Haematological and Biochemical changes of prawn in different water temperatures

A Thesis

By

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DECLARATION

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

Faria Sultana Mita

(Signature of the candidate)

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DEDICATED

TO

MY BELOVED PARENTS

Haematological and Biochemical Changes of Prawn in Different Water Temperatures

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ABSTRACT

This study was conducted to evaluate the effects of different ambient water temperatures on growth, physical appearance, histology (blood, gills) and biochemical compositions of freshwater prawns raised under standardized culture conditions. Prawns averaging 14.5 to 22.0 g were stocked into four controlled groups glass aquarium with aeration during the experiment period. Prawns in all control groups were fed twice daily according to a computer generated feeding schedule. Treatment groups prawns weight was less then controlled group after experiment. During the rearing period, the effect of four water temperatures (26°C, 30°C, 34°C and 38°C) on survivality and mortality rate, number of molts, and growth rate (molt increment and intermolt period) of ***Macrobrachium rosenbergii***, prawns was evaluated in laboratory conditions. The experimental species showed some similarities in the both survival and growth pattern at different temperatures. The survival rate was the highest at 26°C and 30°C and decreasing at the highest temperature (38°C) after collecting haemocyte from the body. The number of molts was increased at higher temperatures for *M .rosenbergii*, corresponding those values to 26°C and 30°C, respectively. The size increment by molting was increased significantly ($P<0.05$) from 30°C to 38°C, whereas a reduction in the growth of prawns was observed at 38°C. Significant differences ($P<0.05$) among temperatures were found in some treatments in the haemocyte of Prawns before and after the experiment. Haemocytes were found to be in large number at 26°C to 30°C but decreasing with increased temperature like 38°C. Non granular and small granular, blood cell were large in number at this species of prawn. Histological changes of gills and haemocyte of *M. rosenbergii* were examined. In histological experiments, a partial changes were observed in prawns gills chamber. In addition the biochemical composition of muscle was investigated during the Histological development of the studied species. In addition, the percentage of biochemical composition data indicated that the protein, moisture content was significantly decreased, while lipid and ash contents were increased during the experiment. The lowest protein content was found in the muscle of prawn at 38°C and the highest at 26°C. It was noted that the changes of components in the prawns muscle was related to the gradual increase of the temperatures at high level during the experiment.

CHAPTER 1

INTRODUCTION

CHAPTER - 01

1. Introduction

1.1 Background of the study

The fisheries sector is one of the most productive and dynamic industries which have a tremendous potentiality for future development in the agrarian economy of Bangladesh. Bangladesh is one of the world's leading fish producing countries with a total production of 45.03 lakh MT in FY 2019-20, where aquaculture production contributes 57.38 % of the total fish production (FRSS, 2020). Average growth performance of this sector is 5.26 % for last 10 years. Aquaculture shows a sturdy and consistent growth, average growth rate is almost 10 % during the same timeframe. It is believed that if the increasing trend of fish production continues, it will be possible to achieve the projected production target of 45.52 lakh MT by 2021 in conformity with the targets of Vision-2021 of the present Government. According to BBS, after 46 years of independence, Bangladesh becomes a self-sufficient country in fish production, with a per capita fish consumption of 62.58 g/day against set target of 60 g/day. Fish and fisheries products contribute 1.39 % to total export earnings (DoF, 2020).

According to FAO report, The State of World Fisheries and Aquaculture 2020, Bangladesh ranked 3rd in inland open water capture production and 5th in world aquaculture production. In 2019-20, this sector contributes 3.57 percent to the national GDP and more than one-fourth (25.30 %) to the agricultural GDP. More than 11 percent of total population of Bangladesh is engaged in this sector on full time and part time basis for their livelihoods. This sector also has high potential for the perspective of economic development of the country. Department of Fisheries awarded Bongabondhu National Agriculture Award 1423 for its outstanding performance during the recent past years. Aquaculture has shown great growth in the last decades. Due to the restrictions on water use, production systems are becoming increasingly more intensive, raising concerns about the maintenance water quality (FAO, 2020).

Bangladesh's agricultural sector contributes 14.2% of GDP, employing 47% of the working population with 17 million people (1.4 million women) depending on fisheries sector for their livelihoods through fishing, farming, fish handling, and processing. Agricultural sector has experienced significant growth over the last couple of decades, with the fisheries sector

following suit. According to the data from the Yearbook of Fisheries Statistics (2019-20), the current CAGR for the fisheries sector is 5.28% over the last 10 years. According to industry estimates, fish production will reach 5.02 million metric ton within 2020-21.

Total shrimp and prawn production including capture has increased from 1,60,000 MT in 2002-03 to 2,57,888 MT in 2019-20. The growing export can be attributed to the sector's steps in ensuring HACCP procedure and traceability regulation according to the requirement of European Union (EU) and USA. The EU and USA accounts for 80% of the total shrimp export in 2015. Other shrimp export destinations include Belgium, UK, Netherlands, Germany, USA, China, France, Russian Federation, Japan and Saudi Arabia.

Prawn-Shrimp is one of the major export items in Bangladesh. Total shrimp and prawn production including capture has been increased from 1.60 lakh MT in 2002-03 to 2.54 lakh MT in 2017-18. Different programs and development projects are also being implemented for the increased production and promotion of shrimp aquaculture. For enhancing shrimp production and promote business-friendly supply chain through adopting good aquaculture practices, cluster farming approach is popularizing. The government has maintained quality standards in all stages of fish and shrimp production, processing and export. More emphasis also provided on the hygienic and safe fish and fishery products supply in the domestic market. DoF has strong monitoring on the food supply chain to ensure food safety of the consumers.

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 (Wang et al., 2019). *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 (APHA, 2005; Ashim et al., 2009).

Giant freshwater prawn is an important crustacean aquaculture species, and Bangladesh is considered as the one of the top producer countries. Prawn aquaculture is substantially important for meeting the world's growing demands for food (DoF, 2020).

