DEVELOPMENT OF BROODSTOCK MATURATION FEED WITH SUPPLEMENTATION OF ARACHIDONIC ACID (ARA, 20:4n-6) AND DOCOSAHEXAENOIC ACID (DHA, 22:6n-3) FOR FEMALE GIANT FRESHWATER PRAWN, *Macrobrachium rosenbergii*

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2023

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by

SAADIAH BINTI IBRAHIM

Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

August 2023

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious, the Most Merciful.

I would like to begin by expressing my humble gratitude to Almighty Allah SWT for guiding me, giving me the strength, and blessing me with the ability to complete this thesis. Peace and blessings be upon Muhammad ﷺ, the last messenger.

I am deeply thankful to my esteemed supervisors, Prof Ng Wing Keong, Prof Chong Shu Chien @ Alexander, and Dr. Teoh Chaiw Yee, for their unwavering guidance and valuable comments throughout my research. Their support has been instrumental in shaping this study.

I extend my heartfelt appreciation to Prof. Dr. Mhd Ikhwanuddin Abdullah for his insightful comments and suggestions, which significantly improved the methodology for data collection.

I am grateful to the Public Service Department of Malaysia (JPA) for sponsoring my tuition fees, enabling me to pursue my academic aspirations.

Special thanks go to the Fisheries Research Division, Fisheries Department of Malaysia, for their generous support and assistance, which proved essential in completing this research.

I would like to express my gratitude to all the industrious training students who assisted me during my PhD research.

I am particularly thankful to Mr. Muhd Zulfadhli Mohd Jaafar (FRI Glami Lemi) for his valuable assistance in sample preparation for fatty acid analysis, Dr. Kua Beng Chu from NaFish for her expert guidance in histology analysis, and Makmal Kesihatan Awam Veterina, Salak Tinggi (Pn Faridah and her team in GC laboratory) for their support and guidance in using GC-FID in their laboratory.

A special acknowledgment goes to all the officers and staff at FRI Glami Lemi and FRI Pulau Sayak, whose direct and indirect contributions were essential in completing this research.

I dedicate this thesis to my beloved parents, Ibrahim Saad (in memory) and Hamisah Hussain, and my loving sister, Sarina Ibrahim, whose unwavering support has been a source of strength throughout my PhD journey.

Lastly, I want to express my deepest appreciation to my loving sons, Muhammad Haikal, Muhammad Hafiy, and Muhammad Hadif, whose patience, sacrifices, and understanding of my PhD research commitment have been my greatest motivation.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF PLATES

Page

LIST OF SYMBOLS

π	Pai
l	major axis (long) of ellipsoidal eggs
h	minor (short) axis of ellipsoidal eggs

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists methods
ARA	Arachidonic acid
CP	Crude protein
DHA	Docosahexaenoic acid
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl esters
GC	Gas chromatography
GFP	Giant freshwater prawn
GSI	Gonadosomatic index
HSI	Hepatosomatic index
LC-PUFA	Long chain polyunsaturated fatty acids
LOA	Linoleic
LNA	Linolenic
MUFA	Monounsaturated fatty acids
OS	Ovarian stage
PUFA	Polyunsaturated fatty acids
PL	post larvae
SFA	Saturated fatty acids

LIST OF APPENDICES

PEMBANGUNAN MAKANAN KEMATANGAN INDUK DENGAN PENAMBAHAN ASID ARAKIDONIK (ARA, 20:4n-6) DAN ASID DOKOSAHEKSANOIK (DHA, 22:6n-3) BAGI UDANG GALAH AIR TAWAR BETINA, *Macrobrachium rosenbergii*

ABSTRAK

Udang galah atau giant freshwater prawn (GFP), *Macrobrachium rosenbergii* (de Man, 1879) merupakan salah satu komoditi penting dalam industri akuakultur di Malaysia. Cabaran utama dalam industri ternakan udang galah adalah bekalan benih berkualiti dan menjadi isu yang semakin kritikal. Untuk mengatasi masalah ini, kajian dijalankan untuk mengenalpasti kesan pemberian makanan pematangan induk yang mengandungi asid arakidonik (ARA, 20:4n-6) dan asid dokosahexaenoik (DHA, 22:6n-3), yang optima terhadap proses pematangan dan prestasi pembiakan *M. rosenbergii* betina. Objektif kajian adalah untuk menilai kesan makanan pematangan dengan nutrisi lengkap yang diperkaya dengan ARA dan DHA terhadap pematangan induk udang galah betina, iaitu penilaian disegi perkembangan gonad, kualiti telur, kuantiti larva, dan perubahan saiz oosit. Kandungan asid lemak dalam badan udang juga dianalisis. Penyelidikan ini menggunakan makanan rumusan pematangan kajian dengan kandungan ARA dan DHA yang berbeza, seterusnya membandingkannya dengan makanan kawalan. Parameter yang dikaji terhadap induk udang galah betina yang diberi makanan kajian termasuk usaha reproduksi, kualiti telur, jumlah larva yang dihasilkan, histologi gonad dan hepatopancreas serta kandungan asid lemak dalam sampel induk kajian. Tiga eksperimen pemberian makanan (*feeding trial*) berkaitan telah dijalankan dalam membangunkan makanan pematangan induk M. rosenbergii betina ini. Eksperimen pertama dijalankan untuk mengetahui kesan diet dengan bentuk

dan kandungan kelembapan yang berbeza terhadap prestasi reproduksi *M. rosenbergii* betina. Eksperimen kedua adalah untuk menentukan aras ARA yang optimum dalam diet yang dapat meningkatkan prestasi pembiakan udang galah betina. Eksperimen ketiga dijalankan untuk menentukan tahap penggunaan DHA yang optimum dalam diet untuk induk betina udang galah. Hasil utama kajian ini menunjukkan bahawa makanan pematangan yang diperkaya dengan ARA dan DHA pada aras optimum secara positif mempengaruhi prestasi pembiakan *M. rosenbergii*. Induk udang galah betina yang diberi makanan pematangan yang dibangunkan menunjukkan pematangan yang lebih baik, peningkatan kualiti telur, dan peningkatan jumlah penghasilan larva. Kesimpulan dari kajian ini menunjukkan potensi penggunaan ARA dan DHA dalam makanan pematangan sebagai strategi yang berkesan untuk meningkatkan kecekapan pembiakan udang galah betina. Kesimpulannya, kajian ini menunjukan bahawa penggunaan 7 mg/g ARA dan 9.22 mg/g DHA dalam diet makanan pematangan boleh meningkatkan prestasi pembiakan *M. rosenbergii* betina dan merupakan strategi yang berkesan untuk meningkatkan prestasi pembiakan udang galah betina. Seterusnya, diet pematangan induk ini dapat digunakan untuk meningkatkan kualiti induk domestikasi dan juga induk liar. Impak yang paling ketara hasil penyelidikan ini adalah dapat meghasilkan benih berkualiti dari stok induk udang galah yang dipertingkatkan kualitinya melalui penggunaan makanan pematangan yang diperkaya dengan ARA dan DHA dan seterusnya menjamin kelestarian industri akuakultur udang galah di Malaysia.

