Immune competence of wild and hatchery produced *Macrobrachium rosenbergii* juvenile exposed experimentally to *Vibrio* spp.

A Thesis By

Marshida Khanom

Student ID: MS 180612

Session: 2018-19

The Thesis submitted to the Fisheries and Marine Resource Technology Discipline in partial fulfillment of the requirements for the degree of

Masters of Science in Aquaculture

Khulna University

Khulna, Bangladesh

September, 2019

Immune competence of wild and hatchery produced *Macrobrachium rosenbergii* juvenile exposed experimentally to *Vibrio* spp.

Approved as to style and content

By

14.10.19

Muhammad Abdur Rouf, PhD

Head and professor

Fisheries and Marine Resource Technology Discipline

Life Science School, Khulna University

Khulna-9208, Bangladesh

September, 2019

DECLARATION

Immune competence of wild and hatchery produced *Macrobrachium rosenbergii* juvenile exposed experimentally to *Vibrio* spp.

The results presented in the thesis with the above mentioned title are entirely of the candidate's own investigations and no parts of the results has been accepted for any degree nor is it being concurrently submitted for any degree.

Candidate

Marshida khanom

Marshida Khanom

Roll No: MS 180612

Session: 2018-19

Co-Supervisor

Shikder Saiful Islam

Assistant Professor

Fisheries and Marine Resource Technology Discipline, Khulna University

Supervisor

A. F. Md. Hasanuzzaman, *PhD*

Associate Professor

Fisheries and Marine Resource Technology Discipline

Khulna University, Khulna-9208, Bangladesh

September, 2019

Dedicated to

My Beloved Parents

Acknowledgement

It is my great pleasure for being able to complete this thesis for which I am completely indebted to the creator of this universe, the Almighty Allah.

No word is appropriate for expressing my sincere gratitude, heartfelt appreciation and profound respect with due solemnity to my supervisor **A. F. Md. Hasanuzzaman,** *PhD,* Associate professor, Fisheries and Marine Resource Technology (FMRT) Discipline, Khulna University, Khulna. His kind and continuous inspiration, scholastic guidance, constructive criticism, constant encouragement and providence of all necessary facilities rationalized me to complete this thesis paper successfully.

I would like to express my sincere gratitude and profound respect to my honorable Co-Supervisor, **Shikder Saiful Islam,** Assistant professor, Fisheries and Marine Resource Technology (FMRT) Discipline, Khulna University, Khulna for his guidance, valuable suggestions and sustained support in preparing this thesis.

My heartiest thanks to my junior Shoaib Muhammod Abir and Md. Bappi Forazi, Lab Attendant of FMRT Discipline, for their helpful cooperation during the study.

Finally, I would like to express my deepest gratitude to my beloved parents and my family for supporting all the way by my side during the preparation of the thesis.

Marshida Khanom

September, 2019

Contents

List of Figures

List of Tables

Abstract

This study was conducted to evaluate the immune competence of wild and hatchery produced *Macrobrachium rosenbergii* juvenile against *Vibrio* spp. It was conducted in plastic aquaria, providing same level of feeding under different treatments; Control wild (C_W), Control hatchery (C_H), Treatment wild (T_W), Treatment hatchery (T_H). In the treatment groups, prawn juveniles were exposed to *Vibrio* spp. at a concentration of 2×10^9 CFU/ml for seven days. In this study, *Vibrio* spp. was isolated from diseased giant freshwater prawn (*M. rosenbergii*). The results demonstrated that there was a significant difference ($P<0.05$) of mortality rate and THC between the control and the treatment groups of wild and hatchery prawns. There was no mortality in the control groups but 25% cumulative mortality rate (CMR) was found in both wild and hatchery treatment groups on day 5, and more than 50% CMR was on day 7 in both hatchery (65%) and wild (60%) groups. Total haemocyte count (THC) was found higher in treatment groups. Differential haemocyte count (DHC) data revealed that there was a significant difference ($P<0.05$) of large granular cell among the control groups of hatchery, treatment groups of hatchery and control groups of wild prawns but no significant difference ($P<0.05$) was found in treatment groups of wild prawns. On the other hand, significant difference was found among control group of hatchery, treatment group of hatchery and control group of wild for Semi granular haemocyte (SGH), Smaller granular haemocyte (SmGH) and Non granular haemocyte (NGH). However, this study pointed out that the prawns had dynamic response regarding THC and DHC, particularly in stress condition, of which association are to be determined through further research.

Chapter 1

Introduction

1. Introduction

1.1 Background of the study

Bangladesh standed 4th position in the world fish production (DoF, 2017) where the contribution of crustaceans is 23% (DoF, 2017); and among these crustaceans, the production of prawn and shrimp is 130296 metric tonne and total contribution is 56.44% (DoF, 2017). This sector plays an important role in supplying nutrition, creation of rural employment, poverty alleviation and earning foreign exchange.

The giant freshwater prawn (GFP) *Macrobrachium rosenbergii* is a decapod shrimp belonging to the family *Palaemonidae*. It has a brown carapace with darker brown rings and its abdomen has a brown mottled pattern. There are bright red markings on each side of its pleural condyle (Wowor and Ng, 2007) and its average size is 320 mm for males and 250 mm for females (Cowles, 1914). *M. rosenbergii* inhabits the freshwater, river mouths and brackish waters with a salinity range from 0.0 to 20.0 ppt; and its distribution is predominant in the tropics and sup-tropics in the Indo Pacific region of temperature ranging from 25 to 30°C, including Bangladesh, India, Malaysia, Thailand, the Philippines, Shrilanka, Myanmar, Indonesia and Vietnam. This species is of nutritional and economic importance as a food source as well as trading commodity in local and export markets.

Fishery is a more environmentally sustainable option for meeting the world's food needs than other animal food sources (DoF, 2017). The increased fish production from sustainable prawn fisheries can be a driver for rural people nutrition and economic development by contributing to income generation and employment, thereby mitigating risks to their livelihoods.

Giant freshwater prawn is an important crustacean aquaculture species, and Bangladesh is considered as the one of the top producer countries (Mazid, 1994; Ahmed et al., 2008; DoF, 2017). Prawn aquaculture is substantially important for meeting the world's growing demands for food.

In Bangladesh, freshwater prawn farming first started in the southwest region in the early 1970s (Mazid, 1994). Around three-quarters of prawn farms are located in the southwest part of Bangladesh including Khulna, Satkhira, Bagerhat, Fokirhat etc. which has been identified as the most important and promising area for prawn culture, because of the availability of wild post larvae, favorable resources and climatic conditions: availability of ponds, low lying agricultural land, warm climate, fertile soil, and cheap and abundant labor (Haroon, 1990;

Ahmed, 2001). Prawn farms are typically smaller than farms in the brackish water shrimp sector; an average 0.28 ha comparing with 4.0 ha of shrimp farms (DoF 2002a; Muir 2003a). There are two prawn farming systems in Bangladesh: pond and gher. Approximately 71% of farmers are involved in gher systems and the remainder in pond systems (Muir, 2003b). Although prawn farming practice is still traditional and extensive in nature, many farmers (20%) practice improved methods. Farmers also practice rice-prawn gher system where farmers use their rice fields for farming prawn and rice (*aman*) concurrently from April/May to January, and for only rice (*boro*) from January to April (Hasanuzzaman et al., 2011). Extensive production typically use slightly modified versions of traditional methods and are called low-density (10,000-18,000 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. Extensive feeding practices generally use supplementary diets consisting of a mixture of locally available feed ingredients, such as rice bran, wheat bran, oil cake and fish meal. Semi-intensive operations practice intermediate levels of stocking (18,000-30,000 post larvae/ha/year) and other inputs. Farms with semi-intensive feeding practices depend on commercially manufactured pelleted feeds.

In Bangladesh, about 3 lakh peoples are directly involved in prawn production in prawn hatchery and hatchery plays an important role in prawn farming industry and to our national economy (Ahmed, 2001). Despite the great potential of fresh water prawn farming in Bangladesh, successful commercial culture in hatcheries faces a number of constrains including lower hatching rate, mortality etc. (Ahmed, 2001).

However, the sustainable expansion of this sector is at risk from the outbreak of several diseases; bacteria are major causative agents for this disease (Walker and Winton, 2010; Debnath et al., 2012). Besides bacterial infection, a wide variety of viral, fungal, and parasitic diseases are also reported to occur in the freshwater prawn culture system, causing remarkable mortalities and economic losses in this sector (Ahamed et al., 2014).

The bacterial infections include melanized lesions (brown or black spot) in GFP, caused by the opportunistic bacteria such as *Vibrio* spp., *Aeromonas* spp., and *Pseudomonas* spp. affecting all life stages of GFP (Pillai and Bonami, 2012). Larval mid cycle disease (MCD) was caused by *Alcaligenes* spp. and *Enterobacter* spp. (Phatarpekar et al., 2002; Anderson et al., 2006). However, vibriosis predominantly caused by *V. cholerae, V. alginolyticus* and *V.*

vulnificus (Jayaprakash et al., 2006; Saurabh and Sahoo, 2008). Black spot necrosis in juvenile and adult GFP caused by *Aeromonas* spp. and *Vibrio* spp. (Lalitha and Surendran, 2004), and chitinolytic bacterial shell disease caused by *Vibrio* spp. (Sharshar and Azab, 2008). All these diseases have been reported to cause severe economic and production loss over the past few years (Faruk et al., 2006; Alam et al., 2007; Paul and Vogl, 2011; Ahamed et al., 2014; Shanker et al., 2014). Sometimes, apparently healthy prawns are also reported to become infected on their shell by *Vibrio, Pseudomonas*, and *Aeromonas* spp. (Sung and Hong, 1997) and reported to cause high mortality (Sung et al., 2000; Sahoo et al., 2007; Shankar et al., 2012).

Several recent research reports demonstrated that the prawn hatchery and prawn post larvae are severely affected by pathogenic bacteria *Vibrio* spp. including *Vibrio parahaemolyticus.* Opportunistic bacteria such as *Vibrio* spp. cause melanised lesions on the carapace of *M. rosenbergii. Vibrio* spp. affecting all life stages of *M. rosenbergii* in hatcheries, has been variously named black spot, brown spot or shell disease, tail root, breakage of antennae, antennule and chelate legs (Lightner and Lewis, 1975; El-Gamal et al., 1986; Lombardi et al., 1996). Bacterial necrosis is very similar to black spot, but affects only IV and V stages of larvae.

Like other crustaceans, prawns depend solely on their innate defense systems to combat the prevailing threats brought by microbial diseases. Though innate immune system, haemocytes directly offend pathogen through the cellular defense mechanisms, for example, phagocytosis, encapsulation, nodule formation, etc. (Rhodes et al., 1985; Johansson and Soderhall, 1989; Bachere, 2000; Rowley, 2016). Besides, haemocytes are also involved in regulation of different physiological functions and humoral defence mechanisms, which includes haemocyte clotting, hardening of exoskeleton, healing of cuticle damage, production of reactive oxygen metabolites, release of molecules stored within the haemocytes. The stored molecules are anticoagulant proteins, agglutinins, phenoloxidase enzyme, antimicrobial peptides, protease inhibitors, carbohydrate metabolism, and protein/amino acid transportation and storage (Johansson and Soderhall, 1989; Cheng et al., 2003; Vazquez et al., 2009; Apines-Amar and Amar, 2015).

1.2 Justification of the study

There are several studies with *M. rosenbergii*, including farming practice (Kendrick, 1994; Williams and Khan, 2001; Ahmed et al., 2008a,b; Wahab et al., 2008; Hasanuzzaman et al., 2011), stocking density (Asaduzzaman et al., 2009) prawn feed development (Chisty and Rahman, 1999; Hossain and Paul, 2007; Hasanuzzaman et al., 2009), probiotics effects (Sayeed et al., 2015; Ghosh et al., 2016; Sumon et al., 2016) in Bangladesh; but, to the best of my knowledge, there is no meticulous scientific work undertaken to compare the immune competence of wild and hatchery juveniles exposed to pathogens (e.g. bacteria, virus).

Taking into account of this, the present study was conducted to evaluate immune response of wild- and hatchery- produced *M. rosenbergii* juveniles once challenged with *Vibrio* spp.

1.3 Objectives of the study

- To determine the survival of wild and hatchery-produced *M. rosenbergii* juveniles exposed experimentally to *Vibrio* spp.
- To estimate total and differential haemocyte count of wild and hatchery-produced *M. rosenbergii* juveniles exposed to *Vibrio* spp.

Chapter 2

Review of literature

2. Review of literature

2.1 Taxonomic and biological features

Macrobrachium rosenbergii is the largest *Macrobrachium* species. This crustacean belongs to the family *Palaemonidae* under the order Decapoda.

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Crustacea
Class: Malacostraca
Order: Decapoda
Infraorder: Caridea
Family: Palaemonidae
Genus: Macrobrachium
Species: *Macrobrachium rosenbergii* (De Man, 1879,
Source: www.fao.org)

The maximum recorded size for males and females are 33 cm and 29 cm, respectively (Jayachandran, 2001). The cephalothorax consists of 5 indistinct segments in the head and eight in the thoracic region (Figure2.1). Cephalothorax with 2 pairs of antennae, 3 pairs of jaws, 3 pairs of maxillipids and 5 pairs of walking legs. Eyes compound. The abdomen has six distinct segmented movable terga (De Man, 1879). Each abdominal segment has a pair of biramous pleopods (swimming legs) (FAO, 2017). The second and largest abdominal tergum laterally overlaps the first and the third. Dorsal surface of abdominal carapace is smooth and rounded. Rostrum is slender and curved upwards. The rostrum extends beyond the antennal scale and has 11-14 dorsal teeth and 8-10 on the ventral side. Mature male prawns are considerably larger than the females and the second pair of chelipeds is much larger and thicker than have the females (Jayachandran, 2001). The head of the male is also proportionately larger and the abdomen is narrower. The left and right chellipeds of *M. rosenbergii* are equal in size, unlike some other *Macrobrachium* species (FAO, 2017). In adult males they become extremely long and reach well beyond the tip of the rostrum. The genital pores of the males are situated at the base of fifth walking leg and in females at the base of the third walking legs. The tip of the telson extends beyond the posterior telson spines (Jayachandran, 2001).

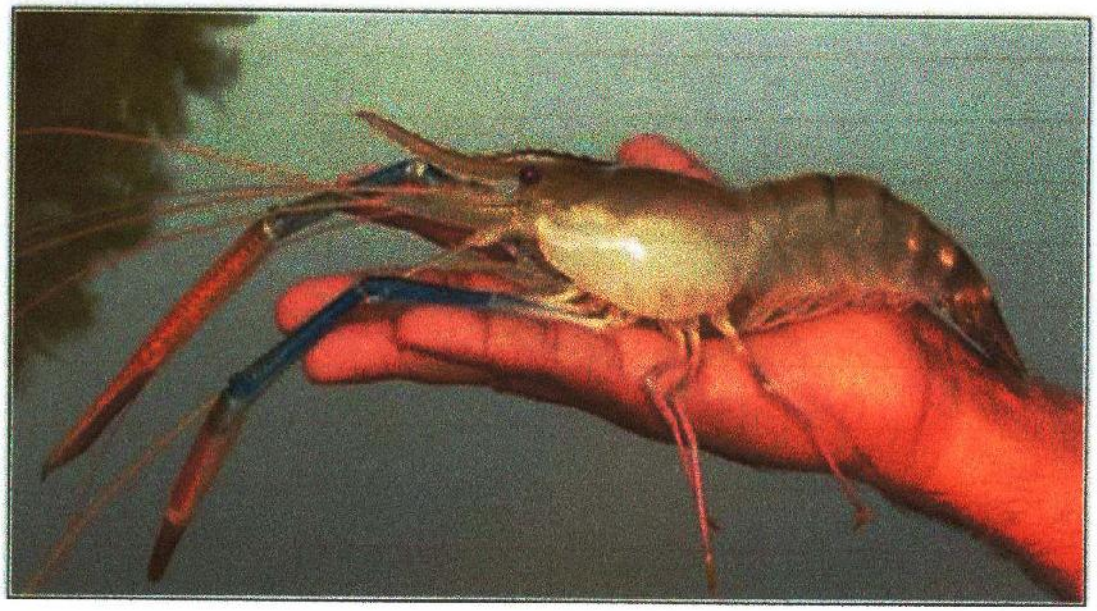


Figure 2.1. Giant freshwater prawn (Source: www.fao.org)

M. rosenbergii is nocturnal, bottom dwelling and sluggish in nature and is territorial. During the day they remain half buried in sediments and prefer shallow, detritus rich and vegetated areas (De Man, 1879).

2.1.1 Life cycle of *M. rosenbergii*

The life cycle of *M. rosenbergii* can be summarized as follows. The mating (copulation) of adults results in the deposition of a gelatinous mass of semen (referred to as a spermatophore) on the underside of the thoracic region of the female's body (between the walking legs). Successful mating can only take place between ripe females, which have just completed their pre-mating moult (usually at night, Figure2.2) and are therefore soft-shelled, and hard shelled males. Under natural conditions, mating occurs throughout the year, although there are sometimes peaks of activity related to environmental conditions. In tropical areas, these coincide with the onset of the rainy season, whereas in temperate areas they occur in the summer (Ismael and New, 2000).

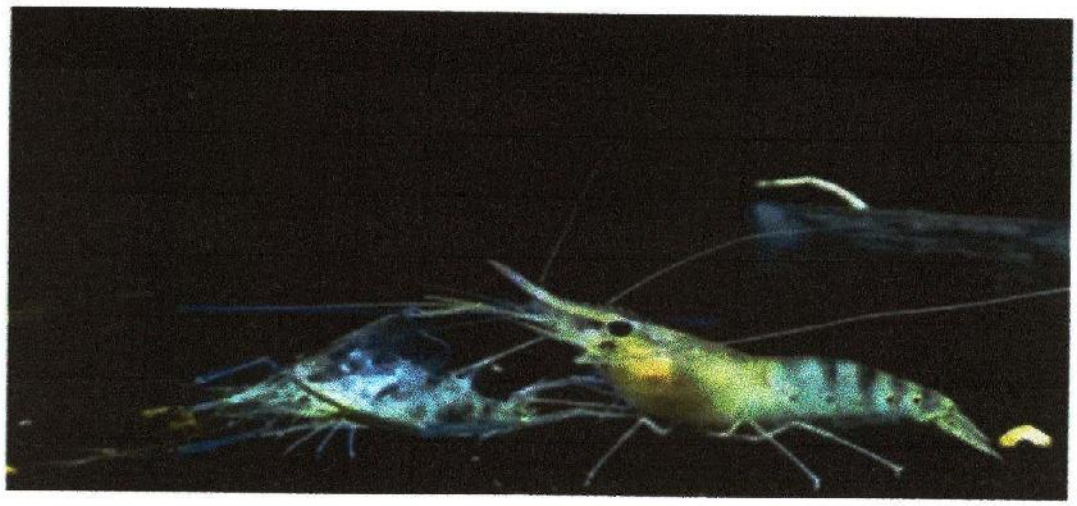


Figure 2.2. Moulting of *M. rosenbergii* (Source: www.fao.org)

Within a few hours of copulation, eggs are extruded through the gonopores and guided by the ovipositing setae (stiff hairs), which are at the base of the walking legs, into the brood chamber. During this process the eggs are fertilized by the semen attached to the exterior of the female's body (Karplus et al., 2000).