Crustaceans are invertebrates that depend on water quality for survival and are increasingly subjected to contaminated or low-quality waters (Silvia et al., 2019). The easy exposure to contaminants or the alteration in physical and chemical water parameters causes stress responses in these organisms (Camargo and Alonso, 2006), such as growth inhibition, reproductive alterations, and changes in immune and behavioral responses (Kumar et al., 2015). In production systems, water quality is a basic requirement to achieve good levels of productivity and the factors that most affect aquatic organisms are nitrogen compounds (Camargo and Alonso, 2006). Among them, ammonia and nitrite are the compounds that most affect the health of aquatic organisms since they quickly reach toxic concentrations. Gills of these organisms are directly in contact with the environment. Therefore, this organ is preferentially subject to the deleterious action of nitrogen compounds (Henry et al., 2012). These compounds can cause damage to the gill structure, as observed in fish including hyperplasia, lamellar fusion, thickening of the epithelium and necrosis (Patrick Saoud et al., 2014).

In Brazil, *M. amazonicum* is one of the native freshwater prawn species with the highest potential for aquaculture (Moraes-Valenti and Valenti, 2009) due to its tasty flesh, its broad regional distribution, and its rusticity, which favors its rearing in captivity. The species presents possibility of intensification in production, with productive systems with high stocking density. Although high concentrations of ammonia and nitrite are not trivial in non-impacted natural aquatic environments (Camargo and Alonso, 2006), the increase in concentration of these compounds in production environments is rather frequent. Furthermore, because of the growing restrictions on water use due to environmental pollution caused by the release of wastewater and excessive water use, aquaculture production systems are becoming increasingly more intensive, with higher stocking densities, higher artificial feed supply and less water renewal or even zero exchange (Ballesteret al., 2017). In this context, concerns about how to deal with higher concentrations of ammonia and nitrite are becoming a real fact. Toxicology studies using lethality assays (LC50) are recommended to provide basic information on the toxic effects of a substance for a living organism, however, they do not provide information on the damage caused to the organism due to poisoning. Thus, histopathology has been a commonly employed tool in

the identification of damage caused to organisms subjected to a toxic agent. Although some studies with histological analyses have assessed the effect of pesticides, heavy metals, viruses (Pazir et al., 2011) and cobalt–60 gamma radiation (Stalin et al., 2019) on the gills of prawns, none has assessed the effects of nitrogen compounds on the gills of freshwater prawns. Therefore, the present study aimed to assess the histological alterations in the gills of *M. rosenbergii* juveniles subjected to different total ammonia and nitrite concentrations and to compare them using the organ index.

1.2 Justification of the study:

There are several studies with *M. rosenbergii*, including farming practice, stocking density, prawn feed development, probiotics effects and immunity in Bangladesh; but, to the best of my knowledge, there is no meticulous scientific work undertaken to compare the blood cell composition with the effects of different temperatures and its biochemical components analysis, histology of gills during or before experiment for variants of temperature.

Taking into account of this, the present study was conducted to evaluate the changes of gills, blood cell composition and biochemical components with the effects of different temperature.

1.3 Objectives of the study:

- To determine the survival rate of *Macrobrachium rosenbergii* exposed to different water temperatures (experimentally).
- To estimate total and differential haemocyte count of *M. rosenbergii* exposed to different water temperatures and their differences between control and treatment groups.
- Histology of gills exposed to different temperatures.
- To determine the ratio of biochemical analysis (Proximate Composition) of *M. rosenbergii* exposed to different temperature.

CHAPTER 2

LITERATURE REVIEW

CHAPTER -02

2.0 Review Of Literature:

2.1Crustaceans:

Crustacean, any member of the subphylum Crustacea (phylum Arthropoda), a group of invertebrate animals consisting of some 67,000 species distributed worldwide (Smith, 2001). Crabs, lobsters, crayfish, shrimp, krill, barnacles, Prawns and wood lice are among the best-known crustaceans, but the group also includes an enormous variety of other forms without popular names. Crustaceans are generally aquatic and differ from other arthropods in having two pairs of appendages (antennules and antennae) in front of the mouth and paired appendages near the mouth that function as jaws. Because there are many exceptions to the basic features, however, a satisfactory inclusive definition of all the Crustacea is extraordinarily hard to frame. Crustaceans are found mainly in water. Different species are found in freshwater, seawater, and even inland brines, which may have several times the salt concentration of seawater. Various species have occupied almost every conceivable niche within the aquatic environment. An enormous abundance of free-swimming (planktonic) species occupies the open waters of lakes and oceans. Other species live at the bottom of the sea, where they may crawl over the sediment or burrow into it. Different species are found in rocky, sandy, and muddy areas. Some species are so small that they live in the spaces between sand grains. If the Arthropods are regarded as a superphylum, then the insects and crustacea would be phyla.

2.2 Taxonomy and Morphology of Freshwater Prawn (*Macrobrachium rosenbergii*):

2.2.1 Taxonomy

*M. rosenbergii*is a large freshwater prawn native to the Indo-West Pacific from northwest India to Vietnam, Philippines, New Guinea and northern Australia. It has been introduced into many countries for aquaculture. Adverse impacts have not been reported so if there are effects they have so far not been noticeable. However, impacts of escapes in tropical river systems might not be noticed that readily. There is the potential for viruses to be introduced via aquaculture stock and a possible risk of interbreeding with local species. This seems to be a very small risk since it has been demonstrated that in the case of *M. carcinus* and *M. rosenbergii* (Graziani et al., 2003)

no successful pairings occurred. There has hitherto been little research into evolutionary and taxonomic relationships amongst species of the freshwater prawn genus *Macrobrachium* Bate across its global distribution. Previous work by the authors demonstrated that the endemic Australian species did not evolve from a single ancestral lineage. To examine whether other regional *Macrobrachium* faunas also reflect this pattern of multiple origins, the phylogeny of 30 *Macrobrachium* species from Asia were documented (Murphy and Austin, 2005).

