BREEDING AND LARVAE REARING OF THE GREEN MUD CRAB, *Scylla paramamosain* (Estampador 1949) IN CAPTIVITY

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By

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LIST OF ABBREVIATIONS, SYMBOLS AND UNITS

ABBREVIATION	ELABORATIONS/ MEANING
<	Less than
>	Greater than
∑	Sum or total
≤	Less than or equal
µ	Micron
µl	Micro litre
µm	Micro metre
°C	Degree centigrade
AD	Artificial diet
ADC	Apparent digestibility coefficient
ALA	Alpha-linoleic acid
ANOVA	Analysis of variances
AOAC	Association of Analytical Chemists
APHA	American Public Health Association
ARA	Arachidonic acid
BL	Body length
C	Crab instars
C1	Crablet-1 stage (Crab instars)
C3	Crab-3 stage
CEMACS	Centre for Marine and coastal studies
CH	Chelae height
CL	Carapace length
CW	Carapace width
CWm	Carapace width at median (50%) sexual maturity

DAH	Day at hatching
DHA	Docosahexaenoic acid
DMRT	Duncan's Multiple Range Test
DNA	Deoxyribonucleic acid
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid
F1	First generation
F2	Second generation
FAME	Fatty acid methyl ester
FAO	Food and Agriculture Organization
g	Gram
GC	Gas chromatography
GPS	Geographical positioning system
GSI	Gonad somatic index
HUFA	Highly Unsaturated Fatty Acid
ICW	Internal carapace width
LSI	Larval stage index
M	Megalopa
MA_{50}	Age at median (50%) sexual maturity
MDS	Moulting death syndrome
MD_{50}	Medium Death (50% death)
Mg/l	Milligram per litre
mm	Millimetre
MT	Metric tons
MUFA	Monounsaturated fatty acid
n-3	Omega-3
ND	Not detected
NF	Natural feed
NFE	Nitrogen free extract

P_0	Base population
PB	Propodus breadth
PL	Propodus length
ppm	Parts per million
ppt	Parts per thousand
RM	Malaysian Ringgit
S1 to S5	Maturity stage-1 to maturity stage-5
SD	Standard deviation
SFA	Saturated fatty acid
SGR	Specific growth rate
SM50	50% sexual maturity
SPSS	Statistical Package for the Social Sciences
TW	Total weight
UFA	Unsaturated fatty acid
USA	United States of America
USM	Universiti Sains Malaysia
Viz., or ie.,	Videlicet, namely, especially
WSSV	White Spot Syndrome Virus
YHV	Yellow Head Virus
Z1 –Z5	Zoea-1 to Zoea-5
OSR	Operational sex ratio
ESR	Estimated sex ratio
GT	Guarding time
♂	Male
♀	Female

PEMBIAKBAKAAN DAN PEMELIHARAAN LARVA KETAM NIPAH, *Scylla paramamosain* (Estampador 1949) DALAM KURUNGAN

ABSTRAK

Kajian perintis pembiakbakaan induk ketam nipah (*Scylla paramamosain*) ini melibatkan beberapa siri eksperimen. Pada peringkat ternakan percubaan larva diberi makan *Artemia* umbrella, rotifer diperkaya dan rotifer daripada peringkat Z1 ke Z2, diikuti dengan *Artemia* nauplii, *Artemia* nauplli diperkaya dan diet komersial, masing-masing daripada peringkat Z3 sehingga megalopa. Indeks peringkat larva (LSI) yang amat tinggi, secara signifikannya menunjukkan bahawa megalopa dan kemandirian anak ketam adalah amat tinggi ($p<0.05$) yang terhasil daripada stok induk yang diberi makan dengan rotifer diperkaya dan diikuti dengan skema pemakanan *Artemia* diperkaya yang mengandungi asid eikosapentaenoik (EPA), asid dokosaheksarnoik (DHA), n-6 hingga n-3 dan EPA tinggi DHA, iaitu 17.32%, 3.82%, 0.20 dan 0.22, masing-masing semasa di peringkat megalopa untuk proses metamorfosis anak ketam secara berturutan. Pertumbuhan ketam bakau memaparkan corak sigmoid bagi kedua-dua jantina dengan pertambahan berat dalam ketam jantan, secara signifikannya adalah amat tinggi ($p<0.05$). Walaupun ketam bakau betina membesar lebih cepat ($p<0.05$) di dalam sangkar-luar, namun kemandirian serta perkadaran intak ketam adalah amat tinggi ($p<0.05$) di dalam sangkar-tertutup dan sangkar-terapung di luar. Ketam bakau jantan mencapai kematangan seks selepas spermatogonia, spermatosit dan spermatozoa, manakala, ketam betina mencapai kematangan sepenuhnya melalui proliferasi, previtelogenesis, vitelogenesis primer, vitelogenesis sekunder dan vitelogenesis tertier. Bagi anak ketam, kematangan seks kali pertama bagi kedua-dua ketam jantan dan betina bermula pada 5 bulan dan 5 hingga 5.5 bulan masing-masing. Umur matang medium (MA_{50}) bagi ketam jantan dan betina adalah 6.5 bulan dan 6.5 hingga 7 bulan masing-masing. Diperhatikan bahawa kematangan adalah lebih cepat dalam ketam jantan, tidak sinkroni pada intraseks dan serentak pada interseks. Ketam bakau mengawan secara lengkap dengan turutan perlakuan seperti memikat, pengawalan sebelum mengawan, penyalinan kulit, kopulasi, dan pengawalan selepas kopulasi. Masa yang diambil dalam setiap peringkat menunjukkan hubungan linear yang kuat ($p<0.01$) dengan sama ada saiz badan ketam jantan atau betina dalam nisbah 1:1. Dapatan nisbah seks operasi jantina (OSR), masa pengawalan (GT) dan perkadaran intak betina semasa "communal mating" mencadangkan bahawa nisbah jantan-betina 2:4 hingga 2:8 sebagai superior dengan kepadatan 1.2 hingga $2/m^2$ masing-masing. Prestasi pembiakan dan kualiti larva adalah sama ($p>0.05$) dalam kalangan stok induk ternakan dan induk semula jadi, kecuali fekunditi yang tinggi dan jumlah larva fototaksi pada induk semula jadi. Dalam kes pembenihan daripada pengawanan tunggal, pembenihan pertama dan kedua mempunyai fekunditi yang amat tinggi dan keguguran telur yang amat sedikit. Asid lemak penting seperti EPA, DHA, jumlah n-3; dan jumlah asid amino penting (EAA) adalah secara signifikannya adalah amat tinggi ($p<0.05$) dalam pembenihan pertama dan kedua larva. Justeru, masa bertahan paling lama ($p<0.05$) terhadap kelaparan berbanding dengan larva dalam pembenihan ketiga. Secara pragmatiknya, stok induk ketam bakau dapat dikembangkan atau boleh diternak dengan jayanya dalam sangkar atau kurungan, yang mungkin menyokong operasi penetasan tanpa henti atau secara berterusan.