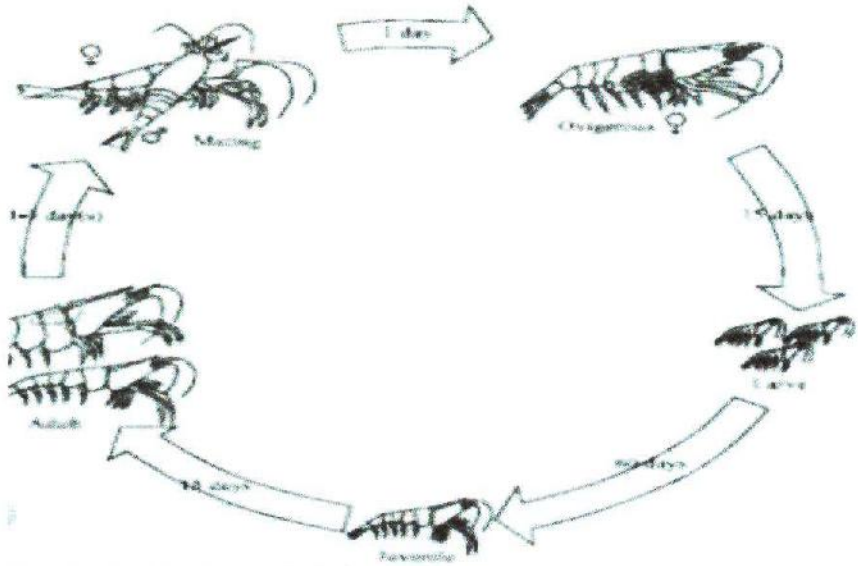


Figure 2.3. Life cycle of *M. rosenbergii* (source: www.fao.org)

The number of eggs which are laid depends also on the size of the female. Female prawns of *M. rosenbergii* are reported to lay from 80,000 to 1,00,000 eggs during one spawning when fully mature. However, their first broods, (i.e. those which are produced within their first year of life), are often not more than 5,000 to 20,000 (Daniels et al., 2000).

Figure 2.4. Matured berried *M. rosenbergii* (Source: Takuji fujimura, reproduced from New and Valenti (2000), with permission from blackwell science)

Under laboratory conditions, where a breeding stock of both males and females was kept, it has been noted that egg incubation time averaged 20 days at 28°C (range 18-23 days). Ovaries frequently ripened again while females were carrying eggs (New, 2000).

As the eggs hatch, a process which is normally completed for the whole brood within one or two nights, the larvae (free-swimming zoeae) are dispersed by rapid movements of the abdominal appendages of the parent. Freshwater prawn larvae are planktonic and swim actively tail first, ventral side uppermost (i.e. upside down). *M. rosenbergii* larvae require brackish water for survival. There are a number of microscopically distinct stages during the larval life of freshwater prawns, which lasts several weeks. Individual larvae of *M. rosenbergii* have been observed, in hatchery conditions, to complete their larval life in as little as 16 days but reaching this stage may take much longer, depending on water temperature and other factors (Pinheiro and Hebling, 1998).

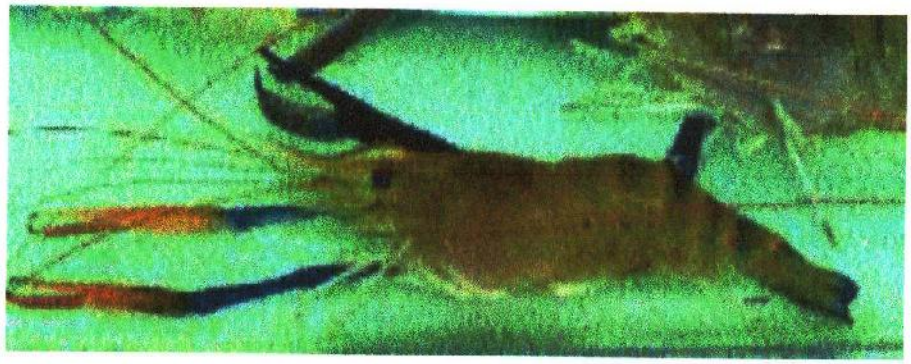


Figure 2.5. Juvenile of *M. rosenbergii* (Source: New and valenti, 2000)

On completion of their larval life, freshwater prawns metamorphose into post larvae (PL). Post larvae exhibit good tolerance to a wide range of salinities, which is a characteristic of freshwater prawns. Post larvae begin to migrate upstream into freshwater conditions within one or two weeks after metamorphosis and are soon able to swim against rapidly flowing currents and to crawl over the stones at the shallow edges of rivers and in rapids. Post larval freshwater prawns are omnivorous and, as they grow, their natural diet eventually includes aquatic insects and their larvae, algae, nuts, grain, seeds, fruits, small molluscs and crustaceans, fish flesh and the offal of fish and other animals. They can also be cannibalistic (Ling, 1969).

2.2 Food and nutritional requirements of *M. rosenbergii*

Prawns are capable of digesting a wide range of foods of both plant and animal origin (Mitra et al., 2005). Characterization of the activities of the digestive enzymes in the alimentary tract indicates the presence of trypsin, amino peptidases, proteases, amylases, chitinase, cellulase, esterases and lipases (Mitra et al., 2005).

The Prawn *M. rosenbergii* contain greater amount of proteins, lipids and unique taste, less fat and has great demand in national and international markets. Shellfish contains potent source of nutrients required for the maintenance and growth of human body (Dong, 2001). Due to low price and efficient availability, the prawns and shrimps have good source of animal protein for low income earners (Adeyeye, 1996). Prawn contains good amount of organic and inorganic constituents. The main constituents are proteins, carbohydrates, lipids in addition to that prawn also contain a significant proportion of minerals (Ca, Mg, P, Mn and Cl) and vitamins A, C and D (Abulude et al., 2006).

The protein content of the cultured prawn was ranged from 72.99 to 74.89% with an average value 74.24% which was greater than that of frozen prawn average protein value recorded as 60.55%. Amino acids are the key stones for protein construction. Proteins are required for normal growth and proper functioning of the body tissues (Diana, 1982). Gomez et al., (1988) and Habashy (2009) reported that the protein composition might be up to 90% depends on the diet composition.

The average carbohydrate content of the cultured prawn was 5.50%. Carbohydrates are first and important nutrients utilized by the body as substrate for the energy production (Heath, 1987). The average content of the lipids were (9.09 ± 0.009%). Ricardo et al., (2003) and New (1986) reported that lipids are the good source of energy producers of the body through metabolic pathways or metabolism and acts as carrier molecules for the non-fat nutrients like A, D, E, K.

The average ash content is 9.71 ± 0.19%. On the other hand the moisture content of the cultured prawn was 77.14 ± 0.19%. The current study findings were more or less similar to the reports of Ferdose and Hossain (2011) reported that the protein, carbohydrates, lipid, ash and moisture contents in cultured prawns were observed as 74.85 ± 0.65, 60.8 ± 0.12, 5.61 ± 0.37, 8.21 ± 0.14, 9.15 ± 0.61%.

Saravana et al., (2010) reported that the protein, lipid, carbohydrate, ash and moisture content in hatchery prawns are 26.26, 2.69, 0.55, 0.86 and 69.82 %.

Diets with 35-40 percent protein, gross energy of 13.37 kJ/g diet and a protein:energy ratio of 30-31 mg protein/kJ are suitable for growth of *M. rosenbergii* in clear water systems that do not have any supply of natural foods (Balazs and Ross, 1976; Millikin et al., 1980). Freuchtnicht et al., (1988) suggested that the optimum protein requirement for freshwater pawn is 30 percent and Reed and Abramo (1989) estimated that the protein requirement of *M. rosenbergii* is greater than 35 percent but less than 38 percent. Many commercial feeds for grow-out contain 24-32 percent crude protein.

Diaz-Herrera et al., (1992) report that carbohydrates are the principal substrates for energy production in larval and post larval *M. rosenbergii*. Dietary protein is optimized at a dietary lipid-carbohydrate ratio of 1:2.5-1:4 (Sahu, 2004).

Quantitative lipid requirements range between 6-8 percent (Rangacharyulu, 1999; Tiwari and Sahu, 1999). Both n-3 and n-6 HUFAs at dietary levels of 0.075 percent are known to increase weight gain and feed efficiency remarkably (Mitra et al., 2005).

M. rosenbergii are requires 60-150 mg vitamin C/kg diet. Levels of 60 mg ascorbic acid and 300 mg α -tocopherol per kg diet are considered sufficient for successful reproduction and larval viability. Manus et al., (2005) reported that vitamin C at 240 mg per kg reduces stress in freshwater prawns. It has been reported that vitamin E at 200 mg/kg diet modulated some of the antioxidants defense system by decreasing lipid peroxidation in the hepatopancreas (Mitra et al., 2005).

Dietary supply of calcium seems to improve growth. In soft water (with Calcium levels of 5 ppm) performance is improved with a dietary supply of 3 percent calcium. The optimum dietary zinc level is 50-90 mg/kg diet (Mitra et al., 2005).

2.3 Distribution

M. rosenbergii is indigenous in the whole of the South and Southeast Asian area as well as in northern Oceania and in the western Pacific islands. The distribution of the giant freshwater prawn, *M. rosenbergii*, is limited to the freshwater zone, river mouths and brackish waters with a salinity range from 0.0 to 20.0 ppt and a temperature range from 25 to 30°C in the tropics and sup-tropics of Indo Pacific region in Malaysia, Thailand, the Philippines, India, Shrilanka, Bangladesh, Myanmar, Indonesia and Vietnam (Sebastian, 1990; Rao, 1991). This prawn are generally found in freshwater, in ponds, rivers, lakes, ditches, canals, depressions, low-lying floodplains and river mouths (Motoh et al., 1980).

M. rosenbergii has been used in research more than any other species and has been introduced many new countries for commercial culture. Fujimura and Okamoto (1972) were successful in producing post-larvae (PL) of *M. rosenbergii* in large numbers in Hawaii in 1972. *M. rosenbergii* is being cultured in commercial quantities in many parts of the world, including Hawaii, Honduras, Mauritius, Taiwan, Thailand and the Philippines. Farming practices have also been developed in Costa Rica, Israel, Malaysia, and Mexico.

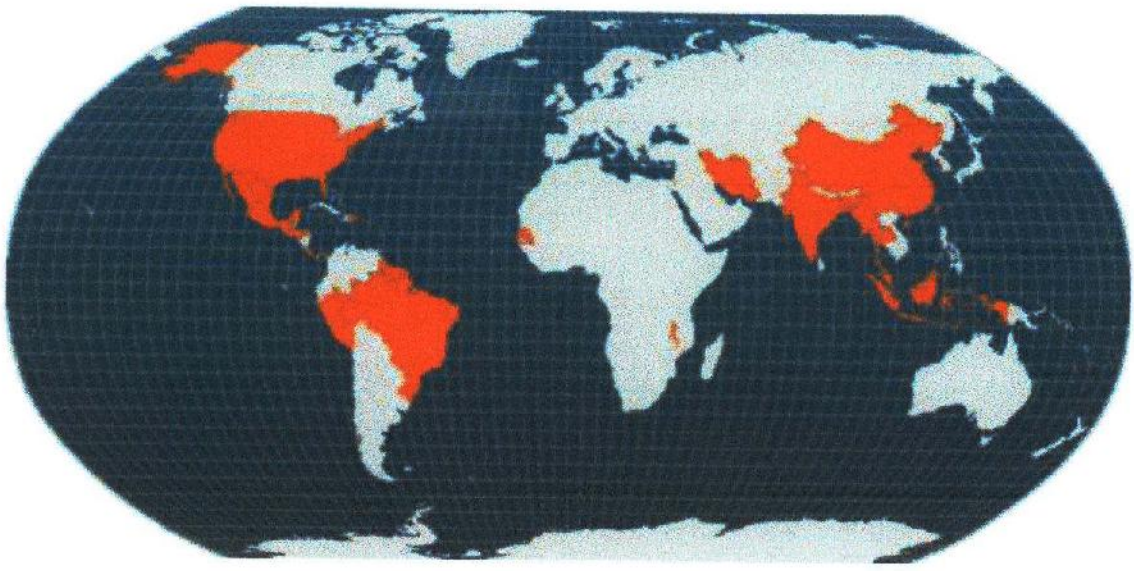


Figure 2.6. Main producer country of *M. rosenbergii* (Source: FAO Fishery Statistics, 2006).

2.4 *M. rosenbergii* aquaculture

The environment for GFP aquaculture should be safe, free from pollution and of moderate temperature 28-31°C (FAO, 1981). Farms must be close to their market so the road access must be good. For successful year-round farming, sites with large diurnal and seasonal fluctuations should be avoided. The optimum temperature range for year-round production is between 25 and 31°C, with the best results achievable if the water temperature is between 28 and 31°C (FAO, 1994).

Freshwater is normally used for rearing freshwater prawns from post larvae to market size. Suitable water quality parameter is given in the following table 2.1 (Boyd, 1979).

Table 2.1. Water quality parameter (FAO, 1981).

Water quality parameter	Recommended range
Temperature	28-31°c
pH	7-8.5
DO	3-7ppm
Salinity	<10ppt
Transparency	25-40cm
Alkalinity	20-60ppm

Hardness	30-150ppm
Nitrate	<10ppm
Nitrite	<2ppm

Prawns will tolerate partially saline water (reports indicate that they have been experimentally cultured at up to 10 ppt; however, they do not grow so well at this salinity). As with hatcheries, the water must also be as predator-free as possible, though standards need not be quite so high. This may be achieved by screening or by the use of well water. Underground water, because of its chemical and microbiological quality and its lack of predators, is undoubtedly the preferred water source. In practice, sites that only have access to surface water supplies (rivers, lakes, reservoirs, irrigation canals, etc.) are the most commonly used.

2.4.1 PL supply and production

Now-a-days, seed (PL) of *M. rosenbergii* is supplied principally from the hatchery; nevertheless PLs are also collected from wild sources.

For successful hatchery operation availability of broodstock is very much necessary. In the tropics, berried (egg-carrying) 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. Berried females can also be obtained from rivers, canals and lakes in areas where they are indigenous (native) (FAO, 1998). Broods should be healthy and active, well pigmented, with no missing appendages or other damage, and carrying large egg masses; egg colour to be from bright orange to brown and finally to greyish-brown a few days before hatching (Daniels et al., 2000). Brood can easily transport by using buckets of water. In case of longer distances, they can be held in tanks or double plastic bags; to prevent the bags being punctured, chelipeds should be tied with rubber bands or covering with plastic tubing and the rostrum should be blunted with scissors inserted into a plastic tube. Prawns can then be transferred to holding tanks which contain freshwater at an optimum temperature of 27-31°C (Daniels et al., 2000).

Broodstock should be disinfected upon arrival at the hatchery by placing them into freshwater containing 0.2 to 0.5 ppm of copper sulphate or 15 to 20 ppm of formalin for 30 minutes (Cavalli, 2000). Continuous aeration should be provided.

Broodstock should be fed at a daily rate of 1-3% of total biomass, adjusted to match consumption. The daily food ration should be given in two equal portions, normally in the early morning and late afternoon (Abramo and New, 2000).

The typical male to female ratio in broodstock holding systems is 1-2 Blue Claws males or 2-3 Opaque Claws males per 20 females, at a total stocking density of 1 prawn per 40 litres (Suwannatous et al., 2000). Within a few hours of copulation, fertilization occurs externally; at hatching, free-swimming zoeae are produced.

A wide variety of feeds including nauplii of brine shrimp (*Artemia* spp.), a freshwater cladoceran (*Moina* spp.), fish eggs, squid flesh, frozen adult *Artemia*, flaked adult *Artemia*, fish flesh, egg custard, worms and commercial feeds are employed by hatcheries, including the 75 to 150 g of *Artemia* cysts will be required to produce the 15 to 30 million required for the daily feeding of a 5 m^3 larval tank initially stocked with 50 larvae/L and expected to provide about 25 PL/L. By day three, tiny feeding can be started, gradually increasing the feeding frequency to five times per day, spread out evenly throughout the day. From day 5 it is started to reduce the frequency of feeding (Durant et al., 1995).

Once prawn larvae become PL, the PL can be harvested and transferred in any suitable container to holding tanks that contain pre-aerated freshwater. These temporary transport containers (e.g. buckets) should not be over crowded with PL. Post larvae should not be left too long in them or oxygen depletion will occur.

2.4.2 PL Nursery

Concrete or fibreglass tanks, usually of 10 to 50 m^2 with a water depth of 1 m, for indoor freshwater prawn nurseries where PL are intentionally reared to a larger size before transfer to outdoor nurseries or grow-out ponds (Chowdhury et al., 1993). Artificial substrates such as layers of mesh over wood/aluminium/PVC frames can be used of various designs and materials to increase surface area; these provide shelter and increase survival rates. Prawns tend to use the edges of substrates, whether they are natural (e.g. leaves, branches) or artificial (Alston, 2000). The water supplies for indoor nurseries can be flow-through or recirculating. Nursery tanks require aeration and may be operated as flow-through or recirculating systems, like hatcheries. Siphon the tank bottoms regularly to remove food wastes, faeces, and decomposing organic matter. General water quality requirements for

indoor nurseries are similar to those for freshwater in hatcheries. Maintain the optimum temperature (27-31°C) (Boyd, 1990).

Stocking density depending on the length of nursing time, should not to exceed a stocking density of 1,000 PL/m^3 in tanks without substrates. It can be 2,000 PL/m^3 in tanks where substrates are provided (Fondren, 1995). The indoor nursery rearing time is not more than 20 days; it must need to reduce the density if anyone want to keep the prawns in indoor nursery for longer periods (e.g. in sub-tropical and temperate regions, where prawns are usually maintained in indoor nurseries until the grow-out ponds reach temperatures of at least 20°C) (Durant et al., 1995).

Feeding once or twice per day is sufficient. Feed such as adult *Artemia*, egg custard based diets (EC), or minced fresh fish; beef liver, fresh feeds should normally be about 10-20% of the total weight of the prawns in the tank (Das et al., 1996).

When prawns are stocked as post larvae, their weight will be about 0.01 g. After 20 days in nursery tanks, the juveniles should be about 0.02 g, and they should reach about 0.3-0.4 g after a total of 60 days at reduced densities. Cannibalism, competition and poor water quality are the main causes of mortality in indoor nurseries. However, survival rates of about 90% can be obtained up to 20 days (Fondren et al., 1995). Some mortality (10-20%) occurs soon after PL is stocked, even when the conditions are ideal. In outdoor system, total survival from stocking (or re-stocking) until removal from the nursery ponds should be at least 75%. The weight of the prawns at the end of the outdoor nursery period should be about 0.8-2.0 g, but the time taken to reach those sizes will depend on local conditions (FAO, 2001).