Taxonomic Tree:

Domain: Eukaryota

Kingdom: Metazoa

Phylum: Arthropoda

Subphylum: Crustacea

Class: Malacostraca

Subclass: Eumalacostraca

Order: Decapoda

Suborder: Natantia

Family: Palaemonidae

Genus: ***Macrobrachium***

Species: ***Macrobrachium rosenbergii***

Scientific name: ***Macrobrachium rosenbergii***

Common name: Giant freshwater prawn (De Man, 1879)

2.2.2 Morphology and Physical appearance:

M. rosenbergii is a striking looking prawn in which the second pair of walking legs can really justify the genus name meaning 'large arms'. In the males these walking legs can have a vibrant shade of blue and can also be twice the body length. The largest males can attain a total length from tip of rostrum to the end of the telson of 320 mm compared to 250 mm for the largest females (Hernández-Sandoval et al., 2018). In general, the body form is typical of a decapod crustacean with the head and thorax fused into a cephalothorax. The rostrum at the front end of the cephalothorax is very prominent with 11-14 dorsal teeth and 8-10 ventral teeth (Mazlum et al., 2011). Another distinctive feature of the adult male is that the moveable finger of the second walking leg or cheliped is covered in tightly packed long setae that give a velvety appearance to the appendage. The first 'walking leg' is not readily visible being very long and delicate in form but tightly folded up under the cephalothorax and functions as a feeding appendage with fine forcep-like chelae at their tips. Males can attain larger size than females and in dominant males the second walking legs are much longer and thicker (Magnuson et al., 1979). The abdomen of the male is narrower and the female, as well as having a wider abdomen, has longer pleura (the overlapping plates of cuticle extending from the exoskeleton) and these combined form a chamber for incubating the eggs carried on the pleopods. The male genital openings are on the fifth walking legs and the females genital pores are on the third walking leg (Kir & Kumlu, 2008). The colours of *M. rosenbergii* can vary according to where the prawns are found but the body can be greenish-grey. In small individuals, delicate striping on the cephalothorax can be seen but these markings are not apparent in tank-reared specimens. The chelipeds of dominant males are bright blue but more yellowish in non-dominant males and females. The ventral side is pale and translucent.

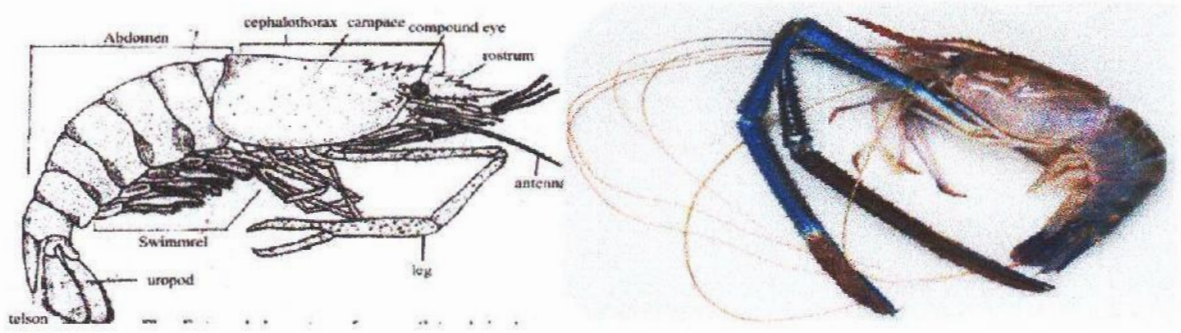


Fig-01: Morphology of prawn (Kir & Kumlu, 2008).

2.3 Distribution:

All the freshwater prawns that have been cultured so far belong to the genus *Macrobrachium* (González et al., 2010), the largest genus of the family *Palaemonidae*. About 200 species have been described, almost all of which live in freshwater at least for part of their life. Fifty three freshwater habitats located in and around Bangalore (South India) were surveyed for studying the distribution and abundance of prawns. They are found in most inland freshwater areas including lakes, rivers, swamps, irrigation ditches, canals and ponds, as well as in estuarine areas. Most species require brackish water in the initial stages of their life cycle (and therefore they are found in water that is directly or indirectly connected with the sea) although some complete their cycle in inland saline and freshwater lakes. Some species prefer rivers containing clear water, while others are found in extremely turbid conditions (Nayak, 2010). *M. rosenbergii* is an example of the latter. There is a wide interspecific variation in maximum size and growth rate, *M. rosenbergii, M. americanum, M. carcinus, M. malcolmsonii, M. choprai, M. vollenhovenii* and *M. lar* being the largest species known. *M. americanum* (Cauque river prawn) is found naturally in western watersheds of the Americas while *M. carcinus* (painted river prawn) is found in those connected with the Atlantic. *M. choprai* (Ganges river prawn) is found in the Ganges and Brahmaputra river systems. *M. lar* (Monkey river prawn) is native from East Africa to the Marquesas Islands of the Pacific (and was introduced into Hawaii). *M. malcolmsonii* (monsoon river prawn) is found in the waters of Bangladesh, India and Pakistan (Pazir,et al., 2011). *M. rosenbergii* (giant river prawn) is indigenous in the whole of the South and Southeast Asian area as well as in northern Oceania and in the western Pacific islands. *M. vollenhovenii* (African river prawn) is naturally distributed in West Africa, from Senegal to Angola. Many *Macrobrachium* species have been transferred from their natural location to other

parts of the world, initially for research purposes. *M. rosenbergii* remains the species most used for commercial farming and consequently is the one which has been introduced to more countries. Viet Nam is also a major producer, according to New (2000b). In addition, there are also valuable capture fisheries for *M. rosenbergii*, for example in Bangladesh, India, and several countries in Southeast Asia. Giant river prawn culture has developed in every continent, particularly in Asia and the Americas. Aquaculture of the giant freshwater prawn, *Macrobrachium rosenbergii* are widely in practice, especially in Asian countries. *Macrobrachium rosenbergii* is among the freshwater prawn species with favorable characteristics for production and possibility of intensification.