DEVELOPMENT OF BROODSTOCK MATURATION FEED WITH SUPPLEMENTATION OF ARACHIDONIC ACID (ARA, 20:4n-6) AND DOCOSAHEXAENOIC ACID (DHA, 22:6n-3) FOR FEMALE GIANT FRESHWATER PRAWN, *Macrobrachium rosenbergii*

ABSTRACT

The giant freshwater prawn (GFP), *Macrobrachium rosenbergii* (de Man, 1879), is one of the important commodities in Malaysia's aquaculture industry. The main challenge in *Macrobrachium* farming is the supply of high-quality seeds, which has become an increasingly critical issue. To address this problem, a study was conducted to investigate the effects of feeding maturation diets containing arachidonic acid (ARA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3) at optimal levels on the maturation and breeding performance of female *M. rosenbergii*. The research aimed to evaluate the impact of maturation diets enriched with ARA and DHA on the maturation of female GFP, including assessments of gonad development, egg quality, larval quantity, and oocyte size. The analysis also included the examination of fatty acid content in the shrimp's body. The study employed different formulations of maturation diets with varying ARA and DHA levels, comparing them with a control diet. Parameters studied in the female GFP given the experimental diets included reproductive effort, egg quality, larval yield, gonad and hepatopancreas histology, and fatty acid content in the study samples. Three feeding trial experiments were conducted to develop the maturation diets for female *M. rosenbergii*. The first experiment examined the effects of different diet forms and moisture content on the reproductive performance of female *M. rosenbergii*. The second experiment aimed to determine the optimal dietary ARA level for enhanced reproductive performance of female GFP. The

third experiment focused on determining the optimum level of DHA in the diet for female GFP. Females provided with the developed maturation diets showed better maturation, improved egg quality, and increased larval production. In conclusion, the study demonstrated the potential of using a diet containing 7 mg/g ARA and 9.22 mg/g DHA to improve the breeding performance of female *M. rosenbergii*, making it an effective strategy to enhance the breeding of GFP. Furthermore, this maturation diet can be used to improve the quality of both domesticated and wild GFP broodstock. The most significant impact of this research is its ability to produce good quality seeds from improved GFP broodstock using the maturation diet enrich with ARA and DHA, thereby ensuring the sustainability of Malaysia's giant freshwater prawn aquaculture industry.

CHAPTER 1

INTRODUCTION

1.1 General introduction

World fish consumption per capita value has been increased since a decade ago, rising from 19.5kg in 2013 to 20.3 kg in 2016 (FAO, 2018). In 2017, world fish consumption per capita had increased further, which around 20.5 kg (FAO, 2020). The preference for fish as a protein source has grown among consumers due to various reasons such as health consciousness and concerns of other meat types such as beef, pork, and poultry. This trend is reflected in the rise of fish consumption compared to other animal proteins.

The production of captured fisheries from inland and marine sectors remained relatively stable, with figures around 90 to 92 million tonnes from 2014 to 2016 (FAO, 2018). However, there was an increase in both world capture fisheries and aquaculture production in 2018, reaching 96.4 million tonnes and 82.1 million tonnes, respectively (FAO, 2020). Aquaculture, with significantly growth seen in both marine and freshwater sectors. Freshwater or inland aquaculture production, for example, rose from 38.6 million tonnes in 2011 to 51.3 million tonnes in 2018 (FAO, 2018, 2020).

Among the various freshwater crustaceans, the giant freshwater prawn (GFP) or *Macrobrachium rosenbergii* stands out as one of the most widely cultured species in Asia and other parts of the world. GFP, *M. rosenbergii* is a notable species being cultured under the freshwater crustacean groups. The GFP production ranked in the fourth among freshwater crustaceans in 2016, it still contributed significantly, accounting for 10.3% of the total freshwater crustacean's production (FAO, 2018).

Numerous studies have been focused on factors contributing to the successful cultivation of GFP. One crucial aspect is the availability of high-quality seeds. Researchers have explored various approaches to improve seeds quality, including enhancing broodstock quality, maturation diet, larval feed, stocking density, feeding regime, and overall larval management (Cavalli et al., 2003; Kitcharoen et al., 2010; Nhan et al., 2010; Habashy, 2013; Chand et al., 2015). The maturation diet is crucial in producing high-quality GFP seeds. Providing broodstock with a well-balanced and complete diet is essential for their development and successful seeds production.

Regarding crustacean maturation diets, researchers have investigated various nutrition requirements, including protein, lipids, and fatty acids, with a particular focus on long-chain polyunsaturated fatty acids (LC-PUFA) (D'Abramo & Sheen, 1994; Zacharia, 2001; Smith et al., 2008, Coman et al., 2007; Glencross & Smith, 2001; Hoa et al., 2009; Huang et al., 2008; Meunpol et al., 2005). Notably, the importance of arachidonic acid (ARA), a type of LC-PUFA, has been highlighted in promoting gonad development and reproductive performance (Coman et al., 2011; Glencross, 2009; Wouters et al., 2001). Despite numerous research efforts focused on developing GFP maturation diets, there is still no specific maturation feed being established and utilised widely in the hatchery.

Traditionally, GFP hatcheries have used fresh feed, such as squid, bivalves, fish fillet, and marine worms, due to their high polyunsaturated fatty acids (PUFA) content. Fresh feed has been reported to positively impact growth, reproductive performance, and larval development (Hoa et al., 2009; Coman et al., 2011, Shanju & Geraldine, 2011). However, the quality of fresh feed can vary between batches and pose the risk of disease transmission. To address these challenges, there is a need to develop and adopt formulated feeds that offer benefits like consistent supply, quick

preparation, known nutrient content, and the ability to include specific additives to meet broodstock nutritional requirements.

In GFP, *M. rosenbergii*, hatcheries, the typical maturation feeds used are fresh food, like fresh squid, bivalves, fish fillet and marine worm, polychaete, which have a high content in polyunsaturated fatty acid (PUFA). Previous studies consistently demonstrated that fresh food positively affected growth, reproductive performance, and larval development (Hoa et al., 2009; Coman et al., 2011; Shanju & Geraldine, 2011). Consequently, most hatchery operators firmly believe that fresh food delivers superior performance compared to formulated diets. However, some hatchery operators choose commercial formulated pellets for their broodstock. These pellets commonly designed for the "grower stage" type, which is not explicitly developed for broodstock. Therefore, it cannot fully meet the nutritional requirement of broodstock for optimal development.

Through the present study, a maturation diet enriched with adequate ARA and docosahexaenoic acid (DHA) can be developed. Implementing this maturation feed can enhance the reproductive performance of *M. rosenbergii,* leading to improved quality of domesticated broodstock. Introducing this developed maturation diet to local hatcheries has the potential to increase the production of good quality fry. Additionally, domesticated broodstock fed with maturation pellets can help reduce dependence on wild broodstock collected from rivers in Malaysia.

1.2 Problem of statement

The aquaculture industry has seen a notable increase in global fish consumption, particularly focusing on the giant freshwater prawn (*Macrobrachium rosenbergii*) due to its significant contribution to freshwater crustacean production. The success of cultivating giant freshwater prawns relies on high-quality seeds, where the maturation diet for broodstock is crucial. Although traditional freshwater crustacean hatcheries use fresh food sources rich in polyunsaturated fatty acids (PUFA) for broodstock maturation, the variable quality of these feeds and the risk of disease transmission necessitate formulated feeds with consistent nutrient content and specific additives to meet broodstock nutritional needs.

This study aims to develop a specialised maturation feed enriched with optimal levels of arachidonic acid (ARA) and docosahexaenoic acid (DHA), essential long-chain polyunsaturated fatty acids (LC-PUFA). The goal is to improve the reproductive performance and overall quality of domesticated *M. rosenbergii* broodstock, leading to increased production of high-quality fry. By reducing reliance on wild broodstock from rivers in Malaysia, this development addresses broodstock scarcity and enables sustainable utilization for multiple larval production cycles. The adoption of this improved broodstock management approach has the potential to positively impact Malaysia's aquaculture sector and meet the growing demand for giant freshwater prawns.