BREEDING AND LARVAE REARING OF THE GREEN MUD CRAB, *Scylla paramamosain* (Estampador 1949) IN CAPTIVITY

ABSTRACT

This pioneer study on the captive broodstock breeding of the mud crab (*Scylla paramamosain*) consisted with a series of experiments. The larvae rearing trial was fed with *Artemia* umbrella, enriched rotifer and rotifer from Z1 to Z2 stages followed by *Artemia* nauplii, enriched *Artemia* nauplii and commercial diet, respectively from Z3 to megalopa. Highest larval stage index (LSI), significantly higher ($p<0.05$) megalopa and crablet survival was achieved from enriched rotifer followed by enriched *Artemia* feeding schemes that had the eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), n-6 to n-3 and EPA to DHA ratios of 17.32%, 3.82%, 0.20 and 0.22, respectively in megalopa for subsequent metamorphosis to crablet. Growth of mud crab displayed the sigmoid pattern for both sexes with significantly higher ($p<0.05$) weight gain in males. The female mud crab grew faster ($p<0.05$) in outdoor tanks, but survival and the proportion of intact crabs were higher ($p<0.05$) under indoor compartment and outdoor floating boxes. The male crabs attained sexual maturity following spermatogonia, spermatocytes and spermatozoa, whilst, the females completed gonad maturity through proliferation, previtellogenesis, primary vitellogenesis, secondary vitellogenesis and tertiary vitellogenesis. The first sexual maturity appeared at 5 months and 5 to 5.5 months for male and female crabs, respectively, after settlement as crablet. The median maturity age (MA_{50}) was 6.5 months and 6.5 to 7 months for male and females, respectively. Faster maturation in male, asynchrony in intra-sexual and simultaneous intersexual maturation was observed. Mud crab completed the mating with sequential behavior of courtship, pre-mate guarding, moulting, copulation and post copulation guarding. Time spent in each step showed strong-linear relationship ($p<0.01$) with either male or female body size under 1:1 ratio. Findings on operational sex ratio (OSR), guarding time (GT) and the proportion of intact females under communal mating suggested that the 2: 4 to 2: 8 male-female ratios as superior that had densities of 1.2 to $2/m^2$, respectively. Reproductive performance and larvae quality seemed similar ($p>0.05$) among domesticated and natural broodstocks, except higher fecundity and amount of phototaxis larvae in natural broodstock. In case of successive spawning from a single mating event, the 1^{st} and 2^{nd} spawning had higher fecundity and lower dropped eggs. The essential fatty acids of EPA, DHA, total n-3; and total essential amino acid (EAA) were significantly higher ($p<0.05$) in 1^{st} and 2^{nd} spawned larvae, thus withstand longer time ($p<0.05$) against starvation than the 3^{rd} spawned larvae. Pragmatically, mud crab broodstock could be developed successfully under captive condition that might support uninterrupted hatchery operation.

CHAPTER- 1

GENERAL INTRODUCTION

1.1 Background

World fish production has increased at a steady rate since the last five decades, whereas, both aquaculture and capture based fisheries have increased dramatically during the immediate past decade (FAO, 2014). Aquaculture has expanded tremendously by means of culture area, farming system, the adoption of new technologies and species diversification, but still to date, the total global aquaculture yield is somewhat lower than capture based fisheries yield, thereby accounting for 46% of total fish food supply (FAO, 2014). The world aquaculture production of edible fish has reached more than double from 32.4 million tons in 2000 to 70.5 million tons in the year 2013 (FAO, 2014). Asia has occupied top world ranking by producing 88.39% of total edible food fish, of which, China has contributed 61.69% as the single largest producer (FAO, 2014). Even though, in 2010, China's aquaculture production has collapsed by about 1.7 million tons as a result of disease, natural disaster and environment pollution, China is still the top ranking nation among Asian countries (FAO, 2014).

In world aquaculture, among the major groups, crustacean aquaculture has diversified rapidly and the production has increased exponentially during the last decade. In 2012, the world aquaculture production was dominated by freshwater finfish (57.9%), whereas, crustacean augmented a production of 6.4 million tons, which was 9.7% of total aquaculture food fish production and 22.4% of that of the value (FAO, 2014). Among crustaceans, only penaeid shrimps, freshwater prawns and crabs are being cultured commercially (Wickins and Lee, 2002) and white leg penaeid shrimp has been regarded as the largest commodity and highly valued species, thereby accounting for 1.6 million metric tons of production in 2002 (Rosenberry, 2003) with 15% of the international trade value of fishery products in the year 2008 (FAO, 2011). During the year 1994 to 1997, shrimp aquaculture had severely faced the outbreak of white spot syndrome virus (WSSV) and yellow head virus (YHV) (FAO, 1997; Hill, 2002), which resulted in significant financial losses in the sector (FAO, 1997; Flegel and Alday-Sanz, 1998). This has caused the farmers to find alternate species of shrimp, which are hardy, practical, commercially important and environmentally friendly (FAO, 1997) to be cultured in extensive shrimp ponds (Christensen *et al.*, 2004).