Nursery ponds for outdoor system are of area from 300 to 2,000 m^2. Some operators cover their nursery ponds with plastic netting to avoid predators, especially dragonflies, whose nymphs predate on freshwater prawn PL (Alston, 2000).

If nursery ponds have no substrates or aeration, stocking rates should be between either 1,000/m^2 of PL, 200/m^2 of small juveniles (0.02 g), or 75/m^2 of 0.3-0.4 g juveniles. It can increase if substrates, aeration and predator protection are provided (Daniels et al., 1995).

2.4.3 Grow-out system

Prawns are cultivated in different farming systems such as extensive, semi-intensive and intensive. In extensive or improved system, prawns are stocked, often from wild sources, with PL or juveniles at 1-4/m^2, and production is less than 500 kg/ha/yr of freshwater prawns.

Supplemental feeding is not normally supplied; locally available feed is given for example uneaten rice, snail, rice bran, chopped stomach etc.

Semi-intensive systems involve stocking PL or juvenile freshwater prawns (usually from hatcheries) at 4-20/m^2 in ponds, and result in a range of productivity of more than 500 kg/ha/yr (Fujimura and Okamoto, 1972). Intensive culture refers to freshwater prawn farming in small earth or concrete ponds (up to 0.2 ha) provided with high water exchange and continuous aeration, stocked at more than 20/m^2 and achieving an output of more than 5,000 kg/ha/yr. 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 (Fujimura and Okamoto, 1972).

In Poly culture, Bighead and silver carp are the usual species of choice (Zimmermann and New, 2000). Freshwater prawns are stocked as1.0-1.2 cm juveniles at 16.5-22.5/m^2, or as 1.5-2.0 cm juveniles at 15-18/m^2. Silver and bighead carps are stocked at 1,500-1,800/ha at a size of 12-15 cm. Production of prawns ranges from 1,500 to 3,000 kg per crop. Production of carp ranges from 750 to 1,500 kg per crop (Johnson et al., 2000). Trash fishes, molluscs, pelleted feeds are given. Feeding rates vary from 15-20% of body weight when the stocked prawns are <1 g, decreasing gradually to 5-6% when the prawns are >10 g; 70% of the daily feed ration is given in a late-afternoon and 30% in the morning. The food is spread evenly around the pond about 2 m from the bank (Johnson and Bueno, 2000). The rearing period is 4-6 months (one cycle per year). Partial seine harvests are taken but the ponds are totally drained before the water temperature drops below 18°C. An average prawn market size of 20 g is sought.

Integrated system, ponds and garden (crop production) canals are typically stocked at 4-6 juveniles/m^2 and paddy fields at 0.5- 2/m^2. Both farm-made and commercial feeds are used in the ponds and, intermittently, in the rice fields. The prawn productivity in rice fields is about 0.1-0.3 mt/ha/yr. Selective harvesting occurs several times before the final drain harvest (FAO, 1995; Zimmermann and New, 2000).

In Bangladesh, freshwater prawn farming first started in the southwest region in the early 1970s (Mazid, 1994). Around 1978, a few well-off local farmers in Fakirhat area of Bagerhat district began to stock prawn post larvae (PL) in carp ponds, and experimented with construction, design, feeding, stocking and other technical aspects as well (Kendrick, 1994). Finally by around 1987, a few pioneers adopted successfully the first prawn cultivation in Ghers converted from low lying lands and rice fields. Since 2000, the increased demand for prawn in the international market has attracted many fish farmers to prawn cultivation in freshwater and low-saline waters in different parts of Bangladesh, mainly Noakhali, Patuakhali, Mymensingh and Bagerhat districts (Asaduzzaman et al., 2007; Hasanuzzaman et al., 2009). There are two prawn farming systems in Bangladesh: pond and gher. Approximately 71 % of farmers are involved in gher systems and the remainder in pond systems (Muir, 2003b). The farmers practice monoculture, poly-culture with fin-fishes, as well as concurrent and crop-rotation with rice in rice fields.

Rice-prawn gher (RPG) farming system is an indigenous agricultural technology solely developed by farmers during mid-1980s (Barmon et al., 2007). On an average 23597.72 prawn PLs (ranging from 15,000 to 30,000) are annually stocked in a gher of one hectare. Farmers stock PL continuously throughout the year but primely during May to June when PLs are available. The average feeding rate found in the study areas was 4306.58 kg/ha. This feeding rate is extensively varied with 9750 kgha-1 in gher farming system of prawn reported by Ahmed et al., (2008b). Various types of feed are used in the study area but most farmers are found to use homemade feed e.g. cooked rice, rice bran, oil cake; which is different from Barmon et al., (2006) and Ahmed et al., (2008b) who reported snail-meat used commonly in prawn farming system. The average production of prawn was found to be about 439.79±90.46 kgha-1. Survival rate differs from gher to gher, and the average survival rate (%) was estimated as 68.23±12.26. The average price of prawn was recorded as US$ 7.86 per kg.

In pre-stocking management in Bagerhat, Bangladesh, a great portion of farmers mainly in extensive and improved extensive farming strategies not perform all of the pond preparation measures although major portion of farmers use lime but the lowest liming was at 24.67 kg/ha/year in extensive and at 143.33 kg/ha/year in semi-intensive culture strategy but according to Hasanuzzaman et al., (2011), sometimes farmers were reported to use lime at the rate of 105.68 kg/ha/year and in Avoynagar upazilla, Bangladesh, it was at 232.18 kg/ha/year (Samad et al., 2014). In the study it was found that, 94% (highest) of farmers practiced

bottom tilling for paddy cultivation in integrated culture (rice-prawn and/or rice-fish-prawn) and 28.57% (lowest) in mixed culture (fish-prawn) belonging to improved extensive culture strategy, although tilling helps to mix the lime and fertilizer with the soil during pond preparation and thus increases the productivity of land by increasing the natural food (FAO, 2002).

Among the responded farmers, fear of disease was existed associated with the use of organic fertilizers. As a result they limited the use of organic fertilizers; it was the similar finding to Wahab et al., (2011). This was found that, in case of mixed culture belonging to semi-intensive culture, average application of organic fertilizer at the rate of 18.82 kg/ha/year (lowest) and in integrated culture belonging to the extensive culture strategy it was at 71.60 kg/ha/year (highest); here is a dissimilarity with the findings of Ahmed (2001) and Hasanuzzaman et al., (2011) when the application of organic fertilizers were respectively at the rate of 1,500 kg/ha/year and 410.22 kg/ha/year but according to Ahmed (2013) it was at 100-150 kg/ha/year. In case of inorganic fertilization, in the present study, average use of inorganic fertilizers was at 208.07 kg/ha/year (highest; emphasizing the paddy culture) in integrated culture occupying the extensive culture strategy and at 83.54 kg/ha/year (lowest) in case of monoculture occupying the improved extensive culture strategy. That is around the rate, stated by Ahmed (2013) and Hasanuzzaman et al., (2011) respectively at 45-85 kg/ha/year and 180.34 kg/ha/year.

Average stocking density of PL (post larvae) varies in extensive, improved extensive and semi-intensive culture strategies respectively at 9,609 PL/ha, 11,502 PL/ha and 22,847 PL/ha in the study area, which is also in line with a density of 1,500-15,000 PL/ha reported by Alam et al., (2007), and a density of 7,411-39,520 PL/ha reported by Barmon and Karim (2007). According to Ahmed et al., (2008a) prawn farmers practiced a stocking density of 19,830-21,155 PL/ha. The average stocking densities were found to be 30,000/ha (2.5 PL/m^2) in semi-intensive farming, 20,000 (2.0 PL/m^2) in improved extensive and 15,000 (1.5 PL/m^2) in extensive systems (Ahmed, 2013). In the three culture strategies, 5.71%, 48.57% and 71.43% farmers perform the acclimatization consciously, correspondingly in extensive, improved extensive and semi-intensive culture strategies, where the assumed survival rate was correspondingly <55%, <60% and <60%, it is closely similar to the survival rate 50 to 60% that was found by Ahmed et al., (2008a,b). Stocking period of PL ranges from 6-10 months in study area and the average production was lowest in extensive culture, that was 193 kg/ha/year and in that order 284 kg and 488 kg/ha/year in improved extensive and semi-

intensive culture strategies but according to (DoF, 2010), the yield ranges from 400-500 kg/ha/year.

2.5 Disease of *M. rosenbergii*

2.5.1 Disease occurs in natural environment

Viral diseases such as *Macrobrachium* hepatopancreatic parvovirus disease and white tail disease, rickettsia like disease or idiopathic diseases such as idiopathic muscle necrosis, balloon disease, appendage deformity syndrome, exuvia entrapment or moult entrapment disease and mid cycle disease are reported in freshwater prawn (Lacroix et al., 1994).

The known diseases list and clinical signs are listed in Table 2.2.

Table 2.2. Disease occurs in wild *M. rosenbergii* (Lombardi et al., 1996).

Disease	Symptoms	Causative agents
Idiopathic muscle necrosis	Whitish colour of muscular tissues becoming necrotic with reddish appearance; affects all life stages.	*Aeromonas* spp., *Pseudomonas* spp. and *Vibrio* spp.
Balloon disease	Swelling of the branchiostegal region; hypertrophy of some gill filaments.	*Enterococcus* spp.
Mid cycle disease	Affects larvae which exhibit lethargy, reduced feeding and growth; spiralling swimming.	*Alcaligenes* spp. and *Enterobacter* spp.
Exuvia entrapment disease	Presence of localized deformities, and failure to complete moulting. Quite all stages, and particularly late larval stages.	Nutritional deficiencies
Moult Death syndrome	Mortality occur just after moulting	Virus, bacteria etc

Appendage deformity syndrome	Deformities (rostrum, antennae, legs, etc.) and mortalities.	*Vibrio* spp.

2.5.2 Disease occurs in Hatchery

The known diseases of *M. rosenbergii* larvae in hatchery are caused by bacteria, protozoa and nutritional deficiencies (Table 2.3). All the disease-causing organisms are probably present in the rearing water and only affect larvae when they are stressed due to inadequate feeding, overcrowding and poor water quality. These types of infections are termed 'opportunistic (New and Singholka, 1985).

Table 2.3. Major known diseases occur in Hatchery (Spotte, 1979).

Disease	Symptoms	Causative agent
Mid-cycle Larvae Disease (MCD)	Spiral swimming behaviour and poor feed consumption. The larvae assume a bluish-grey coloration.	The cause of the disease is not known, but it is infectious and has an incubation period of about five days.
Bacterial Necrosis (BN)	Larvae affected by this disease turn bluish and stop feeding. The intestinal tract will be found to be empty. Small black spots and lesions can also be seen on the exoskeleton. Stage 4 or 5 larvae may suffer 100 per cent mortality, but older larvae and PL seem to be resistant.	Several opportunistic bacteria
Exuvia Entrapment Disease (EED)	EED affects Stage XI larvae and early post-larvae. Infected larvae are unable to extricate themselves from their exuvia during moulting.	Nutritional deficiencies

	The larvae generally have malformed appendages. Mortality usually ranges from 20-30 percent.	
Microscopic Epibiont Diseases (MED)	A variety of protozoa may be found attached to the exoskeleton of larvae. Some species may attack the eggs of broodstock. Others interfere with the feeding and moulting of larvae.	Several Protozoans
White tail disease	Lethargy, anorexia and opaqueness of abdominal muscle in post-larvae and adults. This opaqueness gradually expands on both sides (anterior and posterior) and leads to degeneration of telson and uropods in severe cases. Some infected animals without uropods have been observed. Whitish appearance of the tail is prominent. The mortality recorded is 100% within two or three days after the appearance of the clinical sign of opaqueness	Fungal and bacterial attack

Dugan and Frakes (1973) and Dugan et al., (1975) found black spot as the most prevalent disease of adult *Macrobrachium*, which included mechanical damage to the exoskeleton, attack by chitin classic bacteria, and finally invasion by a lethal freshwater phycomycetous fungus.

Sandifer and Smith (1975) described bacterial infections of gills, pleopods and uropods of juvenile *M. rosenbergii.*

Fujimura (1966) described a condition in *M. rosenbergii* larvae characterized by small opaque white patches beginning at the base of appendages and then spreading throughout the

entire body, producing sporadic heavy larval mortalities. Ling (1969) considered this to be a systemic fungus infection but no description of the causative organism is available.

Smith et al., (1974) reported quite a different fungus infestation of larvae, apparently associated with accumulation of decomposing food in the tanks. The fungus grew on the anterior appendages and tails of larvae, and caused some mortality.

Several reports showed that histopathological changes in giant freshwater prawns were found in several organs including gills, hepatopancreas and heart (Fontaine and Lightner, 1974; Smith et al., 1984; Sung and Song, 1996). The tissue exhibited focal necrosis, haemocytes infiltration, hyperplasia. Muscular damage and mild or massive haemocyte reaction occurred at 1-6 hours after injection (Jiravanichpaisal et al., 2009).

SamCookiyaei et al., (2012) demonstrated that total haemocyte counts (THC) did not significantly increase once *M. rosenbergii* injected with 1×10^6 CFU/50µl of *A. hydrophila* concentration. Vazquez et al., (1997) also did not observe THC rising in different groups through time; Vazquez et al., (1997) also found 70% fusiform or hyaline haemocytes, 20% granular haemocytes and 10% are granular haemocytes in the inter moult *M. rosenbergii*.

2.5.3 Disease occurs in grow-out system

Diseases in freshwater prawn ponds are relatively unusual, compared to other forms of aquaculture. Furthermore, diseases have been known to occur in freshwater prawn grow-out when the quality of the water (either of the intake or within the pond itself) is poor (Johnson and Bueno, 2000). All life stages of freshwater prawns in grow-out system are also subject to a disease known as muscle necrosis. Affected prawns show a whitish colour in the striated muscle of the tail and appendages. The necrotic areas may increase in size and become reddish, a colour similar to cooked prawns, due to the decomposition of the muscle tissue. Secondary pathogens (bacteria and the fungus *Fusarium*) have been found to be associated with muscle necrosis. Prawns suffering from chronic muscle necrosis do not survive. Population mortality rates vary from insignificant to 100%. This disease is associated with poor management and occurs particularly when stocking rates and handling stress are high and when environmental conditions are poor (low dissolved oxygen level; temperature fluctuations; and, in the hatchery, salinity fluctuations). Parasites seem to be quite rare in cultured *M. rosenbergii*. Freshwater prawns have been found to be hosts for the bopyrid isopod *Probopyrus*. These attach themselves to the interior of the gill chamber, usually

resulting in a visible swelling. This would normally only is a problem if it became common in a captive broodstock, because it is reported to interfere with egg production. The only other problem that might occur if this parasite became common in grow-out would be its effect on the appearance of prawns sold head-on. The general body surface of the prawn can serve as a substrate for filamentous bacteria and algae, and single or colonial protozoa (Johnson and Bueno, 2000). Moulting temporarily frees prawns from these fouling micro-organisms. The problem is particularly noticeable in large animals, especially blue-claw (BC) males, which moult less often. Although these organisms do not invade the tissues they make it difficult for the prawns to move and to feed, particularly in the larval and post larval phases. Extreme infestation on the gills can impair their function, and may cause mortalities in juvenile or adult prawns. Heavy infestation over the exterior surface can also reduce the market value of prawns. Infestation by filamentous algae has been observed to occur in grow-out ponds with high transparency (above 40 cm).

2.6 Immunity system of freshwater prawn

In invertebrates, the physical barriers are the first obstacle to detain pathogenic micro-organisms (Soderhall, 1982). *M. rosenbergii*, like other crustaceans, possesses an innate immune system which provides defense against pathogenic agents and consisting of cellular and humoral components which work individually or cooperatively to protect the species from invading pathogens such as *V. parahaemolyticus* (Young et al., 2002; Lee et al., 2006). An enormous number of innate immune-related genes such as lectins, antimicrobial peptides, prophenoloxidase and manganese superoxide dismutase (Pan et al., 2005; Zhao et al., 2007; Leu et al., 2011) have been detected. When foreign material is recognized, different immune mechanisms are activated, such as the prophenoloxidase (proPO) system, phagocytosis, and encapsulation processes. Freshwater prawns possess an open circulatory system, where nutrients, oxygen, hormones, and cells are distributed in the hemolymph. The most important role of the circulation haemocytes of prawns is the protection of this species against invading microorganisms by participating in recognition, phagocytosis and melanization (Tzou et al., 2002).

The effector mechanisms for prawns immune responses include the coagulation cascade, which avoids the loss of hemolymph and stimulates oxidative metabolites and production of melanin by activating the proPOsystem (Kawabata et al., 1996; Vargas-Albores and Yepiz-Plascencia, 2000; Sritunyalucksana and Soderhall, 2000). Prophenoloxidase activation

stimulates other important processes in the immune response, such as phagocytosis, encapsulation, and nodule formation (Table 2.4) (Soderhall, 1990; Smith et al., 2001). Activation of such processes seems to be mediated through the specific recognition of glycosylated pathogen-associated molecular patterns (PAMPs) by prawns proteins.

Freshwater prawns possess an open circulatory system, where nutrients, oxygen, hormones, and cells are distributed in the hemolymph. The most important role of the circulation haemocytes of prawns is the protection of this species against invading microorganisms by participating in recognition, phagocytosis and melanization (Tzou et al., 2002).

Table 2.4. Effector defense mechanisms in giant freshwater prawn (Soderhall, 1990; Smith et al., 2001).

Defense mechanisms	Cellular population involved	Target pathogen
ProPO	Semigranulocytes, haemocytes with big refractile granules	Bacteria and Fungi
Antimicrobial proteins	Haemocytes with granules	Bacteria and Fungi
Phagocytosis	Hyalinocytes, semigranulocytes	Bacteria and micro-organisms<10 mm
Encapsulation	Semigranulocytes haemocytes with big refractile granules	Fungal spore and yeast organisms>10 mm
Lectins	Hyalinocytes, semigranulocytes and haemocytes with refractile granules	Distinguish between the self and non-self particles, inducing agglutination and phagocytosis
Clottable protein	Clottable proteins from haemocytes	Bacteria and Fungi

M. rosenbergii possesses some proteins that participate in immune defense by specific recognition of carbohydrate containing molecules, i.e. glycans, glycolipids, glycoproteins, peptidoglycans, or lipopolysaccharides from Gram-negative and Gram-positive bacteria, viruses, or fungi (Vazquez et al., 1997).