1.3 Objectives

The main aim of this study was to investigate the specific long-chain polyunsaturated fatty acid (LC-PUFA) requirements in the maturation diet for GFP, *Macrobrachium rosenbergii.* To achieve this goal, the following objectives were pursued:

- To determine the sources of protein and moisture content of the maturation pellet for better acceptance and performance of female GFP broodstock.
- To determine the optimum level of ARA (C20:4n-6) that results in better development of the reproductive performance of female GFP broodstock using pure ARA oil extracted from fungi *Mortierella* sp.
- To determine the optimum level of DHA (C22:6n-3) that results in better reproductive performance of female GFP broodstock using DHA from algae and pure ARA oil supplementation.
- To produce maturation feed enriched with ARA and DHA using available sources of additive for better reproductive performance of female GFP, *M. rosenbergii* broodstock.
- To produce premix of essential nutrients and additives of ARA and DHA at optimal level that ready to use in the maturation pellet for female GFP broodstock.

The whole experimental approaches addressed in this thesis and their interconnectivity are summarised in the schematic conceptual in Figure 1.1.

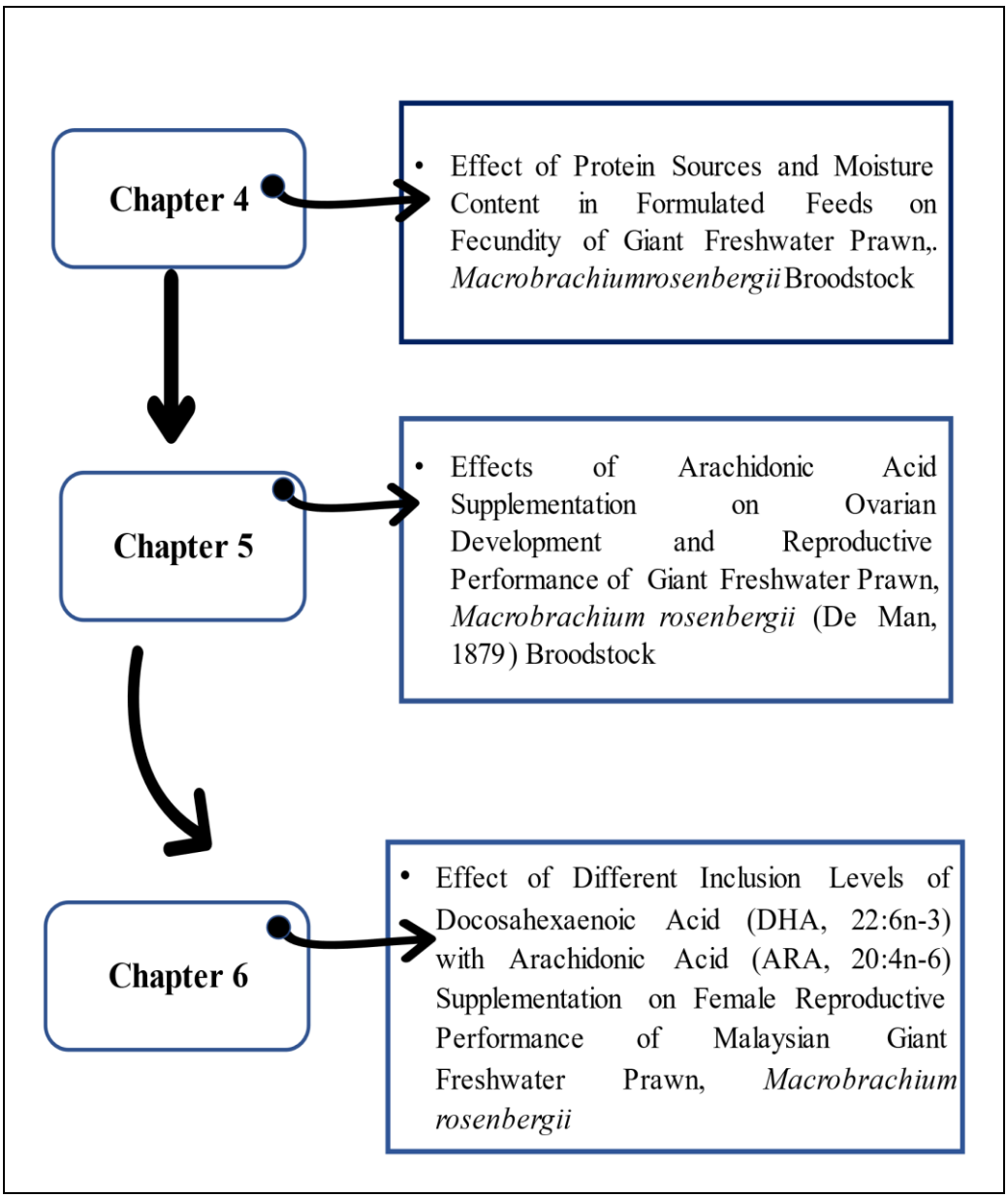

Figure 1.1: Schematic representation of the research frame

CHAPTER 2

LITERATURE REVIEW

2.1 Biology of the giant freshwater prawn, *Macrobrachium rosenbergii*

The giant freshwater prawn (GFP), scientifically known as *Macrobrachium rosenbergii* (de Man, 1879), holds significant cultural and commercial importance. In the Malay language, it is referred to as "udang galah", while it is also known by various names such as giant river prawn, Malaysian prawn, and freshwater scampi in India. In West Bengal, it is called "Golda Chingdi" or "cherabin" (Plate 2.1). Over the past four decades, the GFP farming has experienced remarkable growth since its inception in the 1970s, becoming one of the most promising and sought-after freshwater prawn species in the aquaculture industry.

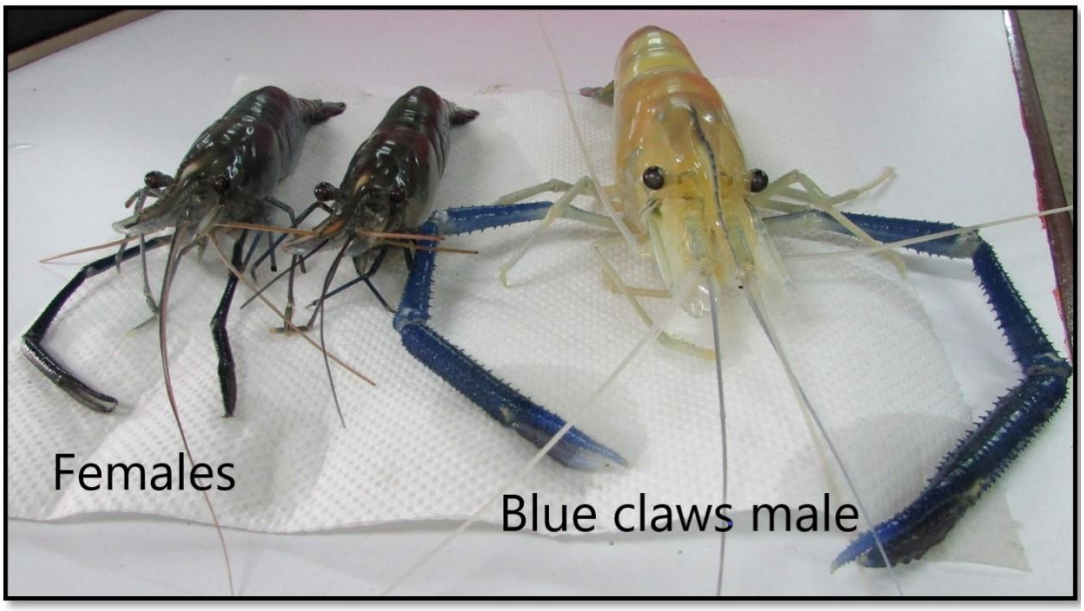

Plate 2.1: Adult blue claw male and female the GFP, *Macrobrachium rosenbergii*

The GFP cultivation is not limited to one region, it is now a global practise, particularly in Southeast Asian countries like Thailand, Vietnam, the Philippines, and Malaysia. Additionally, GFP is being cultured in South Pacific nations, Northern Oceania and Western Pacific islands. The demand for this species in Malaysia remains consistently high throughout the year. Presently, the price of the GFP from pond culture ranges from RM65 to RM100 per kilogram, reflecting a significant increase of over 100% compared to the 1990s, when it was approximately RM30 per kilogram. This remarkable surge in price reflects the growing demand from local consumers, particularly those involved in recreation fishing ponds, and a smaller proportion allocated for exports (DoF record, 2020).