Apart from shrimp, mud crab aquaculture has subsequently attracted the attention within a short time and this has accelerated the production tremendously. The global mud crab production of 10,000 tons in 2001 (FAO, 2002; FishStat, FIGIS) has drastically increased to 140,321 tons, which contributed a total value of $396 million in the year 2010 (FAO, 2011). The global production of mud crab had increased rapidly during the early 21st century, after showing fluctuations during the later part of the 20th century. According to FAO (1997), the global production decreased to almost half in 1995 (3,000 tons) compared to that of the production in 1993 (6,000 tons) which had been reportedly associated with the production fluctuations of major crab production in some countries. Sri Lankan's export of the crab had decreased from 973.75 MT in 1985 to a minimum level of only 45.02 MT in 1990 (Jayamanne, 1991). In the Philippines, mud crab production increased gradually from 1983 and in 1989 the total production was recorded as 1610 MT, it declined in 1990 to the level of 179 MT but further increased 2782 MT in 1995 with an average production of 920 kg/ha (Fortes, 1997). Such type of regional fluctuations in crab production was primarily associated with the indiscriminate destruction of natural habitat, especially the mangroves (Hill, 2002; Sombat, 1991; Walton *et al.*, 2006; Shelley and Lovatelli, 2011). However, the global production of mud crab further increased radically to 173,068 tons in 2012 (FAO, 2014; FishStat, FIGIS) and this was mainly associated with the over exploitation of the resources around the Indo-Pacific Ocean (Le Vay *et al*., 2007); indiscriminate harvests of all size groups to gain more incentives (Takeharu, 2001; Kosuge, 2001) and partiall support from hatchery produced seeds to grow out which added a considerable aquaculture production to the total yield (Shelley and Lovatelli, 2011).

Mud crab is an export oriented commodity being captured, cultured for long duration or fattened within short time and exported in live forms, whereas soft-shell crabs are exported in frozen form. Mud crab has rapidly drawn the attention of farmers due to its giant size, fast growth, a hardy nature and its relatively high immunity to diseases (Keenan, 1999; Shelley and Lovatelli, 2011). With its high quality flesh, luxurious taste and richness in protein, vitamins and minerals (Radhakrishnan, 2000) mud crab is considered as a valuable item for seafood restaurants in many countries. Mud crab has a thriving market in China, Japan, Korea, USA, Hong Kong and Thailand (Ferdoushi *et al.*, 2010), while mud crabs from Bangladesh are exported to Taiwan, Singapore, Hong Kong, Malaysia, Thailand and USA (Chandra *et al*., 2012). The demand of live mud crab, soft-shelled crab and crab meat has been increasing tremendously worldwide (Cholik, 1999; Keenan, 1999; Wickins and Lee, 2002).

However, as an emergent species in aquaculture, mud crab (*Scylla* spp.) has become a non-conventional fishery commodity playing a vital role in foreign exchange earnings, income generation and livelihood improvement for poor people globally in the coastal region, especially in South-East Asian countries. Thus, mud crab has become the key research interest to many crustacean research groups.

1.2 Mud crab aquaculture, research and constraints

At the beginning, mud crab was considered as monospecies of *Scylla serrata,* according to Stephenson and Campbell (1960). On the basis of morphometric and genetic characteristic, Keenan *et al*. (1998) further revised the classification into four distinct species, viz, *Scylla serrata, S. olivacea, S. paramamosain* and *S. tranquebarica*. *Scylla serrata* is large in size and has the widest distribution. *Scylla* species are mainly discovered in areas of the Indo-West Pacific Ocean to the Red Sea as well as along the seas of Australia, Taiwan, Philippines, South Africa, Japan, the Pacific Islands (Keenan *et al.,* 1998) and other tropical and sub-tropical regions. *Scylla* species are large in size and able to tolerate a wider range of ecological conditions (Hill, 1979). Naturally, mud crabs are moderately resistant to diseases and they grow faster by consuming a wide variety of foods (Williams and Primavera, 2001).

In the natural life cycle, mud crabs are euryhaline, usually preferring to inhabit in the intertidal mud flats, shallow lagoons, rocky sheltered estuaries, and bushy mangrove forests (Hill, 1974; Robertson, 1989; Le Vay, 2001). The juveniles are relatively abundant in intertidal mud flats within estuarine mangroves, whereas the adults are more plentiful in the estuarine subtidal regions (Le Vay, 2001; Shelley and Lovatelli, 2011). This type of different habitat selection is mainly associated with the growth and maturity of mud crab through moulting (Du Plessis, 1971). The maturity stages of mud crab have previously been studied by many researchers (Poovachiranon, 1992; Robertson and Kruger, 1994; Quinitio *et al.*, 2007; Islam *et al.*, 2010; Islam and Kurokura, 2012; Azmie *et al.*, 2012) on samples collected from wild sources. All the collected samples were not individually identified, thus might have possibilities of mixture of different age groups, originating from different parents or have more than a single species. However, all those studies have been conducted from the stock management point of view (Goshima *et al.*, 2000; Conan *et al*., 2001) for minimum legal size implementation. It is strongly believed that a clear concept of first sexual maturity of both male and females of the same age group would enable the hatchery manager's to manage successful copulation (mating). Any mistake in determining the sexual maturity period might cause unsuccessful mating or eventually cause the death of the female due to the aggressive nature and cannibalistic behavior of the partner as the female remains in a vulnerable state during moulting (Churchill, 2003; Shelley, 2008). On the other hand, an understanding of gonad maturation stages is a prerequisite for breeding and the establishment of hatchery management protocol such as when and what should be done. However, there are still no documented records of maturity study of hatchery reared same age group of animals.

Mature broodstock move to the estuary and inshore areas in search of mates for copulation. Mating takes place in the estuarine environment as the female remains in its soft shell condition at pubertal moulting (Churchill, 2003, Shelley, 2008). However, the mating of mud crab is a complex and risky process for the female and there is no documented information on the mating process, male-female interaction and male-female density or ratio for successful mating. During mating, the sperms are transferred into the spermathecae of the female, stored therein for a long time, even after moulting (Bliss, 1983). Female mud crabs are capable of fertilizing three successive batches of eggs from the stored sperms without further mating (Churchill, 2003; Shelley and Lovatelli, 2011). This capability of the female mud crab is therefore an opportunity for the hatchery managers to save time in searching or rearing of the new broodstock. This will minimize the cost of purchasing new broodstock or for the rearing of the males. However, still to date, a majority of hatchery managers or even researchers have to only use the females for the first spawning. This is either due to their ignorance about successive spawning or because they believe that successive spawning would not produce good quality larvae. However, there are still no published documents on the successive reproductive performance and larvae quality from a single mating except for some preliminary reports on reproductive performance made by Quinitio *et al.* (2010).