Moreover A 9.5-kDa lectin, specific for N- and O-acetylated sugar residues, has been identified in the hemolymph of the freshwater prawn, *M. rosenbergii*; the lectin of the *M. rosenbergii* prawn recognizes O-acetyl groups of carbohydrates in the cell wall of bacteria, such as *Aeromonas* spp. and *Bacillus cereus* (Vazquez et al., 1994)

M. rosenbergii possesses some unigenes (arginine kinase 1, anti-lipopolysaccharide factor, inhibitor of apoptosis protein, cascade, heat shock protein 21, lectin 1, Serine proteinase inhibitor, Alpha-2-macroglobulin, Transglutaminase, beta-1,3-glucan binding protein, Tachylectin, Crustacyanin-like lipocalin, Ubiquitin-conjugating enzyme and NF-kappa B inhibitor alpha), (Reiner et al., 2003) that plays role in their immunity system. Many of the aberrantly expressed genes found in the *V. parahaemolyticus* infected groups compared to the control group are known to belong to various processes clustered under the animal immune system. These immune genes are grouped into 11 functions, including antimicrobial proteins, proteases and proteinases, signal transduction, blood clotting system, cell death, cytoskeletal, heat shock proteins, oxidative stress, pathogen recognition immune receptors, prophenoloxidase system and other immune genes (Schmittgen et al., 2008).

In *M. rosenbergii*, phagocytosis involves a double mechanism for foreign material recognition: one of these mechanisms is determined by lectin-specificity for O- and N-acetylated carbohydrates and the second mechanism seems to be unspecific, although both allow for the phagocytosis of pathogen agents (Kondo et al., 1992; Vazquez et al.,1997).

In addition, experiments carried out with larvae of the *M. rosenbergii* demonstrated that larvae fed on diets containing inactive *Vibrio* spp. bacteria have a greater resistance to bacterial infection than the control group not previously fed with the bacterium (Bechteler and Holler, 1995). In vitro studies of phagocytic activity in crustaceans have been evaluated by flow cytometry analysis in the prawn, *M. rosenbergii* (Lee et al., 2001; Sierra et al., 2001; Soria et al., 2006) that showed the production of reactive oxygen in its haemocytes.

The haemocytes of *M. rosenbergii*, which participate in the encapsulating process, show acid or neutral mucopolysaccharides and glycoproteins (Hall et al., 1999).

Iwanaga and Lee, (2005) reported clottable proteins in *M. rosenbergii*; these molecules favor recognition and neutralization of non-self cells or particles.

The *Macrobrachium* species consists of molecules related with antimicrobial activity against Gram-positive bacteria and fungi (Destoumieux et al., 1997; Destoumieux et al., 1999; Destoumieux et al., 2000). These proteins are stored in prawns granulocytes and are released in response to bacterial stimuli.

Chapter 3

Materials and Methods

3. Materials and Methods

3.1 Prawn Post larvae collection and rearing

For conducting this study, two types of prawns (hatchery and wild) were collected. Hatchery prawn post larvae (PL) were collected from BFRI, Bagerhat and wild prawns PL from Pashur River, Dacope, Khulna. These PLs were reared separately for 60 days in the experimental pond complex of FMRT Discipline, Khulna University, Khulna. Then the experiment was conducted in the Laboratories of Fisheries and Marine Resource Technology (FMRT) Discipline, Khulna University.

3.2 Experimental design

The experiment was conducted by challenging the test *M. rosenbergii* against *Vibrio* spp. following the given experimental design (Table 3.1).

Table 3.1. Experimental design

Control		Treatment	
Wild PL	**Hatchery PL**	**Wild PL+ Pathogen (*Vibrio* spp.)**	**Hatchery PL+ Pathogen (*Vibrio* spp.)**
C_WR_1	C_HR_1	T_WR_1	T_HR_1
C_WR_2	C_HR_2	T_WR_2	T_HR_2
C_WR_3	C_HR_3	T_WR_3	T_HR_3
C_WR_4	C_HR_4	T_WR_4	T_HR_4

C_W=Control wild; C_H=Control hatchery; T_W=Treatment wild; T_H=Treatment hatchery; R=Replicate

3.3 Media preparation

10g/l of alkaline Peptone water, 5g/l of yeast extract and 10g/l of NaCl were added with required amount of distilled water then heat and pressure were used for the preparation of culture media.

3.4 Bacteria culture

3.4.1 Alkaline Peptone Water (APW)

4.5 grams of Alkaline peptone water were suspended in 225 ml distilled water and slightly heated to dissolve completely and sterilized by autoclaving at 15lb pressure at 20 minutes.

3.4.2 Thio-sulphate Citrate Bile Salts Sucrose Agar (TCBSA)

4.45 grams of TCBS agar were boiled first with 50 ml of distilled water to dissolve and then boiled for 1 minute. Then the media was dried to 60°C for 15 minutes and was poured into petri dishes and allowed to solidify.

3.4.3 Procedure

Before conducting the challenge test, the pathogenic bacteria (i.e., *Vibrio* spp.) were isolated from diseased prawns which displayed typical symptoms of inactivity, black spot on the carapace, broken antennae and antennule (Figure 3.1). All the diseased GFP were collected from the farmer's pond of Manikkhali, under the Upazilla Mollahat, District Bagerhat in Bangladesh. Diseased prawns were brought to the Fish Pathology Laboratory of FMRT Discipline, Khulna University. The diseased prawns were dissected, and then the gut and intestinal tract were pooled for the isolation and culture of target pathogenic bacteria. Approximately 1 g pooled tissue was homogenized with 1 ml distilled water and kept in a 2 ml Eppendorf tube. To avoid cross contamination, separate surgical blades, dissection tray, and autoclaved dissecting instruments were used for each of the individual. All samples were properly labeled and stored at −4°C temperature. Then 25 g of the sample was added with approximately 225 ml of alkaline peptone water in a "Warring" blender flask. Then the sample containing alkaline peptone water was blended for 1 min. Then the sample was incubated at 37 ± 1° C for 6-8 hrs. Thio-sulphate Citrate Bile Salt Sucrose Agar (TCBSA) was used for selective isolation of *Vibrio* spp. (Pfeffer and Oliver, 2003; Nakayama et al., 2005). At the end of the incubation period, a loopful of the alkaline peptone water was streaked onto Thio-sulphate Citrate Bile Salt Sucrose (TCBS) agar plates. The plates were then incubated at 37°C ± 1°C for 24 hrs. At the end of incubation, plates were checked for the characteristic colony of *Vibrio* spp. On TCBS agar colonies appearing as large (2-3 mm), smooth, green and slightly flattened were selected.

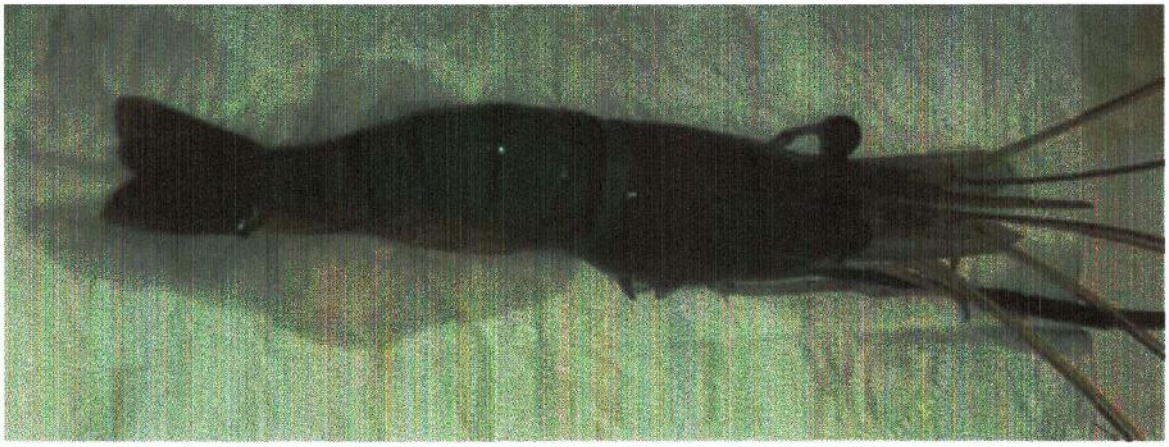


Figure 3.1. Diseased prawn collected for isolation of bacteria

3.4.4 Enrichment of pathogen and preservation

A pure culture of collected bacteria was done at Fisheries Microbiology and quality control laboratory of FMRT Discipline, Khulna University. Stock solutions of bacteria were prepared by adding autoclaved glycerol (80%) to the bacteria culture at equal volume, vortexed and subsequently stored at -80°C to use for long time. Before each experiment, fresh bacteria cultures were done by adding 1% bacterial stock solution (V/V) to autoclaved LB_{10} medium and incubated for 24 hours at 30°C on a shaker under constant agitation.

3.5 Pathogen challenge test

The pathogen challenge test was conducted for determining survival of both wild and hatchery produced prawns juvenile in tetra replicate by an inoculation of 2×10^9cfu/ml *Vibrio* spp. into the water of the tank; 2×10^9cfu/ml *V. parahaemolyticus* induced diseases experimentally in prawns (Khuntia et al., 2008). Prawns were feed (at 5% of their body weight) two times daily with a formulated grow-out prawn diet. Survival was checked daily. During the 7 day challenge test period, the survival of prawn was recorded by the following formula:

Survival rate (%) = $Ni - Nf / ((Ni)) \times 100$

Ni = Initial number of animals.

Nf = Final number of animals.

3.6 Haemocyte count

3.6.1 Haemolymph collection

Haemolymph (100 µl) was collected from the ventral sinus of the cephalothorax, beneath the first abdominal segment using a 1 ml sterile syringe (25-gauge, 0.45 mm) containing 0.9 ml of an anticoagulant solution (0.114 M trisodium citrate and 0.1 M sodium chloride, at pH 7.45 and with an osmolality of 490 mOsm/kg).

3.6.2 Total Haemocyte count

The total haemocyte numbers per ml (THC) were determined following the procedures of Cheng et al., (2006) using a Neubauer hemocytometer chamber (Model: ART-1280, China). A drop of the haemolymph suspension was placed on a haemocytometer to measure the THC using a trinocular research microscope (Labomed, Model: iVu-1500, Lx-400, USA). THC was measured according to the following formula:

$$\text{Total Haemocyte count/cubic millimeter} = \frac{(\text{cell counted}) \times (\text{blood dilution}) \times (\text{Chamber volume})}{\text{Area of Chamber Counted}}$$

3.6.2.1 Cell counting using Haemocytometer

Total haemocyte counts (THC) were performed with an improved Neubauer haemocytometer using a modification of the method described by Bain and Bates (2001) for analysis of blood samples. First, a special cover slip was taken on the improved Neubauer chamber. The WBC pipette containing Giemsa stained 0.5 ml sample were mixed well with 0.90 ml anti-coagulant and ½ drops were discarded outside first, then 1 drop sample was taken in the edge of the special cover slip and allowed to run under the cover slip throughout the Neubauer chamber. When it dispersed over the chamber, the haemocyte cells were visualized under light microscope. The Neubauer haemocytometer had four large corners of 16 squares. So it possess a total 4×16=64 squares (volume of each large corner square=1mm×1mm×0.1mm=0.1cm×0.1cm×0.01cm =0.0001cm^3 or 10^{-4} cm^3). To calculate total haemocyte count (THC) of haemolymph, the number of haemocyte cell found in these 64 square was multiplied with 50 (as per calculating formula) and that was the total number of haemocyte in per cubic millimeter.

3.6.2.2 Sequential procedure of Total Haemocyte count

1. The cover-slip and haemocytometer were ensured as grease-free and clean (alcohol used to clean).
2. A well-mixed blood cell (with anticoagulant) suspension and equal volumes of Giemsa stain were mixed (not too vigorous) e.g. mix 100µl Giemsa stain with 100µl cell suspension.
3. Giemsa stained blood cell mixture was pipetted (approximately 10µl) at the edge of the cover-slip and allowed to run under the cover slip.
4. The haemocytometer grid was visualized under the microscope, where the cells within the square and cells touching middle line of top and left were counted but the cell touching middle line of bottom and right sides were excluded.
5. Counting around 40 to 70 cells to obtain an accurate cell count - therefore counting more than one large corner square.

3.6.3 Differential Haemocyte count

Differential haemocyte counts were performed following the methods of Celi et al., (2013). Briefly, a drop of haemocytes cell suspension was smeared on a slide to prepare a monolayer and allowed to air dry, and then, the monolayer was fixed with few drops of absolute methanol and inundated for 6 min. After that, the methanol was removed keeping the slide in inclined position for a few seconds. Then, the haemocyte monolayer was stained with Giemsa solution (1:10 dilution for 10 min) and dehydrated with 70% ethanol (1 min) and immersed in xylene (6 min) (Celi et al., 2013). Then, the cells were observed under the microscope. Cell types were identified using the simple morphological criteria reported in Brehelin et al., (1989). At least 200 cells on each slide were counted in random areas, and numbers and relative proportions of haemocyte types, that is, the differential haemocyte count (DHC), were calculated by using the following equation (Vazzana et al., 2015):

DHC (%) = {(Number of different haemocyte cell types) / (Total haemocyte cell counted)} × 100

For each slide, an examination of a complete head-to-tail subsection of the smear was performed. Haemocytes were differentiated based on morphology and metachromatic staining of cytoplasm, cytoplasmic granules and nuclear chromatin (Hrubec and Smith, 2000; Campbell and Ellis, 2007) and the relative proportions of each cell type expressed as a percentage of the total haemocytes counted. Thus the prawn haemocyte differential counts were presented as non-granular haemocyte NGH), Large granular haemocyte (LGH), Small granular haemocyte (SmGH), Semi granular haemocyte (SGH).

3.6.3.1 Cell counting from slide

The slide is fixed well on the mechanical stage of the BM-180 Microscope, Boeco, Germany. The SP4/0.10 (160/0.17) LED has an illumination system using LED light to acquire clear images. The slide is placed before a 10X lens for adjustment of the cell image. Then it was observed in 40X lens. The light is adjusted as per requirement of the image clearness. The cells are counted and placed the data in the DLC (Differential Count Chart) for further analysis.

3.6.3.2 Determining Blood dilution

Since, 0.10ml (100 µL) blood was mixed with preloaded 0.90ml (900 µL) anticoagulants and made 1ml of sample. So, the dilution of blood sample was 1 in 10.

3.6.4 Determining chamber volume

Determining volume of each large corner square=1mm×1mm×0.1mm=1mm×1mm×$\frac{1}{10}$mm=

$\frac{1}{10}(10 \times 10 \times 1)\ mm^3 = \frac{1}{10}(100)\ mm^3 = \frac{1}{10} \times 100\ mm^3 = 10\ mm^3$}

$$THC/ml = \frac{(\text{cell counted}) \times (\text{chamber volume}) \times (\text{blood dilution factor})}{\text{chamber area}}$$

3.7 Statistical analyses

All types of statistical analysis were carried out with both Microsoft office Excel (version 2007) and SPSS (version 20). Generalized linear model was used to determine the survival rate difference, total and differential haemocyte count data.

Chapter 4

Result

4. Result

4.1 Physical appearance of prawns in the experiment

At the beginning of the challenge test, all the prawns were very healthy and fresh but after 2 days, some abnormal symptoms were observed in treatment groups compared to control groups, and gradually the abnormal symptoms were prominently evident (Table 4.1, 4.2; Figure 4.1, 4.3).

Table 4.1. Physical appearance of wild prawns in the experiment.

Characteristics	Control groups	Treatment groups
Movement	Normal	Slow and gremlin on day 1
Body color	Natural	Slightly fade on day 2
Shell condition	Normal	Small reddish pore and lesion on the shell on day 3
Tail	Normal	Small reddish pore, lesion and breakdown of telson on day 3
Appendages	Normal	Loosen and breakdown of appendages on day 4
Carapace	Normal	Small black spot on the carapace on day 3
Mortality	No occurrence of mortality	Occurrence of mortality on day 1

Figure 4.1. Disease symptoms in wild prawn

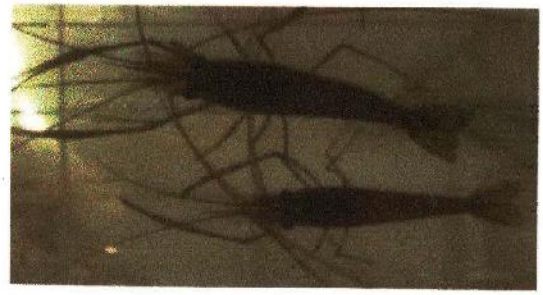


Figure 4.2. Healthy wild prawn

Table 4.2. Physical appearance of hatchery prawns in the experiment.

Characteristics	Control groups	Treatment groups
Movement	Normal	Slow and gremlin on day 2
Body color	Natural	Slightly fade on day 3
Shell condition	Normal	Small reddish pore and lesion on the shell on day 3
Tail	Normal	Small reddish pore, lesion and breakdown of telson on day 3
Appendages	Normal	Loosen and breakdown of appendages on day 4
Carapace	Normal	Fade carapaces and large black spot on the carapace on day 3
Mortality	No occurrence of mortality	Occurrence of mortality on day 4

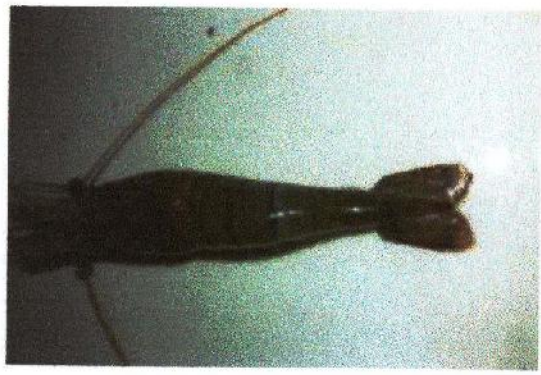


Figure 4.3. Disease symptoms in hatchery prawn

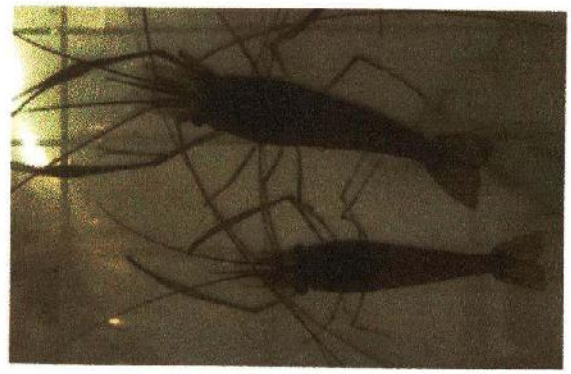


Figure 4.4. Healthy hatchery prawn

4.2 Mortality of prawns in the experiment

There was no mortality found in the control group during the 7 days challenge test period. First mortality occurred on day 1 in the treatment groups of wild prawns and the mortality rate was 20% (Table 4.3). On the other hand, first mortality occurred in the treatment groups of hatchery prawns on day 4 and the mortality percentage was 30 %. From the experiment it was observed that on day 5 higher mortality (75%) occurred in the wild treatment groups whereas higher mortality (77.77%) occurred on day 7 in the hatchery treatment groups. 25% cumulative mortality rate (CMR) was found in both wild and hatchery treatment groups on day 5 and more than 50% CMR was on day 7 in both hatchery (65%) and wild (60%) treatment groups (Figure 4.5).