Fig -02: Distribution site of Prawn in World Map view (marked Places)

2.4 Feed and nutritional requirement:

Almost all crustaceans have the same basic mouthparts although the diversity in form and function is enormous. The main part of the prawn diet is made up of plant material, microorganisms, small shellfish and worms (Murphy and Austin, 2005). In aquaculture, prawns are fed artificial diets. Most digestion is rapid and completed in about six hours. Prawns search the bottom for food with their pereopods which have chemo-sensory hairs attached. Any food that is found is held by those head appendages known as maxillipeds and the teeth or mandibles

are used to bite or tear pieces off. The maxillipeds can be used to push large pieces of food away as the mandibles grasp it, thus tearing the food into manageable portions.

2.5 Culture system of prawn:

Prawn culture methods outlined by those promoting the growth of prawns assumes that natural food organisms are present when ponds are stocked with post-larvae or small juveniles. Fertilization is recommended to produce blooms of algae and zooplankton, but many prawn producers fail to achieve the necessary zooplankton numbers to provide adequate food for the prawns. Often, plastic tanks and plastic lined ponds fail to produce prawns because these artificial systems lack natural food organisms. Earthen ponds will eventually establish zooplankton blooms if fertilization is applied, but the timing of prawn stocking must be delayed until a suitable bloom is present.The system for prawn production involves three phases: hatchery phase, nursery phase, and grow-out phase. Mature prawns from the grow-out phase are then used as spawners for the hatchery phase. Hatcheries have been developed in Texas, Mississippi, Kentucky, Florida, South Carolina and Hawaii. Most of them have"high health" facilities with the promise of certified disease free stock (Parrinello et al., 2015). However, there is always a concern that viral diseases may be encountered if due diligence is not practiced by the prospective prawn buyer. The nursery phase has developed as a means to grow prawn post-larvae to the juvenile stage.

2.6 Site requirements for freshwater prawn production

A good site for prawn production would be a site similar to that needed for construction of other types of aquaculture ponds. The terrain should be flat to gently sloping but well drained. Ponds constructed below the water table are not easily drained and would not allow efficient prawn harvesting. The best site would have soils with at least 25% clay content within two feet of the surface. However, pond liners may be utilized in soils with lower water holding ability. Water availability is important since freshwater prawn ponds are filled each year. The water source can be from ground water or surface water. Location of freshwater prawn ponds near population centers is good for marketing (Romano & Zeng, 2013). The pond site should also be convenient to the manager's center of operations for labor and security reasons.

2.7 Pond Construction

Pond construction planning must take into account engineering requirements, biological needs of the species that is being cultured, and cost of construction. When exceptions are made in these areas, catastrophic results can occur. Some examples of pond failures include: dam failure due to improper compaction or soil type, rapid sedimentation and levee erosion when levee slope is too steep (Saquetto et al., 2019), and business failure when pond construction costs are excessive for the potential return from aquaculture.

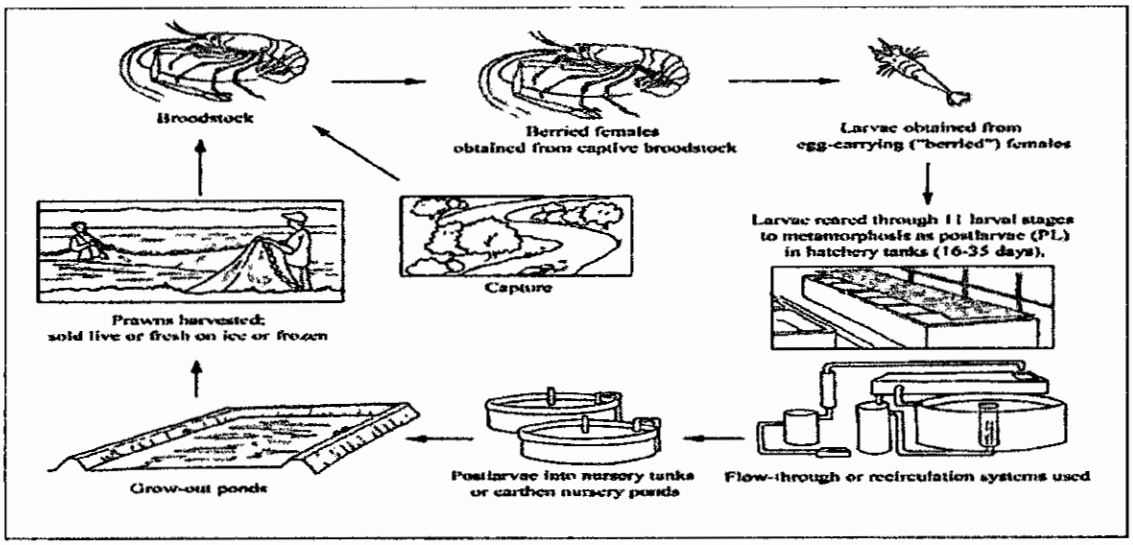


Fig -03: Prawn culture System (Flow Chart) (Stalin et al., 2019)

2.8 Life cycle of prawn:

This species lives in tropical freshwater environments is influenced by adjacent brackish water areas. It is often found in extremely turbid conditions. Gravid females migrate downstream into estuaries, where eggs hatch as free-swimming larvae in brackish water. Before metamorphosis into postlarvae (PL), the planktonic larvae pass through several zoeal stages. After metamorphosis, PL assume a more benthic life style and begin to migrate upstream towards freshwater. Larvae swim actively tail first, ventral side uppermost. From PL onwards prawns swim forwards, dorsal side uppermost. From metamorphosis onwards prawns can also walk, not