2.1.1 Nomenclature

About 200 freshwater prawn species have been described, and GFP, *Macrobrachium rosenbergii* is one of the earliest species to be discovered, recorded and illustrated as reported by New (2002). The nomenclature of GFP, *M. rosenbergii* is listed below.

Kingdom	: Animalia
Phylum	: Arthropoda
Subphylum	: Crustacea
Class	: Malacostraca
Order	: Decapoda
Sub-order:	: Pleocyemata
Infraorder	: Caridea
Family	: Palaemonidae
Subfamily	: Palaemoninae
Genus	: *Macrobrachium*
Species	: *rosenbergii* (de Man,1879)

Sources: (Singh, 2011)

2.1.2 Distribution

The GFP, *M. rosenbergii,* boasts a widespread global distribution. Originating from Asia, it has been successfully introduced to various countries, including Hawaii, Argentina, and Australia, establishing thriving populations beyond its native range. This species is prevalent across the tropical and subtropical waters of the Indo-Pacific region, where it inhabits a diverse array of environments. The GFP can be found in countries such as Malaysia, Thailand, the Philippines, India, Sri Lanka, Bangladesh, Myanmar, Indonesia, and Vietnam, where it contributes to both natural ecosystems and aquaculture industries.

The distribution of the GFP worldwide is graphically depicted in Figure 2.1, illustrating the extensive range of this remarkable species and its significant presence in different regions. As the GFP continues to be an economically and ecologically important aquatic species, understanding its global distribution remains crucial for effective management and conservation efforts.

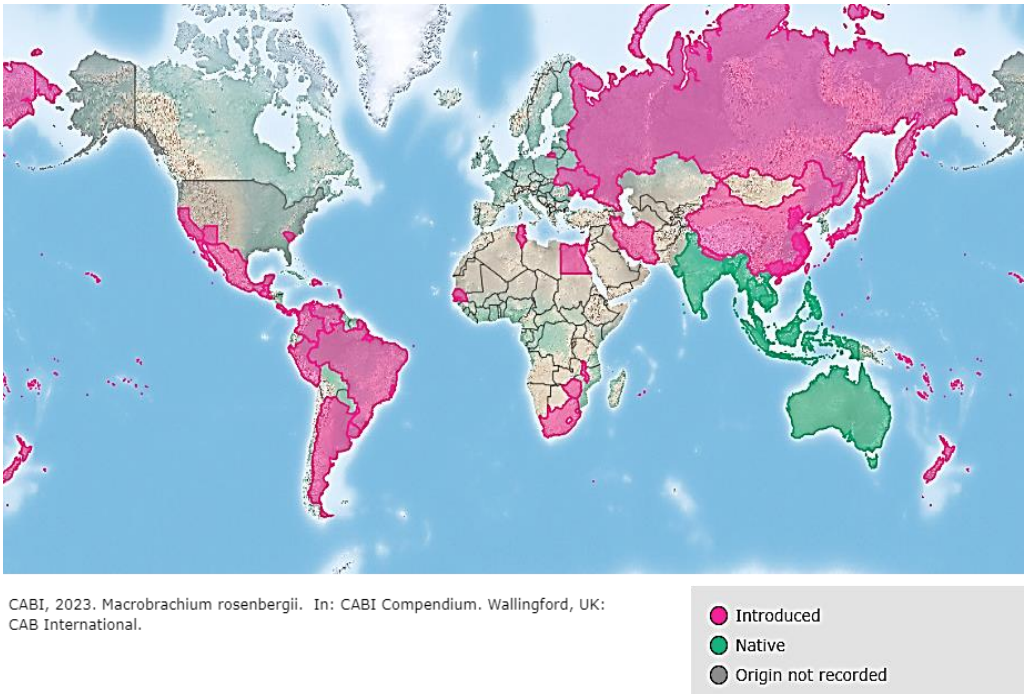

Figure 2.1: The distribution of *Macrobrachium rosenbergii* in the world including as native and introduced species as coloured in green and red in the above map. Retrieved May 24, 2023, from https://www.cabi.org/isc/datasheet/96269.

2.1.3 Morphology

The GFP, *M. rosenbergii* exhibits distinct morphological features that set it apart from other species within the *Macrobrachium* genus. Its unique characteristics, particularly in its outer shell and body segments, distinguish it as a remarkable freshwater crustacean.

External Morphology: The basic morphology of a GFP, consists of three main parts: the head, abdomen, and tail. The abdomen comprises five pairs of swimming legs, with one pair attached to each abdominal somite (Figure 2.2). The first pair of swimming legs are responsible for feeding, efficiently directing food into the mouth. The second pair, known as claws, which are considerably more significant than the rest. These formidable claws serve both as a means of self-defence and for catching food. Another prominent feature is the rostrum, located at the tip of the head. The tail

section consists of two uropods and one telson. For illustration purposes, the morphology of GFP male is presented in Figure 2.3.

Differences Between Male and Female GFP: Distinguishing between male and female GFP can be achieved through several observable morphological differences. Generally, males tend to be more extensive compared to females, with adult males reaching to 33 cm in total length, while adult females typically measure up to 29 cm (Cowles, 1914; Holthuis, 1950, 2000; New, 2002; Wowor & Ng, 2007). Adult males also possess a robust second leg (claw), often blue and orange. Furthermore, adult male can be classified into three types which are blue claw males (BC), orange claw (OC), and small claw (SC), as depicted in Plat 2.2. Blue claws are the dominant and the most prominent type (Plat 2.2 and Plat 2.3). Conversely, adult female prawns generally exhibit smaller bodies size with smaller claws. According to New (2002), adult females GFP can be further categorized as virgins (V or VF), berried females or those carrying eggs (BF or BE), and open brood chamber females or spent females.

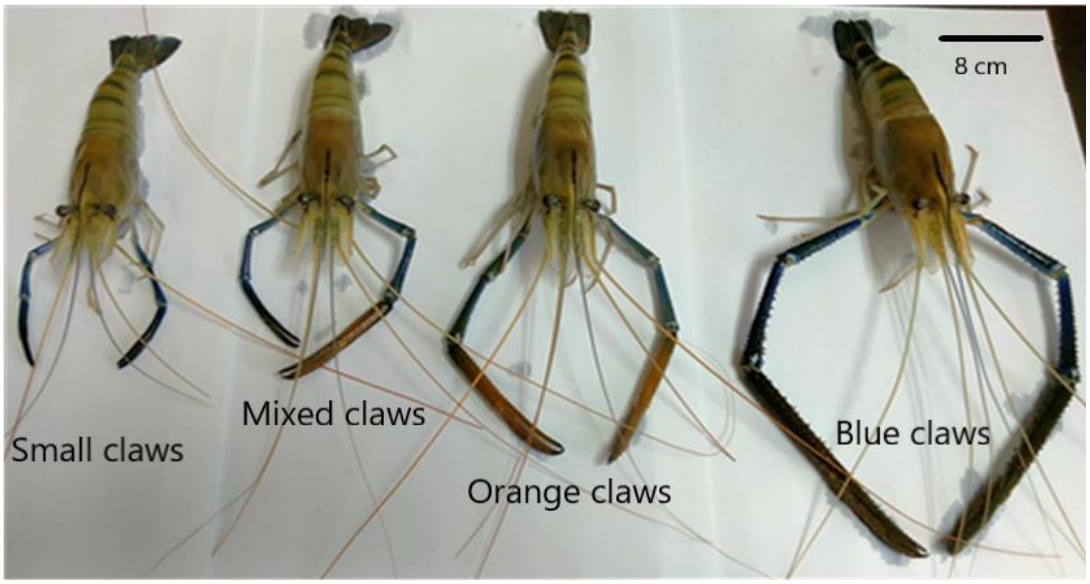

Plate 2.2: External morphology of the different types of claws for *Macrobrachium rosenbergii.* (Mixed claws is not common)