Immediately after mating, the female will migrate to the sea where spawning and hatching takes place (Ong, 1966; Shelley and Lovatelli, 2011), while mature females migrate out of estuaries into the deep sea to spawn and stay there until the eggs hatch (Hill, 1975; Heasman and Fielder, 1983; Hill, 1994; Shelley, 2008). The newly hatched larvae will stay in the deep sea until it reaches into crablet and migrate back to the estuarine environment, intertidal mudflats and mangroves (Keenan, 1999) to grow further. This type of catadromous behavior and frequent change in habitat is the main constraint of sufficient data collection on the crucial phases of the mud crab life cycle.

Virtually, mud crab aquaculture was first started traditionally in China more than 100 years back and gradually spread over to other Asian countries for the last 30 years (Keenan, 1995; Shen and Li, 1994). Meanwhile, mud crab aquaculture has been diversified worldwide with the adoption of farming technology, including the grow out in earthen ponds (Duc, 1997; Kennan, 1995; Tuan and Hai, 1992; Trino *et al.* 1999; Trino *et al.* 1999a; Catacutan *et al*., 2003), pen culture (Trino and Rodriguez, 2002; Agbayani, 2001; Ikhwanuddin *et al*., 2011), the fattening in communal basis (Csavas, 1995), individually fattening in boxes or cages (Cholik and Hanafi, 1992; Liong, 1992; Felix *et al.*, 1995; Sivasubramain and Angel, 1992; Begum *et al.,* 2010; Shelley and Lovatelli, 2011) and soft shell shedding (Sivasubramain and Angel, 1992; Shelley and Lovatelli, 2011). The survival rate has increased from 40% up to 75% for the grow out (Cholik and Hanafi, 1992; Macintosh *et al.*, 1993) and more than 90% for the individual fattening (Begum *et al.,* 2010). All these led to a drastically increased global production of 173,068 tons in 2012 (FAO, 2014; FishStat, FIGIS) and has turned the industry as a promising sector globally.

Despite the emerging industry, the bottleneck that hinders further expansion and commercialization of mud crab aquaculture is the dependence on natural juveniles as seed (Williams and Primavera, 2001). This is because the natural seeds are not sufficient to support the present size of the mud crab industry (Cowan, 1984; Liong, 1992) thereby making the industry unreliable and unsustainable (Le Vay, 2001). Hatchery technology, *ie*, a sustainable seed production protocol in commercial scale, is the pre-requisite for sustainable development that will help to further flourish mud crab aquaculture (Camacho and Apya, 2001; Shelley and Lovatelli, 2011). In fact, seed production technology for some of the commercial marine fin fish species (Phelps, 2010) and that of some crustacean (Penaeid shrimp and giant freshwater shrimp) has been established successfully (Mahmud, 1993; Uddin *et al.,* 2013). Until today, the seed production of mud crabs seems difficult due to the low and inconsistent quantities of crablets production. In many Asian countries, the seed production of mud crab is still under experimental stages, including Malaysia. Despite the low survival rate of larvae from early zoea to crablet stage, Vietnam, China, Philippines, Japan and Australia are trying to produce crablets in commercial scale (Shelley, 2008). Although, in recent years, the average survival rate has increased (Wang *et al.*, 2005) it is still not sufficient for the global demand, it is even far back from the recent need of the seed producer countries though it has a big support from natural seeds.

The reasons behind this low rate of seed production is mainly associated with its sole dependence on natural broodstock (Keenan, 1999; Shelley, 2008), the unavailability of required good quality broodstock in due time (Robertson and Kruger, 1994) and coupled with unpredictable reproductive performance and unreliable larvae quality of the natural broodstock (Churchill, 2003; Quinitio and Parado-Estepa, 2008; Thach, 2009). The reproductive performance and larvae quality often vary within species (DeMartini, 1991), depending on the ambient environment where they were grown and developed (Brooks *et al.,* 1997), and especially by temperature as well as seasonal rainfall (Heasman *et al.,* 1985). The maternal factors which vastly affect the reproductive performance and larvae quality involve the diet of the brood, endocrine status at the time of oocyte development and subsequent physiological condition of brood (Brooks *et al.,* 1997). Indeed, egg and larvae quality are strongly regulated by the instinct nutrient which they receive from the maternal side during oogenesis and embryogenesis (Luquet and Watanabe, 1986). However, nutrition in broodstock affects the egg and larvae quality in fish (Lavens *et al.,* 1999; Kabir, 2012), in crustaceans (Lavens *et al.*, 1991; Palacios *et al.*, 1998) and in crabs (Millamena and Quinitio, 2000; Djunaidah *et al.*, 2003; Veronica *et al*., 2007a). Thus, the biochemical composition of eggs and larvae has been regarded as a prospective indicator for the assessment of eggs and larvae quality (Brooks *et al*., 1997; Bell *et al.*, 1997).

Despite the potential roles of the biochemical composition of eggs and larvae to determine larvae quality, a majority of the studies focused on macro-nutrients, i.e. proteins, fats and carbohydrates (Brooks *et al*., 1997). Of course, the level of lipid plays a vital role in egg formation, while the composition or quality of lipid ie, fatty acid composition, regulates the quality of eggs and larvae of fish (Harel *et al.*, 1994; Carrillo *et al.*, 1995; Kabir, 2012) and also of crustaceans (Harrison, 1990; Jones *et al.*, 1997; Churchill, 2003). Indeed, one of the major nutritional factors that has been found to significantly affect the reproductive performance of fish is the essential fatty acid contents (Watanabe *et al*., 1984b; Kabir, 2012). The percentage of morphologically normal eggs and viable larvae has been found to have an increased level of *n*-3 HUFA (Fernandez *et al*., 1995; Jones *et al.*, 1997; Churchill, 2003). Besides, the level of protein, the amino acid composition in protein is also considered as an indicator to assess the quality of eggs and viable larvae, as major free amino acids decrease with egg embryogenesis (Veronica, 2004).