only on the sub-stratum but also over damp areas including stones by river edges, up vertical surfaces (small waterfalls, weirs, etc.) and across land. Larvae mostly consume zooplankton (mainly minute crustaceans), very small worms, and larval stages of other crustaceans. Postlarvae and adults are omnivorous, eating algae, aquatic plants, molluscs, aquatic insects, worms, and other crustaceans (Tsing et al., 1989). Males and females have different growth rates and males exhibit heterogenous individual growth (HIG); these are vitally important factors in grow-out management. Three distinct male morpho types (and a number of intermediary types) exist: small male (SM), orange claw males (OC), and blue claw males (BC). The normal male developmental pathway is SM → OC → BC. BC males have extremely long second pereiopods; those of OC males are golden coloured; SM have small, slim, almost translucent claws. The type and behaviour of the males affects the growth rates of other prawns. The transition from rapidly growing OC to the slowly growing BC morphotype follows a "leapfrog" growth pattern. An OC metamorphoses into a BC only after it has become larger than the largest BC in its vicinity. The presence of this new BC male then delays the transition of the next OC to the BC morphotype, causing it to attain a larger size following its metamorphosis. BC males dominate OC males, regardless of their size, and suppress the growth of SM. Male prawns are readily identified by the large copulatory organ, known as the petasma (Reddy, 2014), found between the foremost pair of swimming legs. Corresponding structures are not present in the female and the foremost pair of pleopods is identical with the other four pairs. Females possess a structure known as the thelycum between the last pair of pereopods which provides an anchor site for the male sperm packet received during copulation. Males and females can thus be readily identified on the presence or absence of a petasma or a thelycum. Ovaries containing eggs can often be seen through the shell in the head and tail of mature female prawns. Prawn life cycles in waters can be considered to be of three types:

A. Estuarine – the entire life cycle is completed in waters of less than sea water salinity. An example of this type would be the inshore greasy back prawn;

B. Marine – the entire life cycle is completed in oceanic waters. An example of this type would be the royal red prawn

C. Mixed – post larval and juvenile stages of the prawn's life cycle occur in estuarine waters of less than sea water salinity whilst the adolescent, adult, egg and larval stages occur offshore in oceanic waters. Examples of this most common type include eastern king and school prawns.

Fertilized eggs are shed onto the bottom in oceanic waters where they remain for a short period until they hatch. The first larval stage called a nauplius emerges from the spherical egg and starts swimming up towards the surface. This naupliar stage goes through several moults, getting larger with each moult, and ultimately changes to a protozoea. The prawn similarly moults and grows through several protozoea then mysis stages before developing into a postlarva (Valdez, 2006). The mysis stage is the first stage that somewhat resembles the appearance of the adult prawn. The development time from spawning to post larva for prawn species with 'estuarine' or 'mixed' life cycles is between two and three weeks. Hatching success and larval survival are affected by water temperature and salinity and are greatest in waters of the same temperature and salinity as that where spawning took place. Up to the mysis stage the larvae are free swimming and form part of the zooplankton found in the open sea. Zooplankton together with phytoplankton is eaten by a multitude of other animals, including larval prawns, and is a vital part of the ocean's food system. After the free swimming, planktonic mysis stage, post larva adopt a bottom existence, reach the shore and enter the rivers and coastal lakes In the estuaries and lakes, the juvenile prawns have to adapt to wide fluctuations in, among other things, salinity and temperature. School prawns appear to be more tolerant of these fluctuations than eastern king prawns. After over-wintering in the estuaries and lakes the juvenile prawns grow rapidly and upon some cue, start to migrate back to the ocean (Negrini et al., 2017). These migrations rise to a peak in the 'dark' period of the summer, lunar cycles and are fished enthusiastically by both amateur and professional fishermen. The physiology of prawns with a 'mixed' life cycle is such that the prawns require the conditions found in the deeper ocean waters to reach maturity. Once they reach oceanic waters, the prawns rapidly mature (Kurmaly et al., 1989), mate and spawn – thus completing the life cycle. The life cycle of prawns is rather short with species such as school prawns living, for the most part, a little over a year while larger species such as eastern king prawns are probably up to two years old, with some individuals perhaps entering a third year of life (Lee et al., 1996). Prawns have specific habitat preferences which differ between species and between life stages for the same species. For instance, eastern king prawns are less tolerant of low salinity than school or inshore greasy back prawns and so whilst in estuaries will be found in

the 'marine dominated' area of the estuary (Lemaire et al., 2002). Generally, prawns prefer a substrate with high mud content. School prawns though are found in muddy substrate in estuaries but in ocean waters prefer fine sand. Eastern king, school and inshore greasy back prawns prefer a vegetated to an unvegetated substrate. In terms of movements during their life times, inshore greasy back prawns would be confined to their 'home' estuary or coastal lake (Lutterschmidt & Hutchison, 1997). Tagged school prawns on the other hand have been reported to have moved up to 120 km from their 'home' estuary, whilst tagged eastern king prawns have been recorded as moving northwards over distances of up to 1333 km (Magnuson et al., 1979). Total prawn catch in any one season therefore depends upon a complicated interaction of many factors including the success of spawning, the effects of environmental changes, the success of larval stages in finding suitable habitat and, the effects of predators and diseases on all stages of Prawn's life cycle.

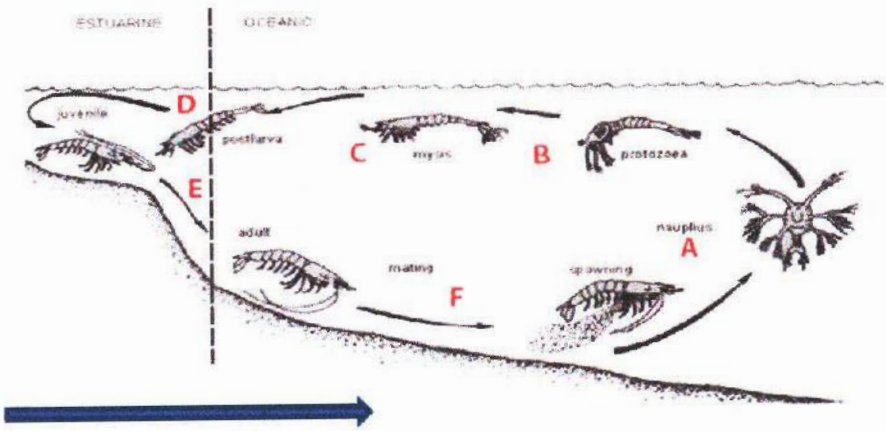


Fig -04: Life cycle of Prawn (Kurmaly et al., 1989)