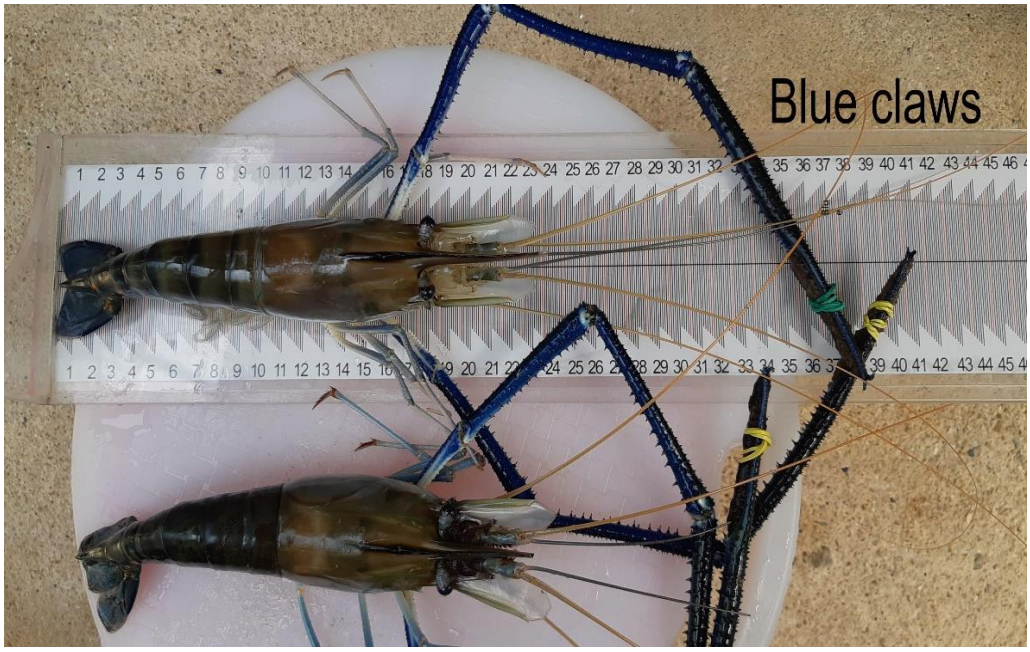

Plate 2.3: Blue claws male is dominant among other claws.

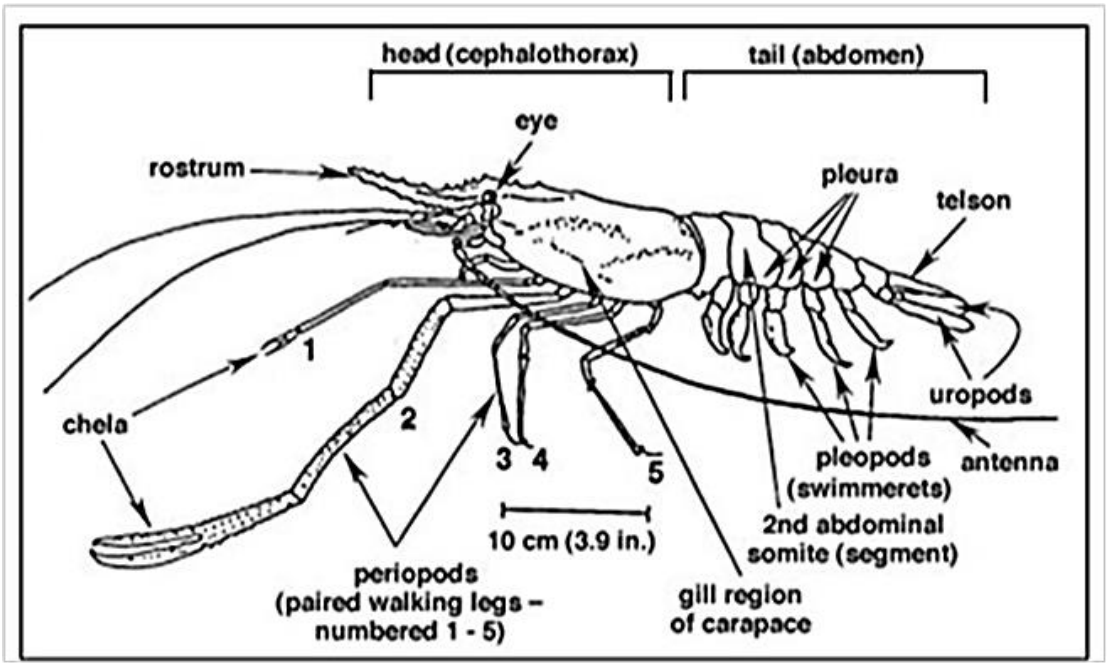

Figure 2.2: The morphology features of the freshwater prawn, *Macrobrachium rosenbergii*. Drawing based on Forster and Wickins (1972) Source: New and Singholka (1985) and New (2002). Retrieved June 23, 2015, from http://www.fao.org/docrep/field/003/AC277E/AC277E02.htm.

Sexual Characteristics: The GFP males and females possess distinct sexual characteristics, making it easy to differentiate between the sexes based on their appearance and internal reproductive organs. For males, the sexual organ is below the abdomen near the last walking leg, as shown in Figure 2.3.

Internal Reproductive Organs: Internal organs and reproductive structures are located in the cephalothorax section (Figure 2.3 and Figure 2.4). In females, the paired ovaries are found dorsally on either side of the proventriculus and dorsal to the digestive gland (Figure 2.3). From the ovaries, a pair of oviducts extend towards and enter the gonopores on the third periopods' (walking legs') (Figure 2.4), as documented by Sandifer & Smith (1985). In males, the internal reproductive structures consist mainly of a pair of fused testes located mid-dorsally in the cephalothorax. Each testis produces a vas deferens (Figure 2.4). The vasa deferentia are simple tubes that end in terminal ampullae containing spermatophores. and release at the gonopores on the coxae of the fifth pereopod. During mating, the ampullae releases the spermatophores, containing the sperm mass (Sandifer & Smith, 1985). Understanding the morphological and sexual characteristics of GFP is essential for scientific research, aquaculture management, and conservation efforts to preserve this remarkable species in various habitats worldwide.