Table 4.3. Mortality (%) of experimental *M. rosenbergii* juveniles.

Day after challenge	Mortality (%)			
	Wild juvenile		Hatchery juvenile	
	Control[a]	Treatment[b]	Control[a]	Treatment[c]
Day 0[a]	0	0	0	0
Day 1[b]	0	20	0	0
Day 2[a]	0	0	0	0
Day 3[a]	0	0	0	0
Day 4[c]	0	20	0	30
Day 5[d]	0	75	0	28.57
Day 6[e]	0	60	0	20
Day 7[f]	0	33.33	0	77.77

Note. Different superscript letters in the same row and column represent significant difference among the treatments (Generalized linear model, $p < 0.05$).

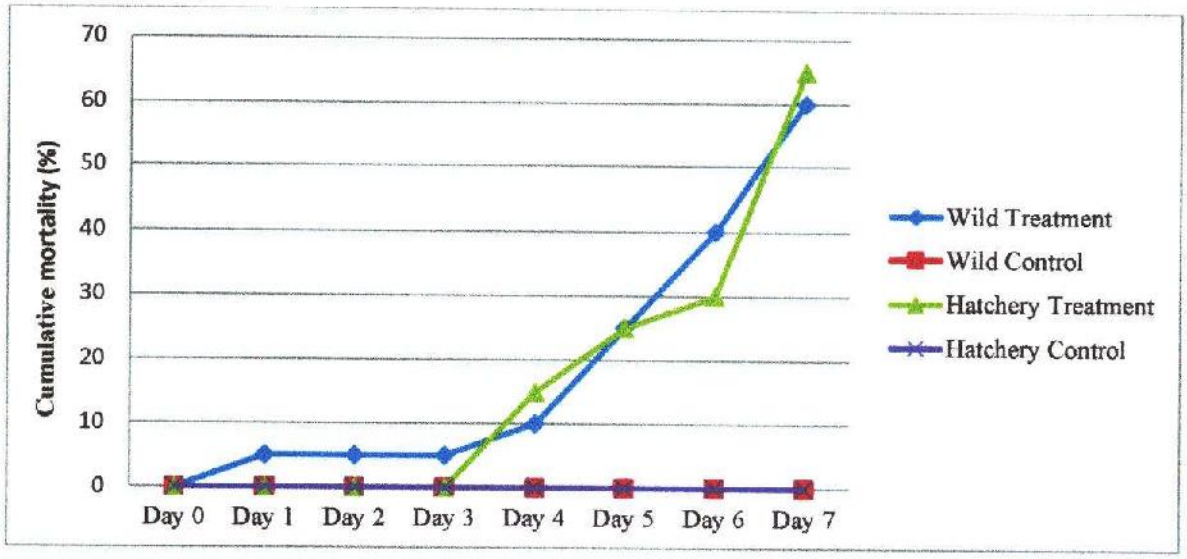


Figure 4.5. Cumulative mortality rate of wild- and hatchery- produced *M. rosenbergii* juvenile during 7 days challenged with *Vibrio* spp.

4.3 Effect of *Vibrio* spp. concentration on prawn mortality

The test model effect demonstrated that concentration (2×10^{9}CFU/ml) of *Vibrio* spp. used in this study have effect on prawns mortality ($P<0.05$). The exposure of prawn juveniles against *Vibrio* spp. with a higher concentration and long duration without considering them might be responsible for their higher mortality.

4.4 Percentage of THC and DHC before challenge test

Four different types of haemocytes were distinguished from differential haemocyte count (DHC) of *M. rosenbergii*, namely; non-granular haemocytes (NGH), semi-granular haemocytes (SGH), large granular haemocytes (LGH) and smaller granular haemocytes (SmGH). Before challenged test the following haemocyte profile was counted in the experiment (Table 4.4).

Table 4.4. THC (cell/ml) and DHC (%) before challenge test.

Tank		THC/ml	DHC			
			Large granular	Semi granular	Smaller granular	Non granular
Hatchery	H_1	3.3×10^5	14.8±4.48	11±2.30	26.2±5.34	48±6.76
	H_2	3.1×10^5	10.3±0.17	4.5±0.28	33.2±5.10	52±3.52
Wild	W_1	3.8×10^5	15.7±4.15	4.77±0.35	24.66±3.08	54.87±7.23
	W_2	3.0×10^5	13.77±0.71	9.44±0.32	21.33±2.14	55.46±7.37

4.5 Total haemocyte count (THC) during challenge test

After inoculating pathogenic species *Vibrio* at a concentration of 2×10^9 CFU/ml, THC was counted and THC data revealed a significant difference ($P<0.05$) between the control and treatment groups. The highest percentage of THC in the wild control group (4.36/ml) was found on day 4 while it was 8.55/ml in wild treatment groups on day 7. On the other hand, the highest percentage of THC in control group of hatchery (5.41/ml) was found on day 6, and at the same day the highest percentage of THC (8.75/ml) was also found in hatchery treatment group (Figure 4.6). The lowest THC in the wild treatment group (4.69/ml) was found on day 1 and in the wild control group (2.13/ml) on day 5. In contrast the lowest THC in both hatchery control groups (2.09/ml) and hatchery treatment groups (5.33/ml) was found on day 1.

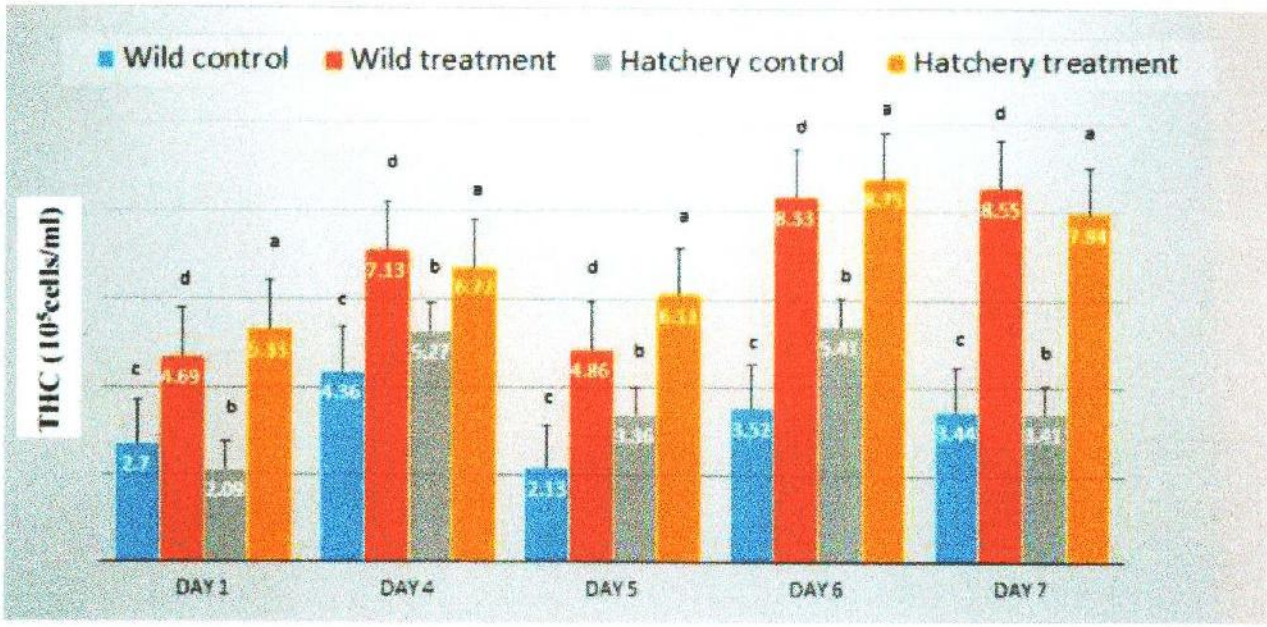


Figure 4.6. THC% in prawns in the experiment

4.6 Differential haemocyte count (DHC) during challenge test

DHC data revealed that there was a significant difference ($P<0.05$) of large granular cell among the control groups of hatchery, treatment groups of hatchery and control groups of wild species but no significant difference ($P<0.05$) was found in the treatment groups of wild species. On the other hand a significant difference ($P<0.05$) was found among control groups of hatchery, treatment groups of hatchery and control groups of wild species for SGH, SmGH and NGH but no significant difference ($P<0.05$) was found in SGH, SmGH and NGH in wild treatment groups. Semi granular cells (SGC) were found to be higher in *Vibrio* treated group (11.25%) in hatchery species on day 1 compared to control and other treatment groups in hatchery species. Smaller granular haemocyte (SmGH) was found to be higher at hatchery control group (32.22 %) on day 1 but is was similar both control groups (27.77 %) of wild species on day 7 and treatment group (27.77 %) of hatchery species on day 1 rather than in other control and treatment groups in wild and hatchery species except control group of hatchery on day 4. Similarly semi-granular haemocyte (SGH) was found to be higher at wild control group (18.06 %) on day 6 and found to be lowest at hatchery treatment group (6.7 %) on day 6 (Table 4.5). Large granular (LG) haemocyte was found to be higher on day 1 in wild treatment group (19.7 %), on the other hand the lowest number of LG cells was found on day 7 in wild treatment group (7.78 %).

Table 4.5. Percentage of DHC (Avg±SD) of prawns during the test period.

Day	Wild								Hatchery							
	Control				Treatment				Control				Treatment			
Day	LG	SG	SmG	NG	LG	SG	SmG	NG	LG	SG	SmG	NG	LG	SG	SmG	NG
1[abcdej]	17.3 ± 1.85[d]	14.7 ± 0.37[b]	27 ± 2.40[b]	41 ± 6.17[b]	19.7 ± 3.48[d]	17 ± 3.75[abc]	24.66 ± 3.08[abc]	38.64 ± 8.24[abc]	12 ± 1.15[a]	9.26 ± 1.72[a]	32.22 ± 5.59[a]	46.52 ± 6.96[a]	18 ± 3.52[c]	11.25 ± 0.72[c]	27.77 ± 4.49[c]	42.98 ± 5.24[c]
Day 4[abfk]	16.6 ± 2.57[d]	12.24 ± 1.59[b]	23 ± 2.02[b]	48.16 ± 3.49[b]	17.1 ± 1.67[d]	12 ± 1.15[abc]	21.22 ± 2.74[abc]	49.68 ± 6.66[abc]	11 ± 1.76[a]	8.12 ± 1.27[a]	27.77 ± 4.17[a]	53.11 ± 10.15[a]	12.4 ± 5.41[c]	9.4 ± 0.34[c]	21.2 ± 2.21[c]	57 ± 7.79[c]
Day 5[acghil]	13.3 ± 1.40[d]	9.7 ± 0.17[b]	23.3 ± 1.93[b]	53.7 ± 5.43[b]	11 ± 0.57[d]	8 ± 2.02[abc]	23 ± 1.33[abc]	58 ± 13.48[abc]	19 ± 0.57[a]	8.22 ± 1.95[a]	21.22 ± 2.74[a]	51.56 ± 3.45[a]	12 ± 1.15[c]	11.2 ± 2.41[c]	18 ± 1.15[c]	58.8 ± 8.38[c]
Day 6[adghim]	11.6 ± 2.20[d]	18.06 ± 3.51[b]	21.34 ± 2.11[b]	49 ± 10.01[b]	8.77 ± 0.71[d]	11 ± 2.51[abc]	21.23 ± 2.74[abc]	59 ± 6.17[abc]	17 ± 3.75[a]	7.11 ± 1.21[a]	25.33 ± 4.28[a]	50.56 ± 3.34[a]	13 ± 1.15[c]	6.7 ± 0.49[c]	19 ± 3.48[c]	61.3 ± 9.32[c]
Day 7[aeghin]	13 ± 1.15[d]	11.5 ± 2.61[b]	27.77 ± 4.49[b]	47.73 ± 3.58[b]	7.78 ± 1.28[d]	14 ± 0.66[abc]	19.12 ± 0.50[abc]	59.1 ± 6.22[abc]	13 ± 2.02[a]	10.2 ± 0.11[a]	27.03 ± 4.60[a]	49.77 ± 6.66[a]	11 ± 0.57[c]	6.33 ± 0.76[c]	21 ± 2.51[c]	61.67 ± 12.05[c]

Note. Different superscript letters in the same row (Cell number) and column (day effect) represent significant difference among the treatments (Generalized linear model, $p < 0.05$).

Chapter 5

Discussion

5. Discussion

The present study was attempted to evaluate the immune competence of giant freshwater prawn (*M. rosenbergii*) against pathogenic *Vibrio* spp. This study demonstrated that unchallenged control prawns had no mortality. Similarly, Menasveta et al., (1998) found the higher survival of control *P. monodon* against *V. harveyi* rather than treatment groups. Lower mortality of *M. rosenbergii* against *Vibrio alginolyticus* was observed by Kumar et al., (2013) upon feeding with *Bacillus licheniformis* (1.0×10^9 CFU/g feed).

The Generalized linear models results reported that there was a significant difference of mortality rate ($P<0.05$) between the control and treatment groups of wild and hatchery species.

During 7 days challenge test period all control prawns both in hatchery and wild were very healthy and alive, no disease symptoms or mortality was found. But, in the treatment groups of wild species of *M. rosenbergii* juvenile some abnormal symptoms were observed from day 2, which might be due to the pathogenicity of *Vibrio* spp. In first stage of 7 days challenge test period in wild species, the health condition was not worse but gradually the abnormal symptoms were prominently evident and lastly mortality of all prawn occurred due to their suppressed immunity against *Vibrio*; this last stage of challenge test period might favor the pathogen to proliferate in the prawn body and to breakdown the host defense mechanism because at this stage the juvenile became very infected and they might lose their immunity power.

Similarly abnormal symptoms were observed on day 2 in hatchery treatment groups and first mortality occurred on day 4, in hatchery species, but first mortality occurred on day 1 in wild species it might be due to the variation of immune reactive function of wild and hatchery species. Compared to hatchery-produced prawns, wild prawns have genetically lower immune response; thus *Vibrio* could easily break down the immune barrier of the host, for this reason first mortality occurred in wild prawns very soon rather than in hatchery prawns.

The basic clinical signs of *A. hydrophila* in *M. rosenbergii* were the presence of one to several focal melanin lesions on the outer body surface. Infected prawns showed melanisation at the site of infection and the disease was usually found focally on the gills, carapace, appendages, uropods, telson, or body cuticle, etc. (Te, 1994; Be, 2002); similar observations were found in the present study.

The haemocyte count is a good tool to monitor health status of crustacean immune response (Evans et al., 1997). Haemocytes not only play a central role in crustacean immune defense but also involved in different physiological functions including carbohydrate metabolism, transport and storage of proteins and amino acids, thereby leading to disease resistance (Le Moullac et al., 1998; Sahoo et al., 2007). In this study, the THC was relatively higher in treatment group compared to the control group in both hatchery and wild species; and there was a significant difference ($P<0.05$) between the control and treatment groups. The increase in THC of *Vibrio* treated prawn in the present study indicates the response of the prawn haemocytes to encounter the invading pathogen, which was similar to the findings reported by Fotedar et al., (2013). Sung et al., (2000), who found a significant decrease in THC in *M. rosenbergii* between 4 and 24 hr after injecting with a sublethal dose (1×10^3 cells/g) of *Aeromonas veronii*. A small rise was observed after 24 h in the group that was injected with 1×10^6 CFU/50µl of *A. hydrophila* concentration (Tonguthai, 1992; Esteve et al., 1994) and this result is similar to the present study, showing an increase of THC just after inoculation of *Vibrio* spp. at a concentration of 2×10^9 CFU/ml.

Menasaveta et al., (2000) reported that THC does not vary significantly in *P. monodon* after treated with probiotics. Hematologic assay showed that total haemocyte counts (THC) did not significantly increase at any time in *M. rosenbergii*.

The circulatory haemocytes of crustacean comprises of non-granular cell (NGC), semi-granular cell (SGC), Smaller granular cell (SmGC) and large granular cell (LGC) (Parrinello et al., 2015). In this study, all of the four types of haemocytes were observed in *M. rosenbergii*, where non-granular cells (NGC) comprise the highest proportion of THC that was very similar to the result of Vazquez et al., (1997); Gargioni and Barracco, (1998); Sierra et al., (2001). In addition, Sumon et al., (2016) reported that NGC was increased, while SGC and LGC decreased in *M. rosenbergii* upon treated with *Clostridium butyricum* for 60 days.

The higher number of NGC in treatment groups compared to other control groups indicated that stress might help in increasing the number of this haemocytes; the stress increased the non-granular circulating haemocytes number, which might have took part in lysis of pathogenic *Vibrio* spp. However, exposing *M. rosenbergii* to ammonia stress resulted in no variation in DHC (Cheng and Chen, 2002a).

In contrast, decreased number of SGC and LGC in control groups, compared to other treatment groups, indicate that pathogen-treated animals degranulate spontaneously and

produced more semi-granular cells and the granular phagocytic cells as their normal cellular defensive mechanism (Bachere, 1995) and similar result found in the present study. Also the present study showed that NGH, SGH and SmGH was significantly different ($P<0.05$) in control groups of hatchery, control groups of wild and treatment groups of hatchery species. This might be due to stress increase the THC and DHC in *M. rosenbergii* that act as immune enhancer for *M. rosenbergii* against pathogenic *Vibrio* spp. But no significant difference ($P<0.05$) was found in NGH, SGH and SmGH in wild treatment groups this might be due to ineffectiveness of stress of similar result also reported by Cheng and Chen, 2002a.

When the body cavity of a crustacean is invaded by a large number of microorganisms, some may be removed directly by phagocytosis, while many others are confined to nodules or clumps of cells. In response to the presence of these infected tissues, it has been suggested that some hemocytes may migrate into connective tissue (Factor and Beekman, 1990), the blood sinuses between the hepatopancreatic tubules and the gills that results in increase or variation of DHC in infected prawn (Fontaine and Lightner,1974; Smith et al., 1984; Sung and Song, 1996). In the present study, significant variation ($P<0.05$) of LGH was found among the control groups of hatchery, treatment groups of hatchery and control groups of wild Factor and Beekman, (1990). On the other hand no significant difference ($P<0.05$) was found of LGH in control and treatment groups of wild prawns.

Vazquez et al., (1997) observed fusiform cells 65.4%, large ovoid cells 22.2% and small round cells 12.4% in different groups of experimental prawns. But in the present study it was observed the highest NG cells 61.67%, SmG cells 32.22%, LG cells 19.7 % and SG cells 18.06%.

Overall, In this study, control groups of *M. rosenbergii* showed no mortality but treatment groups showed mortality; increase THC and DHC in treatment groups indicate that pathogenic organism *Vibrio* caused lysis of these haemocyte sub-types and played a significant role in *M. rosenbergii-Vibrio* spp. interaction.