2.7 Histology of prawn:

The effect of dietary supplementation of probiotic bacterium *Bacillus licheniformis* on the histopathological changes in *Macrobrachium rosenbergii* juveniles (4.0 ± 0.02 g) challenged with known pathogenic strain of *Vibrio alginolyticus* are reported. Two isocaloric basal diets supplemented with probiotic bacteria *B. licheniformis* (1.0 × 109 cfu/g feed) and other without probiotic supplementation were fed to the *M. rosenbergii* juveniles for 45 days (Kumar, et al.,

(9.34%) and carbohydrate (32.69%) contents are found in *S. siamensis*. The highest amino acid content is recorded in S. sirindhornae (784.92 mg g−1 dw) followed by that of *B. thailandensis* (596.12 mg g−1 dw) and *S. siamensis* (439.58 mg g−1 dw). Predominant amino acids found in all three species are lysine, phenylalanine, leucine, tyrosine, and glutamic acid. For fatty acids, palmitic acid C16:0, followed by *S. siamensis* (211.92 μgg−1) and *S. sirindhornae* (128.93 μgg−1). Carotenoid content analysis show the presence of dominant groups consisting of β-carotene, canthaxanthin, astaxanthin, and lutein (Dararat et al., 2012).

2.9 Water quality parameters for prawn culture:

Management of water quality is of primary consideration particularly in ponds with higher stocking rates. The water should be neither too acid nor too alkaline; neutral or slightly alkaline waters are most suitable for fish culture and hence acid water should be limed to make it neutral. Waters with pH values below 5.5 or over 8.5 are not proper for fish culture. When water has poor quality, the animals are exposed to various types of physical, chemical and biological stresses, which will negatively impact the rates of growth, reproduction and survival of Prawn (White, 1983). In addition to favoring the appearance of diseases that could often kill the entire group. Therefore, it is necessary to understand the quality parameters to be controlled to ensure a healthy aquatic environment and favorable to the good development of the Prawns (Azad et al., 2018). Suitable water quality parameters for Prawns culture are given in this table 1:

Parameters (Unit)	**Range**
Water temperature (°C temperature)	18-32
Ammonia [unionised] (mg/l)	<0.3
Dissolved oxygen (mg/l)	5-7
Hardness (mg/l of Calcium Carbonate)	40-100
Alkalinity (mg/l of Calcium Carbonate)	20-60
Salinity (part per thousand)	<10

Water pH (pH)	7.5-8.0

Table -01: Water quality parameters for Prawns culture

2.10 Suitable temperature for Prawns culture:

Water temperature plays a very important role in regulating the activities of the cultured animal. The rate of chemical and biological reactions is said to double every 10°C increase in temperature. This means that aquatic organisms will use twice as much dissolved oxygen and chemical reactions will progress twice as fast at 30°C than 20°C. It follows therefore that dissolved oxygen requirement of aquatic species is higher in warmer than in cooler water.

Many *Penaeid* species are tropical or subtropical species. The optimum temperature is about 25–30°C and hence many of the species such as *P. indicus, P. monodon and P. merguiensis* can be cultured throughout the year while *P. japonicus and P. orientalis* are limited to the summer growing seasons only. The temperature measurement of traditional shrimp farm waters was obtained the temperature of 29.56 ± 0.75°C (Gargioni & Barracco,1998). Meanwhile, temperature of pond waters found of temperature measurement result in the coastal waters and river was 28.56 ± 2.72°C and 26.77 ± 19.94°C, respectively. The temperatures observed were still suitable for shrimp culture. Temperature with range from 20-32°C was temperature values recommended for shrimp culture and 29-30°C were the optimum temperature for cultured shrimp growth (Gupta et al., 2016.)

2.11 Acclimatization:

Acclimatization to a thermal stress will normally occur within 5–7 days of initial exposure to that combination of work and temperature. The PL of *Macrobrachium rosenbergii* was subjected to acclimatization to freshwater for different periods ranging from 2 to 20 days. The best survival rate of 95% was obtained when the acclimatization period was 5 days and the lowest survival of 75% was obtained when the period of acclimatization was only 2 days (Tagliacozzo et al., 2013). When the acclimatization period was 20 days, the survival was 80%. In the case of *Macrobrachium rosenbergii*, the best survival of 93% was obtained when the period of acclimatization was 5 days and the survival was 80% when the period of acclimatization was only 2 days (Camargo,2006).

2.12 Stocking Density:

Stocking density is an important parameter in fish culture operations, since it has direct effects on the growth and survival and hence on production. It is an established fact that growth rate of fishes progressively increase as the stocking densities decreases and vice-versa.The grow-out of freshwater prawns is generally carried out in earthen ponds with fertilization and supplemental feeds, considered a semi-intensive system, and the stocking is accomplished with post-larvae or juveniles, in densities which vary from 4 to 20 ind m-2 (Valenti et al., 2010). Stocking densities for rearing *M. rosenbergii* in super intensive biofloc system still need to be established.

2.13 Length- weight Measurement:

Length-weight relationships (LWRs) and relative condition factor are of great importance in fishery assessment studies since it provide information about the growth of the fish, its general wellbeing, and fitness in a marine habitat. GRO~ is manifested as an increase in size of the prawn and as such is best measured in terms of its volume or weight. But, it is usually gauged from observation of its linear dimension, ie., total length. It has been mathematically proved that there is a fairly constant relationship between total length and weight of the individuals of the species. Therefore, when a knowledge of the growth in volume of weight is required, it is usually calculated from length-weight relationship. The length-weight relationship is also needed for studies on maturity and yield estimates by analytical models (New & Nair, 2012). .