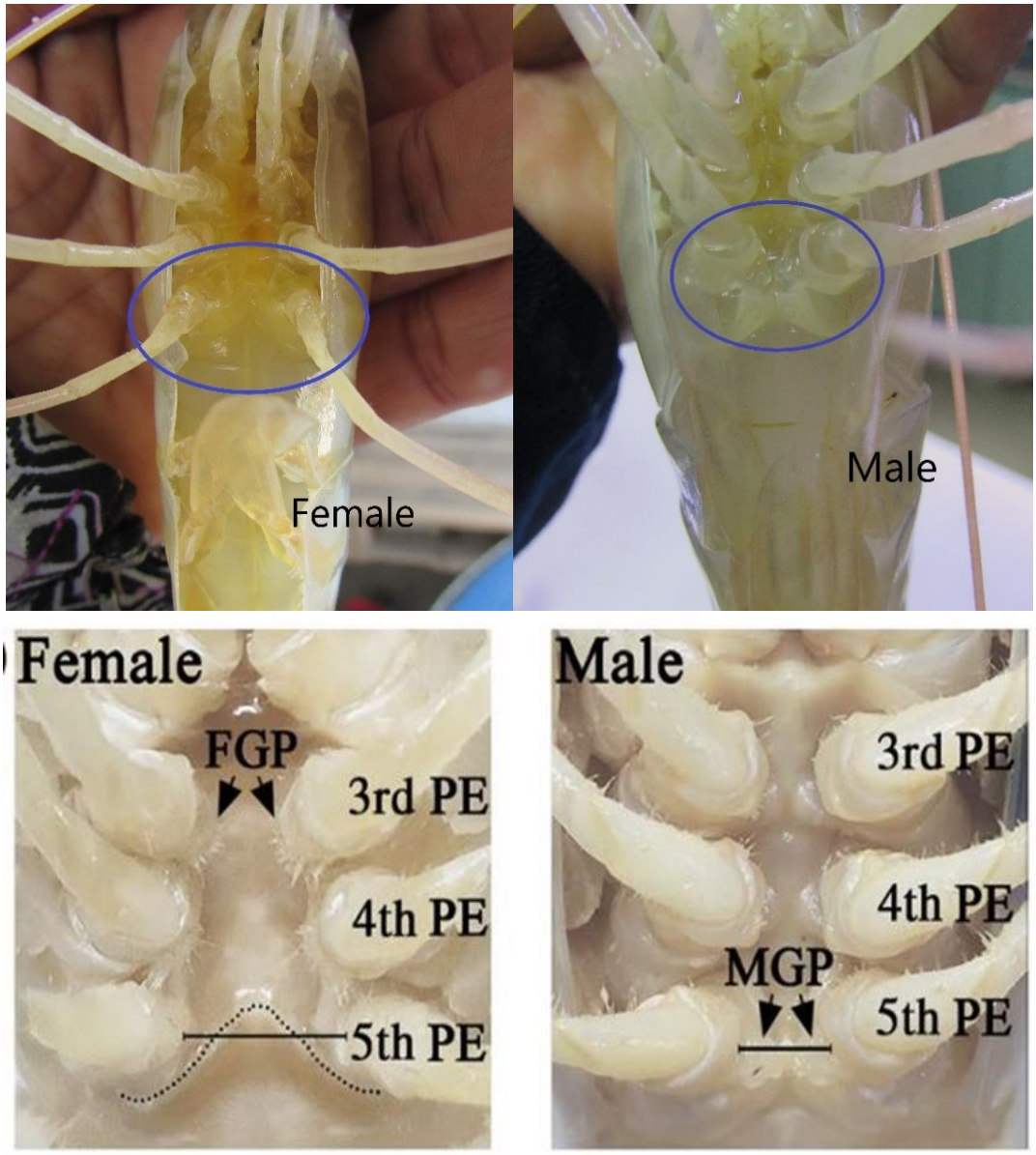

Figure 2.3: Blue circle shows different sexual morphology in adult giant freshwater prawn, *Macrobrachium rosenbergii* between male and female based on the gap between the last pereiopods and MGP existence. FGP = female genital pores; MGP = male genital pores; PE = pereiopods (Shen et al., 2020)

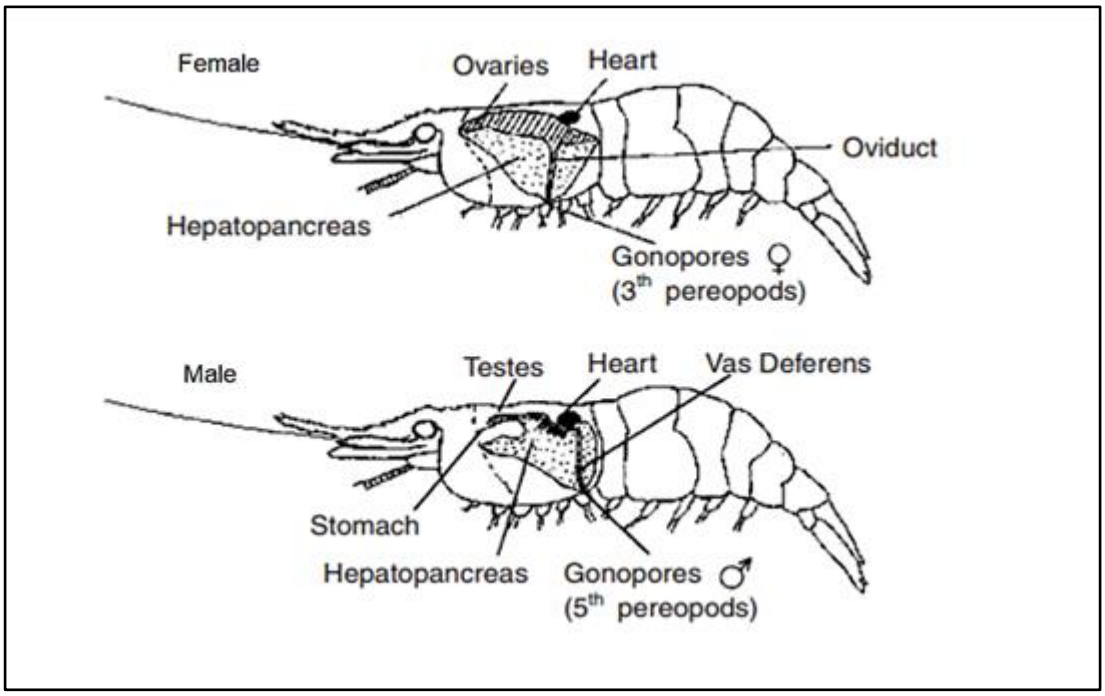

Figure 2.4: The internal reproductive organs of female and male *Macrobrachium rosenbergii* (Sandifer & Smith, 1985).

2.2 Life cycle of the GFP, *Macrobrachium rosenbergii*

The life cycle of the GFP is summarised in Figure 2.5, based on research by Nhan (2009) and New (2002). The complex cycle involves stages in both freshwater and brackish water environments. The life cycle begins with newly hatched larvae, which are found in brackish water environments with salinity levels of approximately 10 – 13 ppt (Wei et al., 2021). Over the course of eleven stages, the larvae undergo metamorphosis until they reach the post-larval stage (PL). Once they reach the PL stage, the prawns migrate upstream, typically taking one to two weeks. They continue to grow and develop in freshwater conditions, progressing through the juvenile and adult stages. As adults, some prawns become broodstock, and engage in copulation, usually in fresh water or very low salinity conditions. After copulation, the female prawns become "berried," carrying eggs (larvae) under their abdomen. These berried females migrate to brackish water areas with salinity levels around

5 – 7 ppt to hatch the larvae. Once the larvae are hatched, the spent females return upstream to freshwater environments, leaving the newly hatched larvae to continue developing in brackish water (New, 2002). This life cycle's intricate process ensures the survival and propagation of the GFP across different environments, involving both freshwater and brackish water stages. Understanding this life cycle is vital for successfully managing and conserving this species, supporting its presence in diverse ecosystems worldwide (New, 2002).

