phylogenetic analysis of the four studied ecotype populations of *Macrobrachium rosenbergii* which has been intensively employed by many authors (Liu et al., 2011; De Jong et al. 2011; Hsu et al., 2013; Xue et al., 2014) as well as testing the effect of cyclical mating on securing variability among their progeny families. Forty three haplotypes of the gene were generated for the parental ecotype populations; most of which were unique. Genetic and nucleotide diversities of these haplotypes ranges were .6575 to .9206 and 0.2511-1.1942% respectively. Another 47 haplotypes were generated for the progeny families. Eighty two of generated for both groups collectively were submitted to NCBI under accession numbers between KM234130 -KM234211. The haplotypes in parental ecotype populations showed high diversity associated with low nucleotide diversity (Table 5.1A). Although differences among these haplotypes are considered as subtle due to only few bases variations still genetic structuring of the ecotype populations is evident and could be attributed to geographical isolation for an enormously long time. this is also corroborated by significant pairwise comparisons of the ecotype populations (Table 5.2 and Table 5.3.), the findings of both the phylogenetic tree and median joining networking (Figure 5.3. and Figure 5.4.) that show haplotypes clustering into two major groups, with those of Perak ecotype population appear in both clusters; while Johor and Kedah

haplotypes exchanges presence and absence in the two clusters. Perak population seem to be artificially introduced to other locations in Malaysia, for historically Perak state, from which the samples of this population were collected, is where hatcheries for this prawn production have been first established. Subsequently at the commencement of the business in Malaysia, all farms in almost all states used to get their fries from Perak hatcheries (i.e. human introduction). Also, an intentional mixing may have occurred or perhaps, some feral prawns may have escaped to rivers mixing with their indigenous populations, for adult prawns are capable to walk, not only on the substratum but also can creep over stones at the shallow edges of rivers and in rapids, and also climb on vertical surfaces (small waterfalls, weirs, etc.) and cross land, provided there is abundance of moisture (New et al., 2010).

Tajima's D neutrality test for parental ecotype populations indicated significant ($p < .05$) departure of Negeri Sembilan ecotype population from mutation- drift equilibrium; meaning that it may be losing variability.The pairwise mismatch graphs for the haplotypes did not show clear monomodal, but sum of squared deviation and raggedness statistics are both small and not significantly distinguishable from simulated data under the assumption of demographic expansion (Figure 5.1 and table 5.5). But results of Fu's neutrality, although negative, failed to support SDD and raggedness results. Seasonal fishing ban was recommended south of latitude 2.9663 N till the southern borders of Malaysia for at least three months a year in order to replenish *M. rosenbergii* populations in the country. On one hand Tajima's D is more powerful when gene frequencies are within moderate levels. On the other hand Fu's Fs test is more sensitive in detecting bottlenecks, genetic hitchhiking and over-dominance of selection which occur when gene frequencies are extreme

(Holsinger, 2012). So, this result may be an evidence for purifying selection at loci with moderate frequencies.

Clear genetic structuring among the parental ecotype populations was evident as a result of significant AMOVA difference among the populations (5.45%, $p < .00001$), haplotypes networking and the phylogenetic tree constructed of only the unique haplotypes of ecotype populations studied. This situation may be considered serious and an actual alarm sign of decreasing diversity that necessitates taking strong decisions to oppose habitat deterioration due to negative human interventions such as overfishing, illegal fishing, logging and pollution were reported by Bhassu et al. (2007) to endanger wild stocks of the species in Malaysia. Therefore, a seasonal ban of fishing was recommended south of latitude 2.9663 N to the Malaysian Singapore border to enable the species to replenish its populations in the area. Moreover, Negeri Sembilan and Johor states from which two ecotype populations were brought are geographically closer to Singapore, which recently witnessed extinction of the species due to pollution (Ng, 1997) as well as high demand for it that lead to its over fishing that may have consequently resulted in decreased population size that affected body size (observed in Johor ecotype population) as a resultant mostly of high levels of inbreeding and overexploitation.

This study clearly revealed that cyclical mating, contrary to mass selection, can secure genetic diversity. As a result AMOVA revealed about double (12.01 vs 5.45) genetic variance in the progeny families compared to the parental ecotype populations. Moreover, pairwise FST comparisons showed about 81% of the progeny families being significantly different compared to 67% in the parental ones. But still close monitoring and regular

checking of populations under aquaculture groups is important for a sudden drift may occur at any time since sorting only siblings in a certain batch is not avoidable under available technology conditions. To conclude application of cohort cyclical mating design is recommended to improve this species in developing countries.

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PUBLICATIONS

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