2.14 Disease:

As is common with all other animals including humans, prawns may suffer from a range of diseases and parasites. Some of these kill the prawn whilst others result in changes in appearance and behavior. One common symptom of stress in a prawn is opaque and white segments in the tail. This can be caused by viruses, bacteria, protozoans or physical trauma. The incidence of disease or parasitic infection is low in the wild but increases under aquaculture conditions when the prawns are in high densities and transmission is thereby enhanced. Viral diseases including taura syndrome, 'white-spot' disease, and 'yellow-head' disease are major health problems with prawn farming worldwide, but Australia remains free of these exotic diseases. However,

infections caused by indigenous prawn viruses such as gill-associated virus do affect the industry (Cheng and Chen 2002a).

2.15 Biosecurity:

Biosecurity is a strategic and integrated approach to analysing and managing relevant risks to human, animal and plant life and health and associated risks for the environment. The term "biosecurity" has been defined differently by various disciplines (Tsing et al., 1989). The term was first used by the agricultural and environmental communities to describe preventative measures against threats from naturally occurring diseases and pests, later expanded to introduced species. Biosecurity is the concept of protecting culture animals from contamination by diseases and of preventing the spread of diseases across boundaries, has become increasingly important with the intensification of aquaculture production systems. A significant challenge to the expansion of aquaculture production is the outbreak of disease. Potential economic losses from disease outbreaks are significant, and can affect the survival of the industry. By implementing biosecurity, the risk of pathological events will be reduced. In general, biosecurity is more easily implemented in small, intensive, and controlled farming systems than in outdoor and large-scale operations. Biosecurity measures in the Prawn-shrimp industry can be seen as a two-pronged approach: excluding pathogens and eliminating pathogens when they are present. Nayak (2010) discussed ways of excluding pathogens from stock (i.e., post larvae and broodstock), especially through the use of quarantine and specific pathogen-free (SPF) certified stocks, and restricting imports of live and frozen shrimp. Excluding vectors and external sources of contamination and preventing internal cross contamination were suggested methods for excluding pathogens from hatcheries and farms (Seenivasan et al., 2012).

2.16: Marketing Status:

Both domestic and international markets exist and are expanding day by day. Peeled, mostly wild-caught *Macrobrachium rosenbergii* have long been exported globally, but farmed shell-on (and normally head-off) freshwater prawns are also a familiar sight in the supermarkets of Europe now. To a lesser extent, this also occurs in the USA (mainly for consumption by Asians or in restaurants serving Asian food) and Japan. India, Bangladesh, Viet Nam and Thailand export a significant proportion of their wild-caught and farmed prawns. Potential for expansion

exists but small-scale producers may need to co-operate in collective marketing to exploit these opportunities. Freshwater prawns are a distinct product from marine shrimp, which have their own favorable culinary characteristics (Banu & Christianus 2016). Farmed production is rapidly expanding in Asia. The rate of expansion in the largest producer, China, has slowed, partly due to the farming of an alternative indigenous species (*Macrobrachium nipponense*) and partly because marine shrimp are now being reared in freshwater (and are sometimes referred to erroneously as freshwater prawns). Chinese production actually fell in 2002 but, as the global market expands, is expected to expand again later. Production in India and Thailand expanded by more than 50 percent per year between 1999 and 2002; this trend is expected to continue (D'Abramo & New 2010). There is also potential for expansion in Bangladesh, a traditional exporter from its capture fisheries. Indonesian production is reported in 2002 for the first time. Viet Nam is a significant producer and exporter of farmed *Macrobrachium*, although its output is masked by being included in the statistical category 'freshwater prawns, shrimps nei'. Statistically, Brazil also appears as a major producer (>4 000 tonnes/yr since 2000) but this is believed to be an error. In total, the output of *M. rosenbergii* from aquaculture expanded during the decade 1993-2002 from 17 000 tonnes to 195 000 tonnes, an APR of 31 percent/yr. Since 1996, Chinese production has formed a large proportion (58 percent in 2002); however, even if it is excluded it becomes clear that production elsewhere grew from 17 000 tonnes in 1993 to nearly 82 000 tonnes in 2002, an APR of 19 percent/yr. Furthermore, from 1999 to 2002, expansion outside China (41 percent/yr) was much faster than in China (13 percent).Further global expansion is difficult to predict, since it depends mainly on the volume of consumer demand (Banu & Christianus, 2016). However, even if a very modest expansion of 10 percent/yr occurs, global farmed production of *M. rosenbergii* will have significantly exceeded 400 000 tonnes by 2010. The farming of other species of *Macrobrachium*, notably *M. nipponense*(already very substantial in China), *M. malcolmsonii* and *M. amazonicum*, is also expected to expand (New, 2005).

CHAPTER 3

MATERIALS AND METHODS

CHAPTER -03

3. Materials and Methods

3.1 Profile of the study area

The study was conducted at the Fish wet Lab, Fish Nutrition and Water Chemistry labs of Fisheries and Marine Resource Technology (FMRT) Discipline of Khulna University. The experiment was conducted in the Fish Wet lab, proximate analysis of *M. rosenbergii* and other analysis were done in Fish Nutrition lab of Fisheries and Marine Resource (FMRT) Technology Discipline of Khulna University.

3.1.1 Prawn collection and rearing:

For conducting this study, Young adult sizes Prawn were collected from Pond complex of Khulna University, Khulna. These Prawns were reared separately for a period of time in the experimental glass aquarium of Fish Wet lab of the FMRT Discipline, Khulna University, Khulna. Then the experiment was conducted in the Laboratories of Fisheries and Marine Resource Technology Discipline, Khulna University. Water temperature in the aquarium were kept in room temperature. Fishes were kept with continuous aeration.

3.2 Experiment Conducting Procedure:

Before conducting the experiment, the length –weight data of Prawns were measured in the Lab.. All samples were properly acclimatized in the aquarium. Before introducing feed, previous uneaten feeds and feces in aquarium were removed by siphoning with a plastic pipe. Then the next procedure had done by the sequence for conducting the proper experiment.

3.2.1 Experimental Design and Tank set up with temperatures:

The experiment was conducted by setting the different temperatures with thermostats to the each aquarium for conducting the experiment, to test the histological and biological changes of *M. rosenbergii* exposed to Different temperatures.