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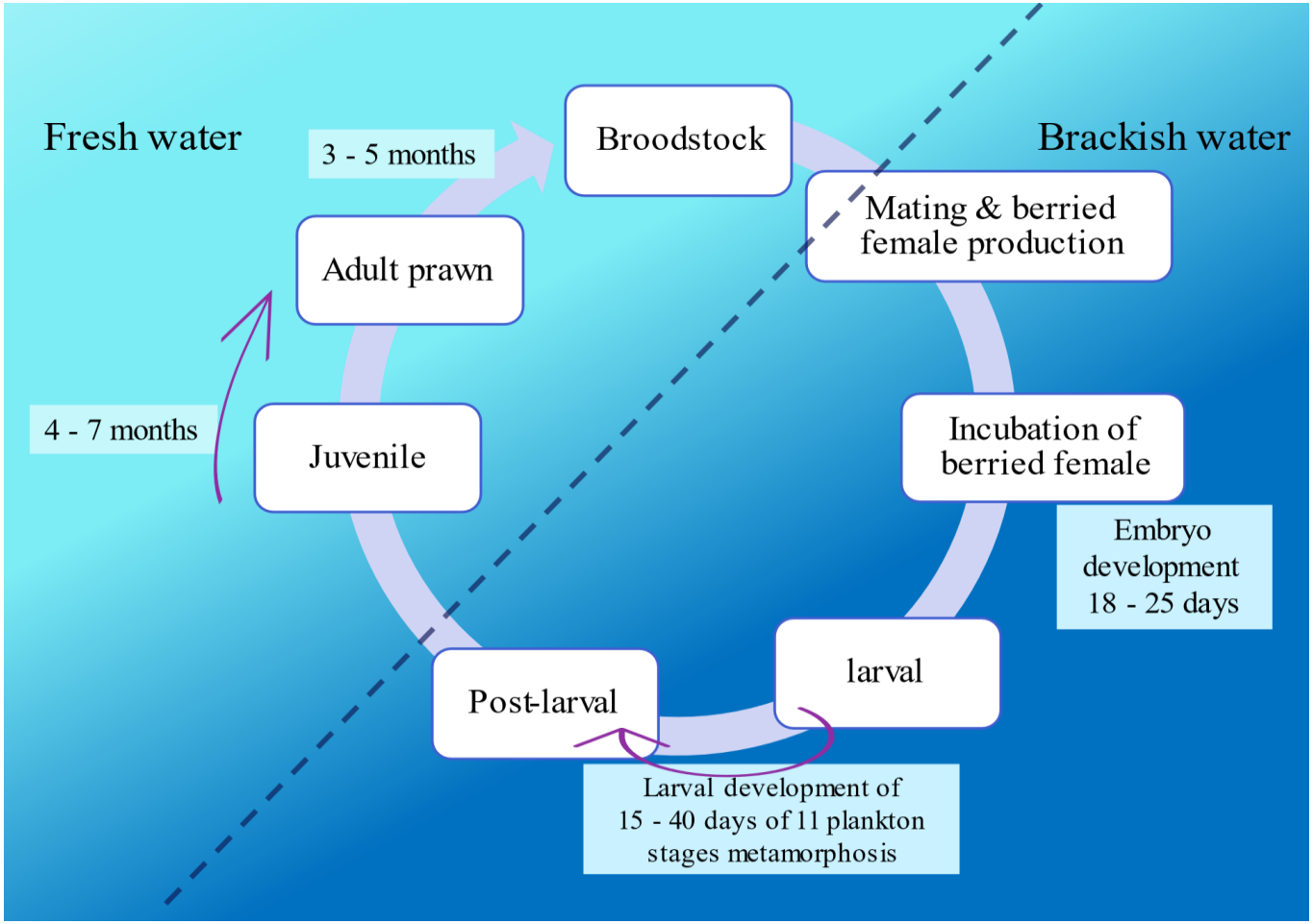


Figure 2.5: The life cycle of *Macrobrachium rosenbergii* (Nhan, 2009)

2.2.1 Broodstock

In freshwater prawn hatcheries, broodstock is sourced from the wild or the rivers for producing freshwater prawn post-larvae (PL). In Malaysia, over 90% of hatchery managers opt for wild broodstock, attributing their ability to produce better-quality larvae. However, the quality of broodstock can vary significantly based on the river's location and the capture methods used. Unfortunately, the availability of wild broodstock is declining, leading to a supply shortage. Moreover, there have been instances where wild broodstock found to be positive for *Macrobrachium rosenbergii* nodavirus (MrNV), making their use inadvisable (personal communication, NaFisH, DoF, 2020).

The maturation of female freshwater prawns undergoes several stages based on the development of their ovaries. These ovarian stages (OS) are estimated by observing the size and colour of the ovaries through the carapace. The most accurate method for determining ovarian stages is by calculating the gonadosomatic index (GSI), which involves measuring the ratio of ovary weight to body weight ((Kamaruding et al., 2017). Based on the GSI value obtained, the ovarian stages are classified accordingly. Various ranges of GSI value have been reported to classify OS, as shown in Table 2.1 (Cavalli et al, 2001; Chang & Shih, 1995; Chengal Reddy et al, 2013; Lee & Chang, 1997; Martins et al., 2007). Ovary development can be observed through the carapace and the OS can be estimated, as shown in Figure 2.6. Proper broodstock selection and accurate determination of ovarian stages are critical for successful freshwater prawn PL production, ensuring the sustainability and viability of this valuable aquaculture industry.

Table 2.1: The ovarian stages based on GSI value of *Macrobrachium rosenbergii*.

GSI value for each ovarian stage (%)					References
I	**II**	**III**	**IV**	**V**	
0.48	0.61	1.77	3.79	7.42	Lee & Chang (1997)
0.52	1.17	2.41	5.77	6.93	Chang & Shih (1995)
0.28	0.43	0.67	4.41	7.91	Martin et al. (2007)
0.76	2.13	4.83	6.03	n. a.	Chengal Reddy et al. (2013)
0.48	1.51	2.79	4.39	5.92	Cavalli et al. (2001)

Note: n.a. = not available

$$\text{Gonadosomatic index (GSI)} = \frac{\text{Wet weight of the gonads}}{\text{Wet weight of the whole animal}} \times 100$$

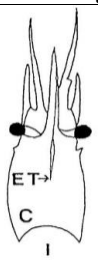
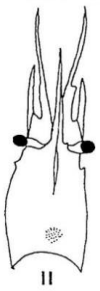
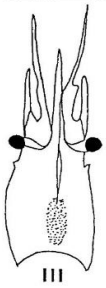
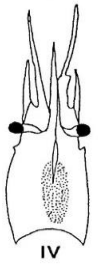
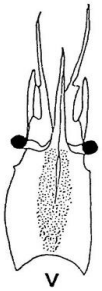
Ovarian stage and description	Drawing	Photograph
Stage I Spent stage: There is no ovarian tissue visible; this is a feature of both non-developed and spent females.		
Stage II Early previtellogenic stage: The ovarian tissue is seen as a little yellow patch towards the carapace's posterior end.		
Stage III Late previtellogenic stage: The ovary, which is orange in colour, can be seen from the back of the carapace to just in front of the epigastric teeth.		
Stage IV Early vitellogenic stage: The orange ovarian tissues had spread to the area around the epigastric teeth.		
Stage V Late vitellogenic stage: The ovarian tissues have spread all the way to the front of the carapace.		

Figure 2.6: Ovarian stages based on observation through the exoskeleton of the prawn *Macrobrachium rosenbergii*. Dot area indicates the ovarian tissues. C = carapace; ET = epigastric tooth (Chang & Shih, 1995) and illustration with description (Kangpanich et al., 2016)

2.2.1(a) Mating and Incubation

Matured females with ovarian stage V can be used for mating by adding hard-shell males into the tank for copulation at the ratio of 1:3 (1♂:3♀) as recommended by researchers (Alam & Alam, 2014; Habashy, 2010; Ratnayake et al., 2011). Mating usually occurs within 24 hours following a female prawn's pre-spawning moult (Habashy, 2013; Wickins & Beard, 1974). The incubation period of the berried female is approximately 20 days, typically ranging from 18 to 24 days (Habashy, 2013; Wynne, 2013; Arshad et al., 2006).