In these circumstances, domesticated broodstock development might be the best solution for smooth and uninterrupted management of hatchery protocol to support the extended demand of seeds. It has been strongly believed that a captive broodstock would augment sufficient precise information on biological perspectives, including growth, maturation, mating and spawning, as well as on nutritional requirement. Only captive broodstock could allow nutritional requirement study and enhance genetic selection for resistance to disease (Bachere *et al*., 1995) which might upgrade the egg and larvae quality and ensure quarantine indeed. Broodstock development of many commercial fish species (Nguyen *et al.*, 2010; Kabir, 2012), some of the marine fish such as grouper (Mathew, 2009; Ranjan *et al.*, 2014) and crustacean, such as giant freshwater prawn (Mohanta, 2000) has been established and has provided a significant contribution for the successful hatchery operation. However, broodstock management is still definitely one of the most poorly researched areas for those species newly introduced in commercial farming, such as mud crab. Studies on broodstock development of mud crab is still limited and scanty except for the partial observation made by Churchill (2003) and Quinitio *et al*. (2010), who collected gravid broods of *Scylla serrata* from wild or pond sources and managed their breeding after a short rearing.

However, majority of the research and development on the mud crab aquaculture has been done especially on *Scylla serrata*. The green mud crab *Scylla paramamosain* as a potential candidate providing lion share of production in the South-East Asian countries is poorly researched yet. Little is known about captive broodstock development, breeding and larvae rearing of *Scylla paramamosain* in Malaysia, even globally. By taking into consideration as a whole, this study aims to develop protocols for the development of broodstock of the green mud crab (*Scylla paramamosain*) in captivity and the management of breeding as a baseline guide with the emphasis on reproductive performance and larvae quality assessment through morphological, physical and bio-chemical component evaluation.

1.3 Broader research themes

The aim of this study is to domesticate the green mud crab (*Scylla paramamosain*) broodstock under exclusively captive/hatchery conditions, maturation and breeding and to observe the reproductive performance and larvae quality.

1.4 Specific objectives

The specific objectives of this study are:

i) To determine the effect of different natural and commercial diet on larvae rearing of the green mud crab *S. paramamosain*;

ii) To assess the impact of different grow out protocols on growth, survival and suitability as broodstock of the green mud crab *S. paramamosain* in captive condition;

iii) To determine the age and size at first maturity and gonad developmental stages of the green mud crab in captive condition;

iv) Assessment of mating behavior, male-female interactions and optimum male-female ratios in captive mating of the green mud crab (*Scylla paramamosain*); and

v) To evaluate the reproductive performance and larvae quality of green mud crab *S. paramamosain* successively spawned from the single mating event in captive condition.

CHAPTER- 2

LITERATURE REVIEW

2.1 General information

2.1.1 Taxonomic classification, morphology and identification

Mud crab, under the genus *Scylla*, is known as mangrove crab or swimming crab. It is also known as portunid crab, based on its family. The taxonomic classification of mud crab (Stephenson and Campbell, 1960) is as bellows:

- Phylum: Arthropoda
  - Class: Crustacea
    - Subclass: Decapoda
      - Infraorder: Brachyura
        - Family: Portunidae
          - Genus: *Scylla*
            - Species: *S. serrata*, *S. olivacea*, *S. tranquebarica*, ***S. paramamosain***

Broader carapace with smooth surface and large claws are the main identifying characteristics of mud crab that makes it different from other crab species. Mud crab contains six spines at the frontal margin in between the eyes and nine spines on each anterolateral margin (Stephenson and Campbell, 1960; Keenan *et al*., 1998). The carapace width can reach up to 24 cm and total body weight attains as much as 3.5 kg for *S. serrata*. The body color of the mud crab differs from species to species and generally ranges between dark brown to mottled green (Keenan *et al*, 1998; Shelley and Lovatelli, 2011). Male mud crabs are easily distinguishable from that of the female with their narrow abdominal flap and strong, larger claws (Phelan and Grubert, 2007).

Plate 2.1 Photos showing four distinct species of mud crab (*Scylla sp*)*;* A: *Scylla serrata*, B: *S. paramamosain*, C: *S. olivacea*, and D: *S. tranquebarica* (Keenan *et al.*, 1998)

Table 2.1 Distinguishing characteristics of four species of the genus *Scylla* (modified after Kennan *et al*., 1998)

Species (English name)	Frontal spine		Chelaepods		Color and polygonal marking
	Shape	Height	Carpus spines	Propodus spines	
Scylla serrata (Giant mud crab)	pointed	High	both present	both present	carapace color greenish to black, polygonal marking prominent on legs of both sexes and abdomen of adult female only
S. tranquebarica (Purple mud crab)	blunt	Moderate	both present	both present	carapace color greenish to black, polygonal marking prominent on last two pairs of leg, but weak on the other legs of both sexes
S. olivacea (Orange mud crab)	rounded	Low	inner absent, outer reduced	reduced	carapace color brownish to brownish green, polygonal marking absent
S. paramamosain (Green mud crab)	triangular	moderately high	inner absent, outer reduced	both present	Carapace color light green to green, polygonal marking weak on appendages of both sexes

2.1.2 Ecological and regional distribution

Mud crab is distributed from the Indo-West Pacific Ocean to the Red Sea along with in Australia, Taiwan, the Philippines, South Africa, Japan and the Pacific Islands (Keenan *et al.*, 1998) and other tropical and sub-tropical regions. Among four distinct species, *S. serrata* is largest in size and widely distributed in Japan, Western Indian Ocean and South Pacific Islands. *S. tranquebarica and S. olivacea* are mainly spread over the Indian Ocean, South China Sea and the Western Pacific region, whereas, *S. paramamosain* is restricted within the South China Sea and Java Sea (Le Vay *et al.*, 2001b). In Asian countries, mud crab is distributed in Bangladesh, Myanmar, Sri Lanka, India, Thailand, Vietnam, The Philippine, Malaysia and Cambodia (Kennan, *et al.*, 1998). In Malaysia, *Scylla olivacea* and *Scylla tranquebarica* are plentiful in Sarawak (Ikhwanuddin and Oakley, 1998), but *Scylla serrata* is mainly found in Perak (Takeharu, 2001). Whilst, Darlina *et al.* (2012) stated the absence of *S. serrata* in the Malaysian environment during their study of molecular analysis (DNA) of mud crab samples collected from different locations of Peninsular Malaysian coasts. In Bangladesh mud crabs are found in the southeast and south-west coastal districts, while abundant in Khulna, Bagerhat and Satkhira districts (Zafar and Siddique, 2000) of the south-west coastal region those are adjacent to the Sundarbans mangrove forest but the species composition not yet identified.