Chapter 6

Conclusion

6. Conclusion

The findings resultant from this study revealed that there was significant difference ($P<0.05$) of mortality between wild- and hatchery- prawns exposed to a concentration of 2×10^9 CFU/ml of *Vibrio* spp. Earlier mortality (20% on day 1) occurred in wild-prawns compared to hatchery-prawns, mortality was firstly found on day 4. The haemocytes counts indicated that there was dynamic response of both wild- and hatchery- prawns to the *Vibrio* spp. challenge; predominantly increased THC and DHC were found in the prawns.

However, for better understanding of immune competence of wild- and hatchery-produced *M. rosenbergii* against *Vibrio* spp., more scientific studies are needed:

- Evaluation of immune competency at different life stages of prawns against pathogens.
- Determination of genetic influence (e.g. Heritability in Immune Gene Expression) on immune response of prawns to pathogens.

Chapter 7

References

7. References

Abramo, L. R., and New, M. B. (Eds). (2000). Nutrition, feeds and feeding. Freshwater prawn culture: the farming of *Macrobrachium rosenbergii*. pp. 203-220. Oxford, England, Blackwell Science.

Abulude, F. O., Lawal, L. O., Ehikhamen, G., Adesanya, W. O., and Ashafa, S. L. (2006). Chemical composition and functional properties of some prawns from the coastal area of Ondo state, Nigeria. Electron Journal of Environment, Agriculture and Food Chemistry 5:1235-1240.

Adeyeye, E. I. (1996). Waste yield, proximate and mineral composition of three different types of land snails found in Nigeria. International Journal of Food Science and Nutrition 47:111-116.

Ahamed, F., Fulanda, B., Siddik, M. A. B., Hossain, M. Y., Rahman, M. M., Mondol, Z. F. A., and Ohtomi, J. (2014). An overview of freshwater prawn fishery in Bangladesh: Present status and future prospect. Journal of Coastal Life Medicine, 2(7), 580–588.

Ahmed, N. (2001). Socio-economic aspects of freshwater prawn culture development in Bangladesh. PhD Thesis, Institute of Aquaculture, University of Stirling, Scotland, UK.

Ahmed, N. (2013). Linking prawn and shrimp farming towards a green economy in Bangladesh: confronting climate change. Ocean Coast Manage 2013, 75: 33-42.

Ahmed, N., Brown, J. H., and Muir, J. F. (2008b). Freshwater prawn farming in gher (farm) systems in southwest Bangladesh. Journal of Aquaculture Economics and Management, 12: 207-223.

Ahmed, N., Demaine, H., and Muir, J. F. (2008). Freshwater prawn farming in Bangladesh: History, present status and future prospects. Aquaculture Research, 39(8), 806–819. https://doi.org/10.1111/j.1365-2109. 2008.01931.x

Ahmed, N., Demaine, H., and Muir, J. F. (2008a). Freshwater prawn farming in Bangladesh: history, present status and future prospects. Aquaculture Research, 39: 806-819.

Alam, M. J., Islam, M. S., Pal, H. K., and Karim, M. (2007). Year-round and rotational freshwater prawn, *Macrobrachium rosenbergii* and paddy farming: size quality, production, and economics. Bangladesh Journal of Fisheries Research 11(1): 75-92.

Alston, D. E., and Sampaio, C. M. S. (Eds.). (2000). Nursery systems and management. Freshwater prawn culture: the farming of *Macrobrachium rosenbergii,* pp. 112-125. Oxford, England, Blackwell Science.

Anderson, I. G., Shamsudin, M. N., and Nash, G. (2006). A preliminary study on the aerobic heterotrophic bacterial flora in giant freshwater prawn, *Macrobrachium rosenbergii,* hatcheries in Malaysia. Aquaculture, 81(3–4), 213–223. https://doi.org/10.1016/0044-8486(89)90147-6

Apines-Amar, M. J. S., and Amar, E. C. (2015). Use of Immunostimulants in Shrimp Culture: An Update (pp. 45–71). Research Signpost, Kerala, India: Biotechnological Advances in Shrimp Health Management in the Philippines.

Asaduzzaman, M., Wahab, M. A., Yi, Y., Diana, J. M., and Lin, C. K. (2007). Bangladesh prawn-farming survey reports industry evaluation. Global Aquaculture Advocate, 9: 40-43.

Asaduzzaman, M., Wahab, M. A., Yi, Y., Diana, J. M., and Lin, C. K. (2009). Bangladesh prawn-farming survey reports industry evaluation. Global Aquaculture Advocate, 9: 40-43.

Bachere, E. (2000). Shrimp immunity and disease control. Aquaculture, 191(1), 3–11. https://doi.org/10.1016/S0044-8486(00)00413-0

Bachere, E., Mialhe, E., Noel, D., Boulo, V., Morvan, A., and Rodriguez, J. (1995). Knowledge and research prospects in marine mollusc and crustacean immunology. *Aquaculture*, *132*(1–2), 17–32. https://doi. org/10.1016/0044-8486(94)00389-6

Bain, I. B., and Bates, I. (2001). Practical Haematology, University of Liverpool, Liverpool, UK, 11: 668.

Balazs, G. H., and Ross, E. (1976). Effect of protein source and level on growth and performance of captive freshwater prawn, *Macrobrachium rosenbergii*. Aquaculture, 7: 200-213.

Barmon, B. K., and Karim, M. (Eds.). (2007). Analysis of feeds and fertilizers for sustainable aquaculture development in Bangladesh. Study and analysis of feeds and fertilizers for sustainable aquaculture development, pp. 113-140. FAO Fisheries Technical Paper No. 497. Rome, FAO, 510 pp.

Barmon, B. K., Kondo, T., and Osanami, F. (2006). Economic evaluation of rice-prawn gher farming system on soil fertility for modern variety (MV) paddy production in Bangladesh. Paper prepared for presentation at the international association of agricultural economists conference, Gold Coast, Australia, August 12-18, 2006.

Barmon, B. K., Kondo, T., and Osanami, F. (2007). Economic evaluation of rice-prawn gher farming system on soil fertility for modern variety (MV) paddy production in Bangladesh. Paper prepared for presentation at the international association of agricultural economists conference, Gold Coast, Australia, August 12-18, 2006.

Barracco, M. A. (1998). Morphological and cytochemical studies of the haemocytes of *M. rosenbergii.* Journal of Crustacean Biology 12: 372-382.

Be, L. M. (2002). Investigation on diseases of giant freshwater prawn (*Macrobrachium rosenbergii*) in ponds and rice-prawn farming systems in AnGiang province. Msc. thesis (in Vietnameses).

Bechteler, C., and Holler, D. (1995). Preliminary studies of the immunization of shrimp (*Penaeus monodon*) against *Vibrio* infections. Berl Munch Tierarztl Wochenschr; 108: 462–465.

Boyd, C. E. (1979). Water quality in warm water fish ponds. Auburn, Alabama, USA, Auburn University Agricultural Experiment Station.

Boyd, C. E. (1990). Grow-out systems – water quality and soil management. Freshwater prawn culture: the farming of *Macrobrachium rosenbergii*, pp. 221-238. Oxford, England, Blackwell Science.

Brehelin, M., Tsing, A., and Arcier, J. M. (1989). Hemocytes of penaeid and palaemonid shrimps: Morphology, cytochemistry, and hemograms. *Journal of Invertebrate Pathology*, *53*(1), 64–77. https://doi.org/10. 1016/0022-2011(89)90075-X

Campbell, T., and Ellis, W. (2007). Avian and Exotic Animal Haematology and Cytology. 3rd ed. Blackwell Publishing, Ames, Iowa, USA.

Cavelli R.O. (2000). Reproductive performance of *Macrobrachium rosenbergii* females in captivity. Journal of theWorld Aquaculture Society 32,60-67.

Celi, M., Filiciotto, F., Parrinello, D., Buscaino, G., Damiano, M. A., Cuttitta, A., and Vazzana, M. (2013). Physiological and agonistic behavioural response of *Procambarus clarkii* to an acoustic stimulus. *Journal of Experimental Biology, 216*(4), 709–718. https://doi.org/10. 1242/jeb.078865

Cheng, W., and Chen, J. C. (2002a). Effects of environmental factors on the immune responses of freshwater prawn *Macrobrachium rosenbergii* and other decapod crustaceans. *Journal-fisheries Society of Taiwan,29*(1), 1–20.

Cheng, W., Juang, F. M., Li, J. T., Lin, M. C., Liu, C. H., and Chen, J. C. (2003). The immune response of the giant freshwater prawn *Macrobrachium rosenbergii* and its susceptibility to *Lactococcus garvieae* in relation to the moult stage. Aquaculture, 218(1), 33–45. https://doi. org/10.1016/S0044-8486(02)00415-5

Cheng, W., Juang, F. M., Li, J. T., Lin, M. C., Liu, C. H., and Chen, J. C. (2006). The immune response of the giant freshwater prawn *Macrobrachium rosenbergii* and its susceptibility to *Lactococcus garvieae* in relation to the moult stage. Aquaculture, 218(1), 33–45. https://doi. org/10.1016/S0044-8486(02)00415-5

Chisty, M. A. H., and Rahman, M. M. (1999). Growth performance of Mirror Carp (*Cyprinus carpio* var. specularis, Lacepede, 1803) Prawns Fry Feed with varying Protein Content Feeds, *Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna-9208, Bangladesh.*

Chowdhury, R., Bhattacharjee, H., and Angell, C. (1993). A manual for operating a small scale recirculation freshwater prawn hatchery. Publication No. BOBP/MAG/13. Madras, India, Bay of Bengal Programme.

Cowles, R. (1914). Palaemons of the Philippine islands. The Philippine Journal of Science 9(4):319-405.

Daniels, W. H., Cavalli, R. O., and Smullen, R. P. (Eds.). (2000). Broodstock management. Freshwater prawn culture: the farming of *Macrobrachium rosenbergii,* pp. 41-51. Oxford, England, Blackwell Science.

Daniels, W. H., D'Abramo, L. R., and Parseval, L. D. (1995). Design and management of a closed, recirculating 'clearwater' hatchery system for freshwater prawns, *Macrobrachium rosenbergii* (De Man 1879). Journal of Shellfish Research, 11:65-73.

Das, N. N., Saad, C. R., Ang, K. J., Law, A. T., and Harmin, S. A. (1996). Diet formulation for *Macrobrachium rosenbergii* (de Man) broodstock based on essential amino acid profile of its eggs. Aquaculture Research, 27:543-555.

De Man, J. G. (1879). On some species of the genus Palaemon Fabr. with descriptions of two new forms. Notes Leden Museum, 1:165-184.

Debnath, P. P., Karim, E., Haque, M. A., Uddin, M., and Karim, M. (2012). Prevalence of White Spot Syndrome Virus in Brood stock, Nauplii and Post-larvae of Tiger shrimp (*Penaeus monodon*) Fabricius, (1798) in Bangladesh. Journal of Advanced Scientific Research, 3(3).

Destoumieux, D., Bulet, P., Loew, D., Van Dorsselaer, A., Rodriguez, J., and Bachere, E. (1997). Palaemonidians, a new family of antimicrobial peptides isolated from the prawns *Macrobrachium rosenbergii* (Decapoda). J Biol Chem; 272: 28398–28406.

Destoumieux, D., Bulet, P., Strub, J. M., Van Dorsselaer, A., and Bachere, E. (1999). Recombinant expression and range of activity of palaemonidians, antimicrobial peptides from *Macrobrachium rosenbergii.* Eur J Biochem; 266: 335–346.

Destoumieux, D., Munoz, M., and Cosseau C et al., (2000). Palaemonidians, antimicrobial peptides with chitin-binding activity, are produced and stored in prawns granulocytes and released after microbial challenge. J Cell Sci; 113: 461–469.

Diana, E. (2000). Palaemonidians, a family of antimicrobial peptides from palaemonid shrimp (Crustacea, Decapoda). Cell Mol Life Sci 2000; 57: 1260–1271.

Diaz-Herrera, F., Perez-Cruz, E., Juarez-Castro, G., and Buckle-Ramirez, L. P. (1992). Assimilation and growth efficiencies of postlarvae and juveniles of the Malaysian prawn, *Macrobrachium rosenbergii* fed two balanced diets. Ciencias Marinas, 18: 17–26.

DoF. (2002a). Shrimp Aquaculture in Bangladesh: A Vision for the Future. Department of Fisheries (DOF), Ministry of Fisheries and Livestock, Dhaka, Bangladesh,7pp.

DoF. (2010). Fishery Statistical Yearbook of Bangladesh 2008-2009. Fisheries Resources Service System, Department of Fisheries (DoF), Ministry of Fisheries and Livestock, Dhaka, Bangladesh, 26(1), 41 pp.

DoF. (2017). Yearbook of Fisheries Statistics of Bangladesh 2016-17. Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh: Volume 34 : p. 129. Web: http://www.fisheries.gov.bd

Dong, F. M. (2001). The nutritional value of shellfish. A Washington Sea Grant Programme publication, pp: 8.

Dugan, C. C., and Frakes, T. A. (1973). Culture of brackish-freshwater shrimp, *Macrobrachium acanthurus, M. carcinus* and *M. ohione*. Proc. 3rd Ann. Workshop, World Mariculture Soc. (1972). pp. 185-191.

Dugan, C. C., Hagood, R. W., and Frakes, T. A. (1975). Development of spawning and mass larval rearing techniques for brackish-freshwater shrimps of the genus *Macrobrachium* (Decapoda, *Palaemonidae*). Fla. Mar. Res. Pubs. No. 12, 28 pp.

Durant, M. D., Fondren, M. W., Daniels, W. H., and D'Abramo, L. R. (1995). Effects of stocking density and feed on pond production characteristics and revenue of harvested freshwater prawns *Macrobrachium rosenbergii* stocked as size-graded juveniles. *Journal of the World Aquaculture Society,* 26:38-47.

El-Gamal A. A., Alderman D. J., Rodgers C. J., Polglase J. L., and Macintosh, D. (1986). A scanning electron microscope study of oxolinic acid treatment of burn spot lesions of *Macrobrachium rosenbergii.* Aquaculture 52, 157–171.

Esteve, C., Amaro, C., and Toranzo, A. E. (1994). O-Serogrouping and surface components of *Aeromonas hydrophila* and *Aeromonas jandaei* pathogenic for prawns. *FEMS Microbiology Letters,* 117(1), 85-90.

Evans, L. H., Jussila, J., Jago, J., Tsvetnenko, E., and Dunstan, B. (1997). Total and differential haemocyte counts in western rock lobsters (*Panulirus cygnus* George) under post-harvest stress. *Marine and Freshwater Research, 48*(8), 863–868. https://doi.org/10.1071/MF97216

Factor, M. A., and Beekman, M. (1990). Atomic force Microscopy of peritoneal macrophages after particle phagocytosis. - Journal of Membrane Biology 140: 197-204.

FAO. (1981). Water for freshwater fish culture. FAO Training Series No. 4. Rome.

FAO. (1994). Handbook on small-scale freshwater fish farming. FAO Training Series No. 24. Rome.

FAO. (1995). Pond construction for freshwater fish culture: building earthen ponds. FAO Training Series No. 20/1. Rome.

FAO. (1998). Management for freshwater fish culture: fish stocks and farm management. FAO Training Series No. 21/2. Rome.

FAO. (2001). Farming of Freshwater Prawns: A Manual For the culture of the giant river prawn *(Macrobrachium rosenbergii).* Food and Agricultural organization, Fisheries Technical Paper, 428 pp.

FAO. (2002). Fishstat Plus (v.2.30), 15.03.2002. Rome, FAO.

FAO. (2017). Yearbook of 2006. Fishery and Aquaculture Statistics. FAO, Rome, 57p.

Faruk, M. A. R., Sarker, M. M. R., Alam, M. J., and Kabir, M. B. (2006). Economic loss from fish diseases on rural freshwater aquaculture of Bangladesh. Pakistan Journal of Biological Sciences, 7(12), 2086–2091. http://docsdrive.com/pdfs/ansinet/pjbs/2004/2086-2091.pdf

Ferdose, A., and Hossain, M. B. (2011). Nutritional value of wild, cultured and frozen prawn *Macrobrachium rosenbergii* (De Man, 1879), International journal of Natural Sciences 1(2): 52-55.

Fondren, M. W., Daniels, W. H., D'Abramo, L. R., and Durant, M. D. (1995). Effects of stocking density and feed on pond production characteristics and revenue of harvested freshwater prawns *Macrobrachium rosenbergii* stocked as size-graded juveniles. *Journal of the World Aquaculture Society,* 26:38-47.

Fontaine, C. T., and Lightner, D. V. (1974). Observations on the phagocytosis and elimination of carmine particles injected into the abdominal musculature of the prawns, *Macrobrachium rosenbergii.* Journal of Invertebrate Pathology 24, 141–148.

Fotedar, R., Ambas, I., and Suriawan, A. (2013). Immunological responses of customised probiotics-fed marron, *Cherax tenuimanus,* (Smith 1912) when challenged with *Vibrio mimicus. Fish & Shellfish Immunology, 35*(2), 262–270. https://doi.org/10.1016/j.fsi.2013.04.026

Freuchtnicht, G. W., Bark, L. E., Malecha, S. R., and Stanley, R. W. (1988). The effect of protein level in the feed on growth performance of fresh water prawn *Macrobrachium rosenbergii* individually recorded in clear water flow through aquaria. Presented at the 19th annual meeting of the world aquaculture society, Honolulu, Hawaii.

Fujimura, T. (1966). Notes on the development of a practical mass culture technique of the giant prawn, *Macrobrachium rosenbergii.* Paper presented to the Indo-Pacific Fisheries Council, 12th Session. IPFC/C66/WP47, 4 pp.

Fujimura, T., and Okamoto, H. (Eds.). (1972). Notes on progress made in developing a mass culturing technique for *Macrobrachium rosenbergii* in Hawaii. Coastal aquaculture in the Indo-Pacific region, pp. 313-327. West Byfleet, England, Fishing News Books.

Fujimura, T., and Okamoto, H. (Eds.). (1992). Notes on progress made in developing a mass culturing technique for *Macrobrachium rosenbergii* in Hawaii. Coastal aquaculture in the Indo-Pacific region, pp. 313-327. West Byfleet, England, Fishing News Books.

Gargioni, R., and Barracco, M. A. (1998). Hemocytes of the palaemonids *Macrobrachium rosenbergii* and *M. acanthurus*, and of the Penaeid *Penaeus paulensis. Journal of Morphology, 236*(3), 209–221. https://doi.org/10.1002/(SICI)1097-4687(199806)236:3<209

Ghosh, A. K., Bir, J., Azad, M. A. K., Hasanuzzaman, A. F. M., Islam, M. S., and Huq, K. A. (2016). Impact of commercial probiotics application on growth and production of giant freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879). *Aquaculture Reports, 4,* 112–117. https://doi.org/10.1016/j.aqrep.2016.08.001

Gomez Nakagawa, G. H., and Kasanara, S. (1988). Effect of dietary protein/ starch ratios and energy level on growth of the giant freshwater prawn, *Macrobrachium rosenbergii.* Nippon Suisan Gakkaishi 54:1401-1407.