2.2.1(b) Fecundity

Fecundity is a measure of an organism's reproductive capability. In prawn, it is measured as the number of eggs per female weight (eggs/g of female) and the percentage of effort spent on reproduction per spawning event (egg clutch weight/female weight × 100). According to Malecha (1983), there are three types of fecundity in prawn: spawning fecundity, Pre-Hatch Fecundity (PHF) and Larvae Hatch Fecundity (LHF). Spawning fecundity refers to the number of eggs a female is biologically capable of extruding in a single spawn. Pre-hatch fecundity represents the quantity of eggs carried by the female at any given time between spawning and larval hatch or discharge. Larvae Hatch Fecundity (LHF) is the number of larvae produced from the egg mass during incubation.

Rao (1998) calculated *M. rosenbergii* fecundity by measuring the number of eggs on the pleopods, which ranged from 20,000 to 70,000 eggs. He discovered that 500 larva per grams of prawn body weight was the average hatching fecundity. Cavalli et al. (2001) developed an equation for estimating egg number based on broodstock size or egg fecundity: $Y = 484 + 1454X$, where X is the female broodstock weight in

gram. According to the available information provided, the number of larvae hatched per grams of prawn body weight is normally estimated to be between 500 – 1000 larvae.

2.2.1(c) Spawning Frequency

According to Wickins and Beard (1974), under suitable growing conditions, *M. rosenbergii* may spawn four times in 170 days, with a calculated time of approximately 42.5 days between successive spawning (intermoult). In captivity, with ideal rearing conditions, *M. rosenbergii* may spawn up to five times in 180 days, with a 36-day intermoult interval (Cavalli et al., 2001). Therefore, providing adequate diet and ideal rearing conditions can increase spawning frequency in captive environments (Cavalli et al., 2001).

2.2.2 Larval

GFP larvae are planktonic and swim tail first with the ventral side uppermost, indicating an upside-down orientation. However, newly hatched larval cannot survive in freshwater more than two days and should be transferred to brackish water, typically in the range of 10– 14 ppt (Kangpanich, 2016) or 9 – 19 ppt (Hicks & Pierce, 2011; Wynne, 2013). The metamorphosis process for freshwater prawns encompasses eleven stages, starting from newly hatched larvae and progressing to post-larvae (PL) development. The duration of larval batches to metamorphosis is between 15 to 40 days, varying based on feeding and environmental conditions, particularly temperature (New, 2002; Wynne, 2013). The metamorphosis stages of larvae are presented in Figure 2.7.

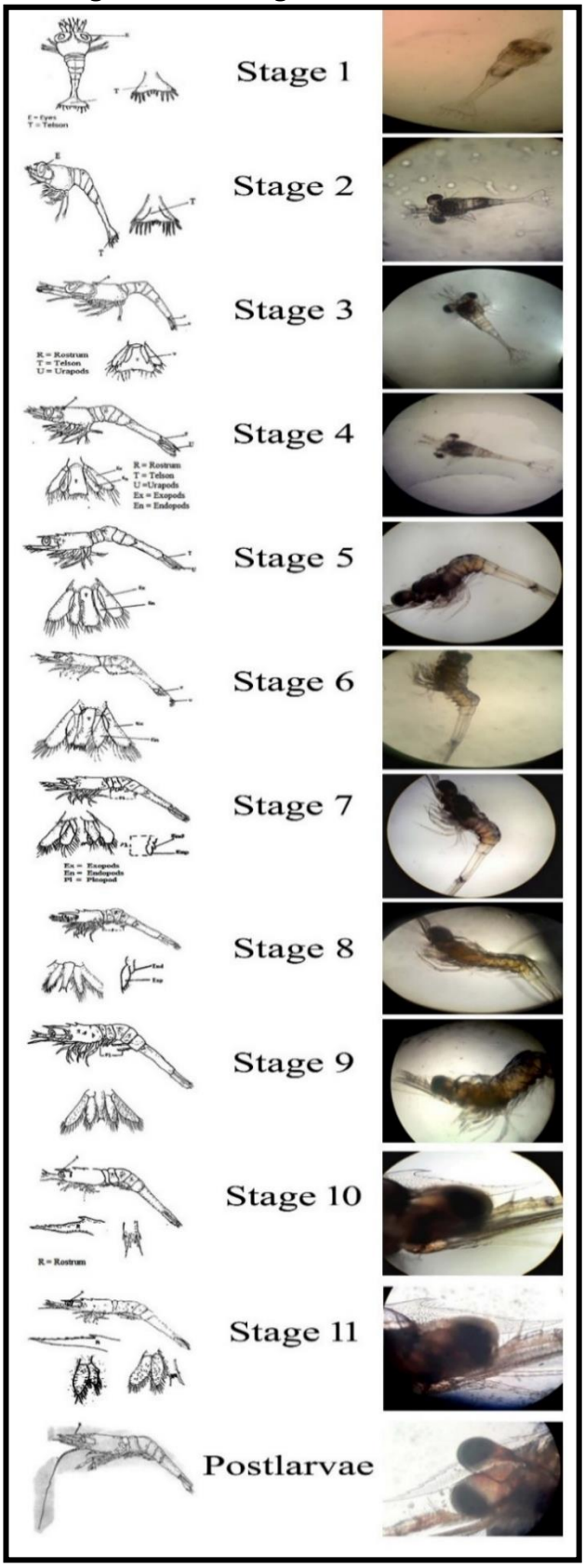
Drawing	Stage	Illustration	Description
	Stage 1		1-day old larvae, eyestalk is absent
	Stage 2		2 days old larvae, eyestalk is developed
	Stage 3		3-4 days old larvae, the tail fan is developed
	Stage 4		4-6 days old larvae, 2 teeth on posterior position of upper rostrum are developed
	Stage 5		5-8 days old larvae, telson is narrow and more elongated
	Stage 6		7-10 days old larvae, pleopod buds are developed
	Stage 7		11-17 days old larvae, pleopod are divided into 2 pendants without setae
	Stage 8		14-19 days old larvae, setae on pleopod are developed
	Stage 9		15-22 days old larvae, other appendixes are developed
	Stage 10		17-24 days old larvae, 3-4 teeth on posterior position of upper rostrum are developed
	Stage 11		19-26 days old larvae, teeth on anterior position of upper rostrum are developed
	Postlarvae		23-27 days old larvae, early postlarvae stage, larvae attach on the pond bottom and swim forward, teeth on both upper and lower rostrum are fully developed

Figure 2.7: The development of metamorphosis of freshwater prawn, *Macrobrachium rosenbergii* larva from newly hatched to post larvae (New, 2002).

2.2.3 Post larvae

Post larvae (PL) play a crucial role in improving prawn culture production. PL obtained from seed producers should be nursed before being introduced to the grow-out pond. The nursing duration typically lasts between 1 – 2 months. The nursery stage can be carried out in tanks, cages in the pond (hapa) and the pond itself. Morphologically, PL resembles adult prawns but are smaller, measuring 7 to 10 mm in length and weighing 6 to 9 mg (Kangpanich, 2016).

Typically, the PL obtained from the hatchery are quite small. As such, it is necessary to nurture these PL until they reach a length of 40-50 mm and a weight of 1-3 grams. Research had been carried out at FRI Glami Lemi, Negeri Sembilan for nursing PL in tanks using a stocking rate of 5 PL / L, fed with commercial feed has recorded a survival rate of 75 – 85% with a final weight of 0.05 – 0.1 g within four weeks of study (Annual Report FRIGL, DoF, 2019). Alam et al. (1997) reported that PL nursery in "hapa" with a stocking density of 100 – 300 PL/m^2, resulted in good growth of 47 – 49 mm body length and body weight 0.6 – 0.7 g, along with an 84 – 88% survival rate within 45 days.

2.2.4 Grow out and farm production.

The normal practice in freshwater prawn farming is a monoculture technique. The duration of the grow out stage varies between 4 – 8 months. Depending on management practices and environmental factors, the survival rate can range between 50 – 70%. The critical management factors and parameters for grow-out stage in the pond included pond preparation, selection of good quality PL, stocking density, feeding management, water quality management, monitoring of prawn condition