2.1.3 Local distribution, habitat and niche selection

Mud crabs are capable to withstand wider ecological variations, mainly salinity and temperature fluctuations (Hill, 1979) and are known to be euryhaline species (Hill, 1974; Robertson, 1989; Le Vay, 2001). Mud crabs are capable to tolerate salinity ranges from 5 to 40 ppt (Shelley and Lovatelli, 2011). In natural habitat, mud crabs ingest varieties of food; grow faster and moderately capable to resist disease (Williams and Primavera, 2001). Diversified habitat selection of mud crab is mostly associated with size and the ontogenetic developmental stages (Churchill, 2003). Mud crabs usually prefer to inhabit in the intertidal mud flats, shallow lagoons, rocky sheltered estuaries, and bushy mangrove forests (Hill, 1974; Robertson, 1989; Le Vay, 2001). Different size groups inhabit separate niches within the mangrove forests and the adjoining sub-tidal areas (Walton *et al.*, 2006). Smaller juveniles occupy within sea grasses, seaweeds and bushy mangrove roots of the shallow waters to further upstream (Chandrasekaran and Natarajan, 1994). Juveniles are plentiful in the estuarine intertidal mud flats, while, adult crabs with carapace width >8 cm are copious in the estuarine sub-tidal areas (Le Vay, 2001). In the case of *Scylla paramamosain*, small crablets of carapace width up to 1.5 cm settle on the outer mangrove edge and move to deep forest as size increased, while larger crabs with carapace width 4.5 cm prefer to live in burrows in the sub-tidal zone with feeding migration to the forest at high tide period, whereas main adult crabs permanently live in sub-tidal, offshore (Shelly and Lovatelli, 2011). Most of the crabs show little movement within the local niche of typical mangrove forest (Hill, 1975; Le Vay *et al.*, 2007). Thus, the boundary of the mangroves and mud flats is identified as an area that can support higher densities of crabs (Shelly and Lovatelli, 2011).

2.1.4 Life cycle and migration

Mature male and female moves to the estuary and inshore areas to find mates for copulation. Mating takes place in the estuarine environment as the female remains in soft shelled condition at pubertal moulting (Churchill, 2003). As the matting is completed the female migrate to the offshore for spawning and stay until hatching the eggs to first zoea (Hill, 1975; Heasman and Fielder, 1983; Hill, 1994). Spawning takes place in the offshore region with favorable salinity of 30 to 31 ppt. Mud crab is highly fecund and individual female can produce approximately 1 to 6 million of eggs per spawn (Shelley and Lovatelli, 2011) depending on size. Hatching and larval development occurs in the offshore region and the larvae spends about 30 days as a planktonic form and complete 6 larval stages (5 zoeal stages, Z1 to Z5; and a megalop stage) which leads to the crab instars (Phelan and Grubert, 2007; Shelley, 2008). The first crablet then enter into the estuarine environment and settle down on intertidal mudflats and mangrove shelters (Keenan, 1999) for grow out and attains maturity.

2.1.5 Natural food and feeding habit

Mud crab prefers to feed on a variety of foods and thus they are opportunistic omnivore (Warner, 1977). However, in some cases, they are passive carnivores and herbivores, scavengers and cannibals, prefer to ingest anything that encounter, including bivalves, worms, fish, as well as vegetative material and smaller crabs (Phelan and Grubert, 2007). Juvenile crabs have frequent movement and feed on small crabs, prawns, small fishes and other tiny invertebrates (Joel and Sanjeevaraj, 1986). Adult crabs have no specific feed items and feeds on carrion, molluscs, prawns, submerged vegetation and detritus (Hill, 1979; Joel and Sanjeevaraj 1986; Paterson and Whitfield, 1997). However, environmental factors such as temperature and physiological factors like moult condition, mostly regulate feeding activity of mud crab (Phelan and Grubert, 2007).

2.2 Mud crab aquaculture and production

Initially mud crab was harvested as a bycatch from the brackishwater shrimp ponds and from other brackishwater fin-fish or integrated aquaculture systems, without stocking of crablets. Such type of mud crab aquaculture was first initiated in China before 100 years back and gradually spread over to other Asian countries (Keenan, 1999; Shen and Lai, 1994).

From past to date, mud crab aquaculture evolved two major types of practice, grow out and fattening/hardening; while the recent addition in mud crab aquaculture is soft shell crab shedding. In grow out system, juvenile crabs of body weight 10 to 100 g each are stocked in earthen ponds or mangrove pens and cultured for 3 to 8 months (Duc, 1997) until reach to marketable size. The stocking density remains 500 to 1000 crabs/m^2 (Keenan, 1999) however, the survival rate is poor (<40%). In Vietnam, during the 1990s, from integrated aquaculture of shrimp, seaweed and mud crab practice, augmented production of 150 kg/ha for mud crab and 150-250 kg/ha for shrimp (Tuan and Hai, 1992). Survival in earthen pond culture is usually lower due to cannibalism and escaping (Liong, 1992). While, in monoculture with densities from 5,000 to 10,000 crabs/m^2 and provision of adequate shelter like seaweed following selective harvesting (Trino *et al.*, 1999) and supply of feeds with low-cost vegetable and animal source (Catacutan *et al*., 2003; Rodriguez *et al.*, 2003) can contribute better harvests of 340 kg crab/ha/year (Tuan and Hai, 1992). In Vietnam, mud crab farming with the stocking densities of 1-1.5/m^2, produce 1.5t/ha of crab per crop with an average body weight of 300 g to 450 g when fed with small fish and molluscs (Nguyen Co Thach, 2003).

Secondly, pen culture has been practiced in deforested and/or reforested mangrove flats with stocking densities of 2.5 to 5 crabs/m^2 which leads to better survival and production (Agbayani, 2001; Trino and Rodriguez, 2002). In Malaysia, mud crab culture is conducted in mangrove ecosystem, but farming is still limited due to the scarcity of seed supply (Chang, 1997). Survival in grow out seems lower (40 to 60%), sometimes exceed 75%, depending on grow out facilities (Cholik and Hanafi, 1992; Macintosh *et al.*, 1993) including regular feeding and provision of adequate shelters.