Habashy, M. M. (2009). Growth and body composition of juvenile freshwater prawn, *Macrobrachium rosenbergii,* fed different dietary protein/starch ratios. National Institute of Oceanography and Fisheries, Fish Research Station, El-Qanatar El-Khairya, Cairo, Egypt.

Hall, M. T., Lansing, Gr., and Kibria, G. (1999). Fish stock assessment program, chapter 7. 2nd Annual Report, Karnaphuli Irrigation and Fishery Development Project, Snell Environ. Michigan. 30pp.

Haroon, A. K. Y. (1990). Freshwater prawn farming trails in Bangladesh. Naga, the ICLARM Quarterly13,6-7.

Hasanuzzaman, A. F. Md., Siddiqui, N. M., and Chisty, M. A. H. (2009). Optimum replacement of fishmeal with soybean meal in diet for *Macrobrachium rosenbergii* (De Man, 1879) cultured in low saline water. Turkish Journal of Fisheries and Aquatic Science, 9: 17-22.

Hasanuzzaman, A. F., Rahman, M. A., and Islam. S. S. (2011). Practice and Economics of Freshwater Prawn Farming in Seasonally Saline Rice Field in Bangladesh, Mesopotamian Journal of Marine Science, 2011, 26 (1): 69-78.

Heath, A. G. (Ed.). (1987). Water pollution and fish physiology. In Physiological Energetic. Boca Raton, FL: 131-163, CRC Press, USA.

Hossain, M. A., and Paul, L. (2007). Low-cost diet for monoculture of giant freshwater prawn (*Macrobrachium rosenbergii* de Man) in Bangladesh. Aqua. Res., 38: 232-238.

Hrubec, T. C., and Smith, S. A. (2000). Age-related changes in hematology and plasma chemistry values of *M. rosenbergii*, Moronechrysops and *Moro-ne saxatilis*, Veterinary clinical pathology, 30:8-15, doi: 10.1111/j.1939- 165X2001.tb00249.x

Ismael, D., and New, M. B. (Eds.). (2000). Biology. Freshwater prawn culture: the farming of *Macrobrachium rosenbergii*, pp. 18-40. Oxford, England, Blackwell Science.

Iwanaga, S., and Lee, B. L. (2005). Recent advances in the innate immunity of invertebrate animals. J Biochem Mol Biol; 38: 128–150.

Jayachandran, K. V. (2001). Palaemonid prawns: biodiversity, taxonomy, biology and management. Science Publishers, Enfield (NH), USA. 624 pp.

Jayaprakash, N. S., Rejish Kumar, V. J., Philip, R., and Bright Singh, I. S. (2006). Vibrios associated with *Macrobrachium rosenbergii* (De Man, 1879) larvae from three hatcheries on the Indian southwest coast. Aquaculture Research, 37(4), 351–358. https://doi.org/10.1111/j. 1365-2109.2006.01432.x

Jiravanichpaisal, P., and Miyazaki, T. (2009). Histopathology, biochemistry and pathogencity of *Vibrio harveyi* infecting Giant Freshwater Prawn Journal of Aquatic Animal Health 6, 2735.

Johansson, M. W., and Soderhall, K. (1989). Cellular immunity in crustaceans and the proPO system. Parasitology Today, 5(6), 171–176. https://doi.org/10.1016/0169-4758(89)90139-7

Johnson, S. K., and Bueno, S. L. S. (Eds.). (2000). Health management. Freshwater prawn culture: the farming of *Macrobrachium rosenbergii*, pp. 239-258. Oxford, England, Blackwell Science.

Karplus, I., Malecha, S. R., and Sagi, A. (Eds.). (2000). The biology and management of size variation. Freshwater prawn culture: the farming of *Macrobrachium rosenbergii*, pp. 259-289. Oxford, England, Blackwell Science.

Kawabata, S. I., Mutua, T., and Iwanaga, S. (Eds.). (1996). The clotting cascade and defense molecules found in the hemolymph of the prawns. Invertebrate Immunology. Fair Haven, CT: SOS; 255–283.

Kendrick, A. (1994). The gher revolution: the social impacts of technological change in freshwater prawn cultivation in southern Bangladesh. Bangladesh Aquaculture and Fisheries Resource Unit, Dhaka, Bangladesh, 67 pp.

Khuntia, L., Sheikha, E. F. A., Levin, E. R., and Xu, J. (Eds). (2008). Molecular Techniques in Food Biology: safety, Biotechnology, Authenticity and Traceability, Wiley & Sons. ISBN: 978-1-119-37460-2: 455-472.

Kondo, M. H., Matsuyama, H., and Yano, T. (1992). The opsonic effect of lectin on the phagocytosis by hemocytes of prawn, *Macrobrachium rosenbergii.* Fish Pathol; 27: 217–222.

Kumar, N. R., Raman, R. P., Jadhao, S. B., Brahmchari, R. K., Kumar, K., and Dash, G. (2013). Effect of dietary supplementation of *Bacillus licheniformis* on gut microbiota, growth and immune response in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). *Aquaculture International, 21*(2), 387–403. https://link.springer.com/article/10.1007/s10499-012-9567-8.

Lacroix, D., Glude, J., Thomas, J. E., and Le Menn, H. (1994). From research to commercialization: lessons from four different strategies in the development of freshwater prawn culture *(Macrobrachium rosenbergii)* in America since 1977. World Aquaculture 25, 5–17.

Lalitha, K. V., and Surendran, P. K. (2004). Bacterial microflora associated with farmed freshwater prawn *Macrobrachium rosenbergii* (de Man) and the aquaculture environment. Aquaculture Research, 35(7), 629– 635. https://doi.org/10.1111/j.1365-2109.2004.01039.x

Le Moullac, G., Soyez, C., Saulnier, D., Ansquer, D., Avarre, J. C., and Levy, P. (1998). Effect of hypoxic stress on the immune response and the resistance to vibriosis of the shrimp *Penaeus stylirostris. Fish & Shellfish Immunology, 8*(8), 621–629. https://doi.org/10.1006/fsim.1998.0166

Lee, P. P., Yeh, S. P., Liu, C. H., Sung, T. G., and Cheng, W. (2006). Effect of saponin on hematological and immunological parameters of the giant freshwater prawn, *Macrobrachium rosenbergii. Aquaculture, 261* (4), 1432–1439. https://doi.org/10.1016/j.aquaculture.2006.08.051

Lee, Y. S., Yeh, S. P., and Soderhall, K. (2001). Early events in crustacean innate immunity. Fish Shellfish Immunol;12(5):421–37.

Leu, J. H., Chen, S. H., Wang, Y. B., Chen, Y. C. S. S. Y., and Lin C Y et al., (2011). A review of the major penaeid shrimp EST studies and the construction of a shrimp transcriptome database based on the ESTs from four penaeid shrimp. Marine Biotechnol;13(4):608–21.

Lightner, D. V., and Lewis, D. H. (1975). Asepticemia bacterial disease syndrome in penaeid shrimp. Marine Fisheries Review 37, 25-28.

Ling, S. W. (1969). Methods of rearing and culturing *Macrobrachium rosenbergii.* FAO Fisheries Reports No. 57 Vol. 3:607-619.

Lombardi, J. V., Hipolito, M., Baldassi, L., and Pires, D. C. (1996). Prevalencia bacteriana em necrose de camaraodeagua doce (*Macrobrachium rosenbergii,* Decapoda, Palaemonidae). Boletim do Instituto de Pesca 23, 13–20.

Mazid, M. A. (1994). Evaluation of Prawn Farming on Socio- Economic Aspects. Fisheries Research Institute, Mymensingh, Bangladesh.

Menasveta, P., Rengpipat, S., Phianphak, W., and Piyatiratitivorakul, S. (1998). Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. *Aquaculture, 167*(3), 301–313. https://doi.org/10.1016/S0044-8486(98)00305-6

Millikin, M. R., Fortner, A. R., Fair, P. H., and Sick, L. V. (1980). Influence of dietary protein concentration on growth, feed conversion and general metabolism of juvenile prawn *(Macrobrachium rosenbergii).* Proceedings of the World Mariculture Society, 11: 382-39.

Mitra, G., Mukhopadhyay, P. K., and Chattopadhyay, D. N. (2005). Nutrition and feeding in freshwater prawn *(Macrobrachium rosenbergii)* farming. Aquafeed Formulation and Beyond, 2: 17-19.

Motoh, H., Oliveros, E. N., and Buri, P. (1980). Early developmental stages of the Giant Freshwater prawn, M. rosenbergii. Res. Crust. (10): 13-34.

Muir, J. F. (2003a). The future for fisheries: economic performance. Fisheries Sector Review and Future Development Study. Commissioned with the association of the World Bank, DANIDA, USAID, FAO, DFID with the cooperation of the Bangladesh Ministry of Fisheries and Livestock and the Department of Fisheries, Dhaka,172pp.

Muir, J. F. (2003b). The Future for Fisheries: Economic Performance. Fisheries Sector Review and Future Development Study. Commissioned with the association of the World Bank, DANIDA, USAID, FAO, DFID with the cooperation of the Bangladesh Ministry of Fisheries and Livestock and the Department of Fisheries, Dhaka, 172 pp.

Munoz, M., Vandenbulcke, F., Saulnier, D., and Bachere, E. (2005). Expression and distribution of palaemonidians antimicrobial peptides are regulated by haemocyte reactions in microbial challenged prawns. Eur J Biochem; 269: 2678–2689.

Nakayama, T., Nomura, N., and Matsumura, M. (2005). Analysis of the relationship between luminescence and toxicity of *Vibrio carchariae* pathogenic to shrimp. *Fisheries Science*, *71*(6), 1236–1242. https://doi. org/10.1111/j.1444-2906.2005.01088.x

New, M. B. (1986). Aquaculture diets of post larval marine fish of the super–family Percoidae, with special reference to sea bass, sea breams, groupers and yellow tail: a review. Kuwait bulletin of Marine Science 7:75-151.

New, M. B., and Singholka, S. (1985). Freshwater prawn farming: a manual for the culture of *Macrobrachium rosenbergii*. FAO Fisheries Technical Paper No. 225 Rev. 1. Rome. [also published in Farsi, French, Hindi, Spanish, and Vietnamese]

New, M. B., and Valenti, W. C. (Eds.), (2000). Freshwater Prawn Culture: the farming of *Macrobrachium rosenbergii*. Blackwell Science, Oxford, England. 443p.

Okamoto, H. (Eds.). (1972). Notes on progress made in developing a mass culturing technique for *Macrobrachium rosenbergii* in Hawaii. Coastal aquaculture in the Indo-Pacific region, pp. 313-327. West Byfleet, England, Fishing News Books.

Pan, C. H., Chen, Y. H., and Chen, J. H. (2005). Effects of Light Regime, algae in water and dietary astaxanthin on pigmentation, growth and survival of black tiger prawn post-larvae. Zoological studies 40(4): 371-382.

Parrinello, S., Enver, T., Napoli, I., Guo, Y., and Burden, J. (2015). Macrophage-Induced Blood Vessels Guide schwann Cell-Mediated Regeneration of *M. rosenbergii.* MRC Laboratory for Molecular Cell Biology, UCL, Gower Street, London WC1E 6BT, UK.

Paul, B. G., and Vogl, C. R. (2011). Impacts of shrimp farming in Bangladesh: Challenges and alternatives. Ocean & Coastal Management, 54 (3), 201–211. https://doi.org/10.1016/j.ocecoaman.2010.12.001

Pfeffer, C., and Oliver, J. D. (2003). A comparison of thiosulphate-citratebile salts-sucrose (TCBS) agar and thiosulphate-chloride-iodide (TCI) agar for the isolation of *Vibrio* species from estuarine environments. *Letters in Applied Microbiology*, *36*(3), 150–151. https://doi.org/10. 1046/j.1472-765X.2003.01280.x

Phatarpekar, P. V., Kenkre, V. D., Sreepada, R. A., Desai, U. M., and Achuthan kutty, C. T. (2002). Bacterial flora associated with larval rearing of the Giant Freshwater prawn, *M. rosenbergii.* Aquaculture, 203(3-4), 279-291. https://doi.org/10.1016/S0044-8486 (01)00705-0

Pillai, D., and Bonami, J. R. (2012). A review on the diseases of freshwater prawns with special focus on white tail disease of *Macrobrachium rosenbergii.* Aquaculture Research, 43(7), 1029–1037. https://doi.org/ 10.1111/j.1365-2109.2011.03061.x

Pinheiro, M. A. A., and Hebling, N. J. (Ed.). (1998). Biologia de *Macrobrachium rosenbergii* (De Man, 1879). Carcinicultura de agua doce. Tecnologia para produçao de Camaroes, pp. 21-46. Brasilia, IBAMA.

Plascencia, W. J. F. (2000). Crustacean Farming: ranching and culture, Blackwell Scientific Publications, Ames, lowa; Oxford.

Rangacharyulu, P. V. (1999). Studies on nutrition and diet development of giant freshwater prawn, *Macrobrachium rosenbergii* post larvae. University of Kalyani, West Bengal, India. (PhD thesis).

Rao, K. J. (1991). Reproductive biology of giant freshwater prawn *Macrobrachium rosenbergii* (de Man) from the lake Kolleru (Andhra pradesh). Indian, J. Anim. Sci., 61(7): 780-787.

Reed, L., and Abramo, L. R. (1989). A Standard Reference Diet for Crustacean Nutrition Research.: III. Effects on Weight Gain and Amino Acid Composition of Whole Body and tail Muscle of Juvenile Prawns *Macrobrachium rosenbergii* 1. Journal of the World Aquaculture Society 20(3):107-113. April 2007.

Reiner, A., Yekutieli, D., and Benjamini, Y. (2003). Identifying differentially expressed genes using false discovery rate controlling procedures. Bioinformatics.19(3):368–75.

Rhodes, C. P., Ratcliffe, N. A., Rowley, A. F., and Fitzgerald, S. W. (1985). Invertebrate immunity: Basic concepts and recent advances. International Review of Cytology, 97, 183–350. https://doi.org/10.1016/ S0074-7696(08)62351-7

Ricardo, L. S., James, T. L., Zelionara, P. B., Bianchini, A., and Luiz Eduardo Maia, N. Y. (2003). Lipids as energy source during salinity acclimation in the prawns *Macrobrachium rosenbergii,* (Dana, 1851) (crustacea-palaemonidae). Journal of Experimental Zoology 295 A: 200-20.

Rowley, A. F. (Ed.). (2016). The immune system of Crustaceans. Encyclopedia of Immunobiology (pp. 437–453, 1st edn). The Boulevard, Langford Lane, Kidlington, Oxford, UK: Academic Press (an imprint of Elsevier). Retrieved from https://www.elsevier.com/ooks/encyclopedia-of-immunobiology/ratcliffe/978-0-12-374279-7. Accessed 15 October 2017.

Sahoo, P. K., Pillai, B. R., Mohanty, J., Kumari, J., Mohanty, S., and Mishra, B. K. (2007). In vivo humoral and cellular reactions, and fate of injected bacteria *Aeromonas hydrophila* in freshwater prawn *Macrobrachium rosenbergii.* Fish and Shellfish Immunology, 23(2), 327–340. https://doi.org/10.1016/j.fsi.2006.11.006

Sahu, N. P. (2004). Nutrient requirement of Indian fishes of commercial importance: Present status and future need. Indian Society of Fisheries Professional Newsletter 'Indofish'. Vol VI (1-2): 7-15.

Samad, M. A., Hossain, M. M., Nima Rahman, A. B. M. S., and Haidar, M. I. (2014). Present status of freshwater prawn culture and management system in Abhaynagar Upazila in Jessore of Bangladesh. Bangladesh Research Publications Journal 9(3): 182-189.

SamCookiyaei, A., Afsharnasab, M., Razavilar, V., Motalebi, A., Kakoolaki, S., Asadpor, Y., and Nekuie Fard, A. (2012). Experimentally pathogenesis of *Aeromonas hydrophila* in freshwater prawn in iran. Iranian Journal of Fisheries Sciences, 11(3), 644-656.

Sandifer, P. A., and Smith, T. I. (1975). Experimental aquaculture of the Malaysian prawn, *Macrobrachium rosenbergii* (de Man) in South Carolina, U. S. A. FAD Tech. Conf. on Aquacult., Doc. FIR:AQ/Conf.76/E.3, 7 pp.

Saravana-Bhavan, P., Radhakrishnan, S., Seenivasan, C., Shanti, R., Poongodi, R., and Kannan S et al., (2010). Proximate composition and profiles of amino acids and fatty acids in the muscle of adult males and females of commercially viable prawn species *Macrobrachium rosenbergii* collected from natural culture environments. Int. J. Bio; 2(2):107-119.

Saurabh, S., and Sahoo, P. K. (2008). Major diseases and the defence mechanism in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). Proceedings of the National Academy of Sciences, India, Section B, 78, 103–121.

Sayeed, M. R., Ghosh, A. K., Islam., S. S. and Bir, J. (2015). Probiotic Tiger Shrimp (*Penaeus monodon*) Farming at Different Stocking Densities and its Impact on Production and Economics, International Journal of Research in Fisheries and Aquaculture.

Schmittgen, T. D., and Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. Nat Proto; 3(6):1101–8.

Sebastian, M. J. (1990). The giant freshwater prawn *Macrobrachium rosenbergii* (de Man). AGG Menon Publ Kerala Agricultural University, Thrissur, India, pp. 1-43.

Shankar, K. M. Viswanatha, B. S., and Bhatta, R. (2014). An economic assessment of fish and prawn health management in Andhra Pradesh.

Shankar, R., Murthy, H. S., Sujatha, H. R., Jayaraj, E. G., Tejpal, C. S., and Chinthamani, V. S. (2012). Effect of nucleotide on growth, immune responses and resistance of *Macrobrachium rosenbergii* (De Man) to *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus (XSV) and *Aeromonas hydrophila* infection. Aquaculture International, 20 (1), 1–12. https://doi.org/10.1007/s10499-011-9430-3

Sharshar, K., and Azab, E. A. (2008). Studies on diseased freshwater prawn *Macrobrachium rosenbergii* infected with *Vibrio vulnificus*. Pakistan Journal of Biological Sciences, 11(17), 2092–2100. https://doi.org/10. 3923/pjbs.2008.2092.2100

Sierra, C., Guevara, J., Lascurain, R., Perez, A., Agundis, C., Zenteno, E., and Vazquez, L. (2001). Sialylation is modulated through maturation in hemocytes from *Macrobrachium rosenbergii*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *130*(2), 179–189.https://doi.org/10.1016/S1532-0456(01)00242-3

Smith, T. I. J., Sandifer, P. A., and Trimble, W. C. (1974). Progress in developing a recirculating synthetic sea water hatchery for rearing larvae of *Macrobrachium rosenbergii*. Proc. Fourth Food-Drugs from-the Sea Conf. (in press).