Amongst all types of mud crab aquaculture practices, fattening is regarded as a popular method, simplest procedure and higher turnover within short duration. In fattening, crabs with insufficient meat content or female crabs without sufficient gonad or with immature gonad (body weight >250 g) are reared for a short period, usually within 10 to 20 days (Csavas, 1995), feeding with trash fish or waste products to build as a value added product. Fattening is done in earthen ponds, fencing by nylon net or bamboo splits with a stocking density of 5 to 15 crabs/m^2, where the production and survival is low in pond culture due to cannibalism and escaping (Liong, 1992). To avoid escaping and cannibalism, fattening is done in sophisticated methods by stocking of individual crabs in separate bamboo made boxes/compartment, net cages or in galvanizing wire boxes (Cholik and Hanafi, 1992; Liong, 1992; Felix *et al.*, 1995; Shelley and Lovatelli, 2011) or in floating cages/boxes (Sivasubramain and Angel, 1992) and this increased the survival rate more than 90% (Begum, *et al.*, 2010) and cannibalism is low as no moulting takes place (Rattanachote and Dangwattanakul, 1992). The majority of cannibalism happened during grow out period due to frequent moulting (Shelley and Lovatelli, 2011).

In mud crab aquaculture, latest addition is the soft shell crab production with sub-adult crabs (around or <100 g) under indoor conditions in plastic or fibre glass tanks; in outdoor floating cages (Sivasubramain and Angel, 1992), plastic boxes or bamboo made compartments floating on the pond water surface to shed single crab in each box/compartment (Shelley and Lovatelli, 2011). Newly moulted crabs are picked out immediately (within 6 hours) before their shell hardened, processed and frozen for export.

Shelley (2008) reported that the production of mud crab in Malaysia has decreased from 623 tons in 1995 to 162 tons in 2005 and mud crab production is dominated by China with a production of 11423 tons in the year 2005. In Bangladesh, mud crab production is season based and a production of 6945.62 tons was recorded in 2010 from Khulna-Satkhira-Bagerhat district, which is the major crab producing area (Chandra, *et al.*, 2012).

The trend in global production of mud crab is shown in Fig. 2.1. Up to 2001 the aquaculture production was lower than the natural harvest. Though the production of mud crab has shown an increasing trend since 1980, but had fluctuations during the late 20th century (1990 to 2002). By that time, over-exploitation in coupled with natural disaster (Tsunami, cyclone, etc.) affected the mud crab production in some Asian countries like Sri Lanka, Japan, Malaysia and Philippine (Jayamanne, 1991; Fortes, 1999; Takeharu, 2001; Shelley, 2008). Since 2003, the natural harvest has increased gradually but the aquaculture production has increased dramatically. Diversification of farming system by means of culture pattern (like grow out, mangrove pen culture, fattening and soft-shell shedding) and cultural areas as well as the partial support of hatchery produced seeds are triggering factors for the drastic increase of aquaculture production in the last decade.

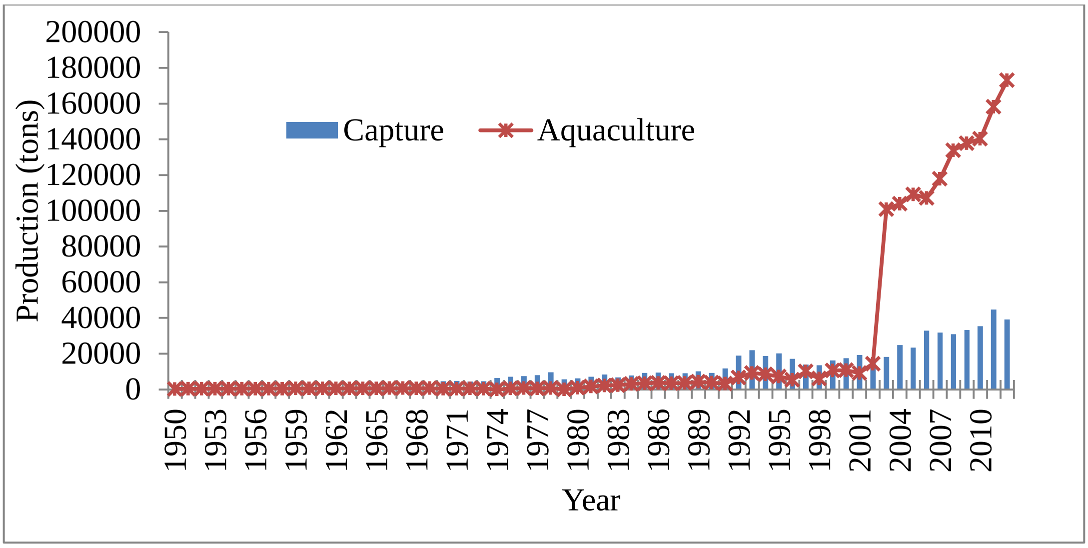

Fig. 2.1 Trend in global production of mud crab (Source: FAO, 2014; FishStat, FIGIS)

2.3 Demand, market price and export of mud crab

All forms of marketable sized mud crabs are sought as luxury seafood items. Mud crabs are exported in live forms and the main markets of the mud crab include China, USA, Japan, Korea and Thailand (Ferdoushi *et al.*, 2010). The market price of crabs varies from country to country, seasons, occasion and especially on size, sex and condition of the crabs; live gravid females demand premium prices (Keenan, 1999; Agbayani, 2001) than other forms in Asian countries. In Malaysia, intact female full with ovary priced from RM 28 to RM 60/kg (personal market survey). A growing market demand for frozen, soft-shelled mud crab have also been noticed in the USA, and demand of mud crab meat for value added in products is mounting internationally (Cholik, 1999; Keenan, 1999; Wickins and Lee, 2002). In Malaysia, about two tones of mud crabs are being imported daily from India, Indonesia, Sri Lanka and Bangladesh (Sivasubramain and Angel, 1992), which indicates a shortage of mud crab production and high requirement to mitigate local market demands and increasing over the years (Muchlisin and Azizah, 2009).

2.4 Broodstock

2.4.1 Grow out and maturation

Like other crustaceans, growth of mud crab occurs through regular moulting. Any interruption in moulting may cause a slowing down in growth, susceptive to disease or eventually may die. Growth of mud crab varies depending on sex, species, while males grow faster than female (Trino *et al.*, 1999; Christensen *et al*., 2004). Mud crabs usually undergo 16 to 17 moults in its total life span of 3 to 4 years, of which 6 times at the larvae stage (Z1 to C1), 2 times at the crablet stage (C1 to C3), 5 to 6 at grow out stage followed by 1 pubertal moulting to attain sexual maturity, whereas, the remaining two/three moults occurs during further reproductive re-maturation (Shelley and Lovatelli, 2011). The intermoult duration increase as the crab grew bigger, like that of other crustacean such as spiny lobster (Ehrhardt, 2008). It has been reported that, generally all species of mud crab attain maturity within one year from settling as crablet (C1), *S. serrata* shows maturity signs at 147 days (Field, 2006 by Shelley, 2008), whereas, *S. paramamosain* within 160 days with carapace width of approximately 10.2 cm (Le Vay *et al.*, 2007). Mean carapace width (CW) measures approximately 8.6 cm at median sexual maturity (CWm) for wild female crabs in Thailand (Tongdee, 2001), while, as estimated by applying a logistic model it was found that 50% of the wild female *S. olivacea* attained first maturity at a mean carapace width (CW) of 9.55 cm (Jirapunpipat, 2008**)** from the same agro-ecological environment. Hamasaki *et al.* (2011) studied the size at sexual maturity of natural *S. paramamosain* and stated that 50% of the females reached maturity (SM50) at 112.0 mm carapace width and for the male the maturity (SM50) happened at the 106.4 mm CW on the basis of allometric growth of chela height and carapace width ratio. Mud crab (*S. serrata*) in East Africa showed a sigmoid pattern in carapace width increment, reached sexual maturity around 9.9 months after settlement with 300 g of weight (Moksnes *et al.,* 2014) and growth in cage culture system was about 40% lower than the natural pond system culture, they added.

It is noteworthy to mention that mud crab attains the functional maturity through a series of step-wise gonad/physiological maturation process (Poovachiranon, 1992; Robertson and Kruger, 1994; Quinitio, *et al.,* 2007; Islam *et al*., 2010; Shelley and Lovatelli, 2011; Islam and Kurokura, 2012; Azmie *et al.*, 2012), which is expressed by several morphological features like abdominal shape and color for the female. Male mud crabs do not show any remarkable external characteristics except mating scars (Phelan and Grubert, 2007) and sudden increase in chelae height and propodus length (Islam and Kurokura, 2012).

Male mud crab of *Scylla olivacea* attains full sexual maturity with the completion of three gonad development stages (Islam and Kurokura, 2012). Five ovarian development stages are identified in natural female *S. paramamosain* (Islam *et al.,* 2010) and *S. serrata* (Quinitio *et al.,* 2007). On the other hand, ovarian development stages are classified into six stages in wild female *S. serrata (*Robertson and Kruger, 1994) and four stages in wild female *S. olivacea* (Azmie *et al.,* 2012). This contradictory observation might happen due to collection of wild animal and improper identification followed by laps of any stages. Thus, the hatchery produced uniform animal is therefore able to provide exact information including age at maturity.

2.4.2 Reproductive behavior

Mud crabs are dioecious in nature and fertilization occurs through direct copulation between sexually matured male and female (Churchill, 2003). The entire mating system is a complex procedure and takes about for a few days of time, whereas, the actual mating (copulation) occurs at night during the high tidal period. The actual mating takes place as much as within 48 hours of pubertal moulting of the female as the shell remains soft (FAO, 2011; Shelley and Lovatelli, 2011), while the male is therefore in the hardened condition. At mating, the male transfer the enviable sperms into the spermathecae of the female and it is stored up to 6 months (Nghia *et al*. 2001a) or as long as the female spawn (Phelan and Grubert, 2007). The female can fertilize two to three successive batches of eggs without further mating with the male (Shelley, 2008). Immediately after mating, the female takes a long migration for searching a suitable spawning environment; ingest diverse type and huge amount of food to nourish the development of the gonad and the ovulation duration can be from 30 to 60 days after mating (FAO, 2011) or more.

2.4.3 Broodstock husbandry, feeding and management

To achieve satisfactory reproductive performance and larvae quality, a good husbandry facility for brood stock is necessary, including maintenance of hygiene, well feeding and minimizing of stress. Gravid brood stock needs to rear for a few weeks to spawn and can be reared in communal basis with a density of $1.5/m^2$ in larger tanks, or separately in small tanks with shallow water depth (80 to 100 cm) for nutritional or genetic study (Shelley and Lovatelli, 2011). Unlike other commercial species, inert or commercial diet for mud crab grow out not developed yet. Most commercial diets developed for shrimp/prawn are being used for nutritional experimental purposes of mud crab (Nghia *et al*., 2001a). Thus, gravid broods are generally fed with fresh natural diet, like small trash fishes, squid, mussel meat, and sometimes with slaughterhouse wastes (Quinitio and Parado-Estepa, 2008; Nguyen Co Thach, 2009). Raw feeds are offered at the rate of 6 to 10% of body weight daily (Millamena and Qunitio, 2000), at 8% of body weight (Trino, *et al.*, 1999), at 5–15% of body weight per day with the frequencies of twice in a day (Shelley and Lovatelli, 2011). Thus produce large amount of detritus and deteriorates water quality. Regular cleaning of uneaten feeds and wastes and high exchange of water following manual or through a biofilter should ensure good water quality and hygiene condition for broodstock management (Quinitio and Parado-Estepa, 2008; Nguyen Co Thach, 2009; Shelley and Lovatelli, 2011).

2.4.4 Spawning behavior and management

It has been reported that, darkened condition that minimizes the stress and provision of sand bed or sand tray helps the proper arrangement of eggs into the pleopods, ultimately, enhance reproductive performance and hatching rates (Shelley and Lovatelli, 2011). Perhaps, a sand bed or tray may influence the moulting and act as catalyst for spawning. Immediately after spawning is completed, the spawner accumulates and aggregate the extruded eggs properly with the walking legs and attach them onto the filamentous setae of pleopods under the abdominal flap (Shelley and Lovatelli, 2011; Phelan and Grubert, 2007; Shelley, 2008). Spawning seasons of the mud crab are mostly temperature dependent. Spawning of the mud crab occurs throughout the year in the tropical region with a peak during the rainy season, whereas, in the sub-tropical areas of Southern China, the peak spawning season has been detected in summer season (Le Vay *et al*., 2001b). The fecundity of mud crab