Smith, T. I. J., Sandifer, P. A., and Trimble, W. C. (1984). Progress in developing a recirculating synthetic sea water hatchery for rearing larvae of *Macrobrachium rosenbergii*. Proc. Fourth Food-Drugs from-the Sea Conf. (in press).

Smith, V. J., and Chisholm, J. R. (2001). Antimicrobial proteins in crustaceans. Adv Exp Med Biol; 484: 95–112.

Smith, V. J., Relf, J. M., Chisholm, J. R., and Kemp, G. D. (1999). Purification and characterization of a cysteine-rich 11.5 kDa antibacterial protein from the granular haemocytes of prawns, *M. rosenbergii*. Eur J Biochem; 264: 350–357.

Soderhall, K. (1982). Prophenoloxidase activating system and melanization – a recognition system of arthropods? A review. Dev Comp Immunol; 6: 601–611.

Soderhall, K. (1989). Prophenoloxidase activating system and melanization – a recognition system of arthropods? A review. Dev Comp Immunol; 6: 601–611.

Soderhall, K., and Vey, A. (1984). Rented *M. rosenbergii* Haemocytes lysate enhancement of fungal spore encapsulation by prawns hemocytes. Dev Comp Immunol; 8: 23–29.

Soderhall, K., Aspan, A., and Duvic, B. (1990). The proPO system and associated proteins – role in cellular communication in arthropods. Res Immunol; 141: 896–907.

Soria, F., Sierra, C., and Bouquelet S et al., (2006). The effect of sugars and free amino acids from the freshwater prawn *Macrobrachium rosenbergii* hemolymph on lectin activity and on oxidative burst. Comp. Biochem Physiol C; 142: 212–219.

Spotte, S. (1979). Fish and invertebrate culture: water management in closed systems. New York, Wiley-Interscience.

Sritunyalucksana, K., and Soderhall, K. (2000). The proPO and clotting system in crustaceans. Aquaculture; 191: 53–69.

Sumon, M. S., Ahmmed, F., Khushi, S. S., Ahmmed, M. K., Rouf, M. A., Chisty, M. A. H., and Sarower, M. G. (2016). Growth performance, digestive enzyme activity and immune response of *Macrobrachium rosenbergii* fed with probiotic *Clostridium butyricum* incorporated diets. *Journal of King Saud University-Science*, *30*(1), 21–28. https://doi.org/ 10.1016/j.jksus.2016.11.003

Sung, H. H., and Hong, T. Y. (1997). The gram-negative bacterial flora in hepatopancreas of giant freshwater prawn *(Macrobrachium rosenbergii):* Antibiotic sensitivities and production of extracellular products. Journal of the Fisheries Society of Taiwan, 24(3), 211–223.

Sung, H. H., Hwang, S. F., and Tasi, F. M. (2000). Responses of giant freshwater prawn *(Macrobrachium rosenbergii)* to challenge by two strains of *Aeromonas* spp. Journal of Invertebrate Pathology, 76(4), 278–284. https://doi.org/10.1006/jipa.2000.4981

Sung, H. H., Kou, G. H., and Song, Y. L. (1996). Vibriosis resistance induced by glucan treatment in prawns *(Macrobrachium rosenbergii).* Fish Pathology 29,11-17.

Suwannatous, S., and New, M. B. (2000). Development of a shallow beach well for hatcheries requiring seawater. *In* M.B. New. ed. *Developments in aquaculture and fisheries science, Volume 10: Giant prawn farming,* pp. 303-307. Amsterdam, Elsevier.

Te, B. Q., and Tam, V. T. (1994). Common diseases in cultured fish and shrimp at Mekong delta – treatment and prevention methods. *Agricultural Publishing House. (in Vietnameses).*

Tiwari, J. B., and Sahu, N. P. (1999). Possible use of soyalecithin as a source of lipid in the post larval diet of *Macrobrachium rosenbergii.* Journal of Aquaculture in the Tropics, 14: 37-46.

Tonguthue, L. (Eds.). (2002). Diseases of the freshwater prawn *Macrobrachium rosenbergii* in Thailand. In Diseases in Asian Aquaculture, Asian Fisheries Society, Manila. pp. 89-95.

Tzou, K. (Eds.). (2002). Diseases of the freshwater prawn *Macrobrachium rosenbergii* in Thailand. In Diseases in Asian Aquaculture, Asian Fisheries Society, Manila. pp. 89-95.

Vargas-Albores, F., and Yepiz-Plascencia, G. (2000). Beta glucan binding protein (BGBP) and its role in immune response. Aquaculture; 191: 13–21.

Vazquez, L., Alpuche, J., Maldonado, G., Agundis, C., Pereyra-Morales, A., and Zenteno, E. (2009). Immunity mechanisms in crustaceans. Innate Immunity, 15(3), 179–188. https://doi.org/10.1177/1753425909102876

Vazquez, L., Lanz, H., Montano, L. F., Vasquez, L., and Zenteno, E. (1994). Biological activity of the lectin from *Macrobrachium rosenbergii.* Lectins; 10: 261–265.

Vazquez, L., Maldonado, G., and Agundis C et al., (1997). Participation of a sialic acid specific serum lectin from freshwater prawn *Macrobrachium rosenbergii* hemocytes in the recognition of non-self cells. J Exp Zool; 279: 265–272.

Vazquez, L., Perez, A., Millan, D., Agundis, C., Martin, G., Cooper, E. L., and Zenteno, E. (1997). Morphology of hemocytes from the freshwater prawn *Macrobrachium rosenbergii. Journal of Morphology, 234*(2), 147–153. https://doi.org/10.1002/(SICI)1097-4687(199711)234:2<147:AID-JMOR3>3.0.CO;2-9

Vazzana, G. R., Ahmed, H., Tasumi, S., Odom, E., and Saito, K. (2015). Biological roles of lectins in innate immunity: molecular and structural basis for diversity in self/non-self recognition. Adv Exp Med Biol; 598: 389–406.

Wahab, M. A., Nahid, S. A. A., and Haque, M. M. (2008). Power point presentation on Freshwater prawn farming in Bangladesh: development trends, challenges and sustainability issues. Conference on freshwater prawns, GIANT PRAWN 2011, Kochi, India.

Wahab, M. A., Nahid, S. A. A., and Haque, M. M. (2011). Power point presentation on Freshwater prawn farming in Bangladesh: development trends, challenges and sustainability issues. Conference on freshwater prawns, GIANT PRAWN 2011, Kochi, India.

Williams, D., and Khan, N. (2001). Freshwater prawn gher farming Systems: indigenous technology developed in southwest Bangladesh. CARE GOLDA Project, CARE Bangladesh, Dhaka.

Wowor, D., Muthu, V., Meier, R., Balke, M., Cai, Y., and Ng, P. K. (2007). Evolution of life history traits in Asian freshwater prawns of the genus *Macrobrachium*(Crustacea: Decapoda: Palaemonidae) based on multilocus molecular phylogenetic analysis. Mol Phylogenet Evol; 52(2):340–50.

Young Lee, S., and Soderhall, K. (2002). Early events in crustacean innate immunity. Fish Shellfish Immunol;12(5):421–37.

Zhao, Z. Y., Yin, Z. X., Weng, S. P., Guan, H. J., Li, S. D., and Xing K et al., (2007). Profiling of differentially expressed genes in hepatopancreas of white spot syndrome virus-resistant prawn *(Macrobrachium rosenbergii)* by suppression subtractive hybridisation. Fish Shellfish Immunol;22(5):520–34.

Zimmermann, S., and New, M. B. (Eds.). (2000). Grow-out systems - polyculture and integrated culture. Freshwater prawn culture: the farming of *Macrobrachium rosenbergii*, pp. 187-202. Oxford, England, Blackwell Science.

Chapter 8

Appendix

8. Appendixes

8.1 Goodness of Fit[a] of THC

	Value	df	Value/df
Deviance	8302980.046	116	71577.414
Scaled Deviance	8302980.046	116	
Pearson Chi-Square	8170333.721	116	70433.911
Scaled Pearson Chi-Square	8170333.721	116	
Log Likelihood[b]	-4152393.953		
Akaike's Information Criterion (AIC)	8304795.907		
Finite Sample Corrected AIC (AICC)	8304796.255		
Bayesian Information Criterion (BIC)	8304807.057		
Consistent AIC (CAIC)	8304811.057		

Dependent Variable: THC

Model: (Intercept), Treatment[a]

a. Information criteria are in smaller-is-better form.

b. The full log likelihood function is displayed and used in computing information criteria.

8.2 Omnibus Test[a] of THC

Omnibus Test[a]		
Likelihood Ratio Chi-Square	df	Sig.
7776780.020	3	.000

Dependent Variable: THC

Model: (Intercept), Treatment[a]

a. Compares the fitted model against the intercept-only model.

8.3 Goodness of Fit[a] of DHC (Large granular cell)

	Value	df	Value/df
Deviance	295.814	116	2.550
Scaled Deviance	295.814	116	
Pearson Chi-Square	301.703	116	2.601
Scaled Pearson Chi-Square	301.703	116	
Log Likelihood[b]	-422.914		
Akaike's Information Criterion (AIC)	853.828		
Finite Sample Corrected AIC (AICC)	854.176		
Bayesian Information Criterion (BIC)	864.978		
Consistent AIC (CAIC)	868.978		

Dependent Variable: LG

Model: (Intercept), Treatment

a. Information criteria are in small-is-better form.

b. The full log likelihood function is displayed and used in computing information criteria.

8.4 Omnibus Test[a] of DHC (Semi granular cell)

Likelihood Ratio Chi-Square	df	Sig.
67.719	3	.000

Dependent Variable: SG

Model: (Intercept), Treatment

a. Compares the fitted model against the intercept-only model.

8.5 Goodness of Fit[a] of DHC (Smaller granular cell)

	Value	df	Value/df
Deviance	410.700	116	3.541
Scaled Deviance	410.700	116	
Pearson Chi-Square	406.405	116	3.503
Scaled Pearson Chi-Square	406.405	116	
Log Likelihood[b]	-497.128		
Akaike's Information Criterion (AIC)	1002.256		
Finite Sample Corrected AIC (AICC)	1002.604		
Bayesian Information Criterion (BIC)	1013.406		
Consistent AIC (CAIC)	1017.406		

Dependent Variable: SmG

Model: (Intercept), Treatment

a. Information criteria are in small-is-better form.

b. The full log likelihood function is displayed and used in computing information criteria.

8.6 Omnibus Test[a] of DHC (Non-granular cell)

Likelihood Ratio Chi-Square	df	Sig.
310.022	3	.000

Dependent Variable: NG

Model: (Intercept), Treatment

a. Compares the fitted model against the intercept-only model.

8.7 Goodness of Fit[a] of mortality rate

	Value	df	Value/df
Deviance	363.871	25	14.555
Scaled Deviance	363.871	25	
Pearson Chi-Square	332.000	25	13.280
Scaled Pearson Chi-Square	332.000	25	
Log Likelihood[b]	-197.867		
Akaike's Information Criterion (AIC)	403.734		
Finite Sample Corrected AIC (AICC)	405.401		
Bayesian Information Criterion (BIC)	409.204		
Consistent AIC (CAIC)	413.204		

Dependent Variable: Mortality

Model: (Intercept), Treatment

a. Information criteria are in small-is-better form.

b. The full log likelihood function is displayed and used in computing information criteria.

8.8 Tests of Model Effects of Mortality

Source	Type III		
	Wald Chi-Square	df	Sig.
(Intercept)	1343.993	1	.000
Treatment	16.015	1	.000
day	51.947	3	.000

Dependent Variable: Mortality

Model: (Intercept), Treatment, day

8.9 List of photographs

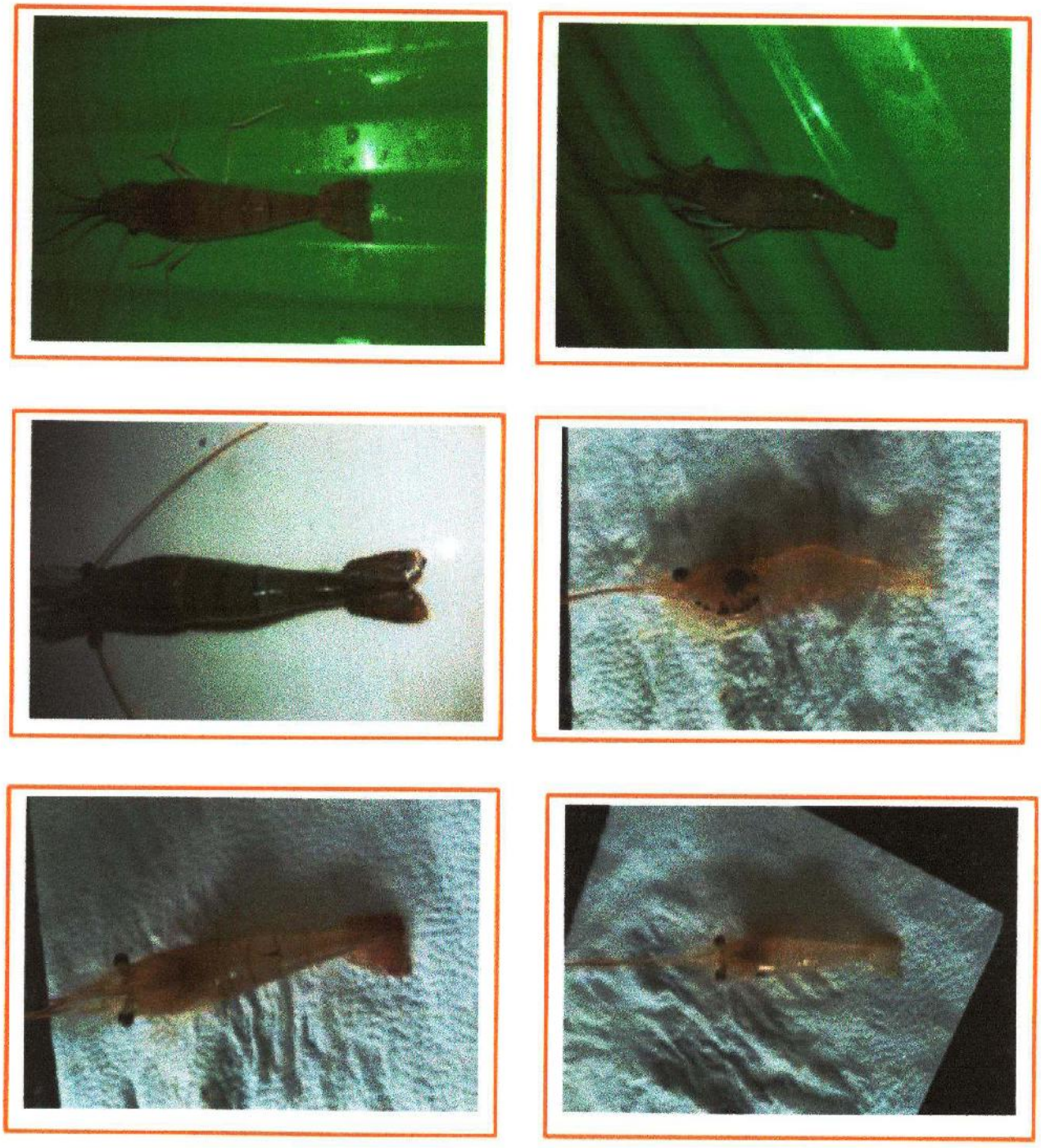


Fig: Physical appearance of prawns during experimental period

Fig: Pathogen

Fig: Experimental tank

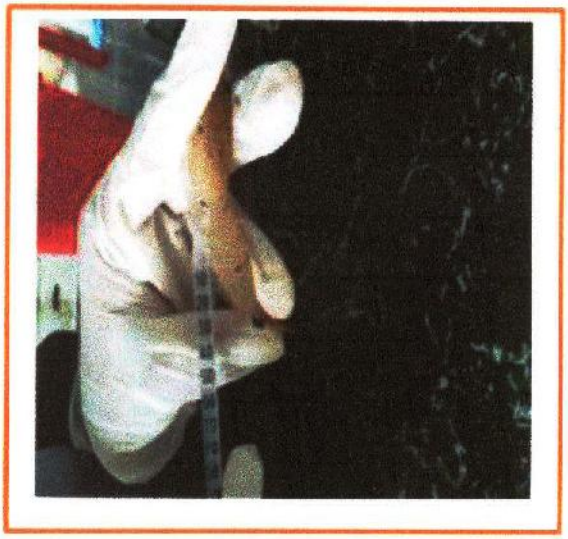
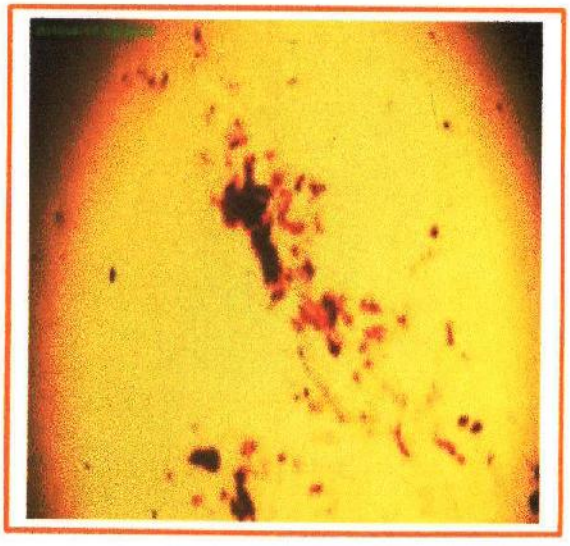


Fig: Haemolymph collection

Fig: Haemocyte analysis



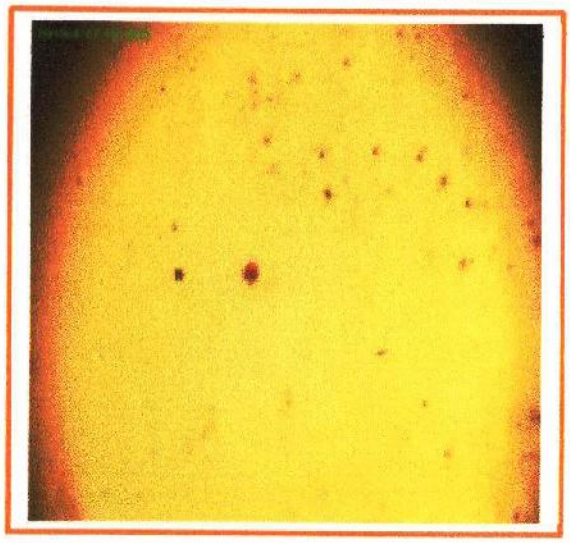


Fig: Differential haemocyte of prawns