After acclimatization in the aquarium, some species of prawn had separated for conducting the next procedure of the experiment. The test temperatures (26, 30, 34 and 38°C) were regulated by using automatic thermostat by following design.

Temperatures (°C)	**26**	**30**	**34**	**38**
Stocking Density (Piece per aquarium)	**4**	**4**	**4**	**4**
Thermostat Number	**1**	**1**	**1**	**1**
Aerator	**1**	**1**	**1**	**1**
Feeding frequency (times/daily)	**2**	**2**	**2**	**2**

Table -02: Experimental Design of treatmented species. Each treatment has three replication. So, four treatment has total 12 replications.

3.3 Feeding rate:

The feeding frequency was 2 times per day (8 AM and 5 PM) at their satiation level. Dry pellet feed had given to the experimental species.

3.4 Length –weight Estimation:

It is important to emphasize that length-weight estimation had done on the experimental species. In the maximum case the length of the experimental Prawns were ranges from 2.5 to 4 inches and weight ranges from 12.5 to 23 grams. This estimation had done on the experiment before conducting the experiment.

3.5 Mortality:

Experimental prawn species were fed with a formulated prawn diet for two times in a day at 5% of its body weight ratio. During the experiment at 8 days challenge test, the mortality of these experimental species were checked and recorded daily.

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 Cuhan & Din (2010) 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) (Cheng et al., 2006). THC was measured according to the following formula:

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

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 anticoagulant 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 itpossess a total 4X16=64 squares (volume of each large corner square=1mm X1mm X0.1mm=0.1cm X0.1cm X0.01cm =0.0001cm3 or 10-4 cm3). 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 coverslip 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 (Taylor, 2009) and the relative proportions of each cell type expressed as a percentage of the total haemocytes counted (Hrubec and Smith, 2000). Thus the prawn haemocyte differential counts were presented as nongranular haemocyte NGH), Large granular haemocyte (LGH), Small granular haemocyte (SmGH), Semi granular haemocyte (SGH) (Campbell and Ellis 2007).

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=1mmX1mmX0.1mm=1mmX1mmX

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

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

3.7 Histological analysis of Gills:

The histological analysis of gills was conducted by randomly collecting a pooled sample of three prawns from each replicate at the final stage, which was quickly crio-anesthetized (−20 °C) for 15 min (to ensure that the prawns were fully anesthetized and unconscious). Further, the anterior gills were dehydrated in ethanol, soaked in paraffin wax, and cut into 5 µm slices. The slices were stained using Giemsa, xylene, hematoxylin and eosin before being attached to slides. Photomicrographs were taken using an advanced research microscope The images were categorized with reference to published depictions of development or changes in *M. rosenbergii* (Abol-Munafi et al., 2020)..

3.8 Biochemical composition Analysis:

The Proximate composition of feed ingredients was analyzed to determine the level of crude protein, lipid, moisture and ash of fish meal, soybean meal, wheat flour and rice polishing.

3.8.1. Crude Protein

The sample was digested with concentrated sulphuric acid (H2SO4) and resolvent followed by distillation with 40% NaOH according to AOAC (1995). Finally, by titration total N2 was determined that was multiplied by 6.25 to determine crude protein.

3.8.2 Lipid

Most method of measuring lipid content depends on extracting the lipid by dissolving it in a suitable solvent.

% of lipid = (Weight of lipid ÷ weight of sample) × 100

3.8.3. Moisture

The moisture content (%) of sample was determined by using an electric moisture determination apparatus, oven (model no. MA 30-000V3, SARTORIUS AG Gottingen, Germany) (Pearson and Eggum, 1976). In brief, approximately 2-5g sample was heated at 105 □C in an oven for 24

hrs and the difference in weight between the raw sample and dried sample was measured to determine the moisture levels of the sample.

3.8.4 Ash

The ash content (%) of sample was determined by complete ignition of the sample at 550□ (6-8 hours) in a digital muffle furnace apparatus (model no. DME-05, made in Korea).

3.9 Statistical analyses

As culture temperature was the only quantitative independent variable used in the present study due to having four variant levels, we have calculated the means using analysis of variance (ANOVA) with SPSS (V. 22). All types of statistical analysis were carried out with both Microsoft office Excel (version 2007) and SPSS (version 22). One way ANOVA was applied per variable among all densities ($\alpha = 0.05$). If significant differences among treatments were observed, a Tukey's test was executed to detect significant groups (Zar, 2010).

CHAPTER 4

RESULTS

CHAPTER - 4

4. Result:

4.1 Physical appearance:

The effects of prawn (*Macrobrachium rosenbergii*) stocking density and the use of commercial diet on rice–prawn culture were evaluated Survival rate was significantly higher ($P < 0.05$) for the 2 prawn m^{-2} density while the other variables did not vary significantly among treatments (Boock et al., 2016). At the beginning of the Challenge test, all the experimental species were healthy and fresh. But after four days in some cases like highest temperature, some changes were observed in treatment groups compared to control groups and gradually the prominent symptoms were evident. Physical appearance of the Prawns in the Experiment:

Characteristics	Control Group	Treatment Group
Locomotion	Normal	Slow and abnormal movement at highest temperature at 38(°C)
Body Color	Normal	Slightly fade
Shell condition	Normal	Shell damages, Moulting had occured
Tail	Normal	In some cases breakdown had observed
Appendages	Normal	Loosen and breakdown
Carapace	Normal	Soft for high temperature
Mortality	Normal	Occurance of mortality had observed at higher temperature after collecting haemocyte from the body.

Table -03: Physical appearance of Prawn during experiment.

4.2 Length – weight Measurement:

Length – weight of controlled and treatmented groups were recorded at the end of the experiment. Due to stress developed by higher temperature , the metabolic rates increased and as a result of the weight and length of the exposed groups was significantly reduced compared to

the control groups. The average length – weight of control group was 3.6 inches and 21 g and the treatment group was 19 g in weight (Kurmaly et al., 1989).

Here, graphical view of Length – weight Measurement of prawn in our present study :

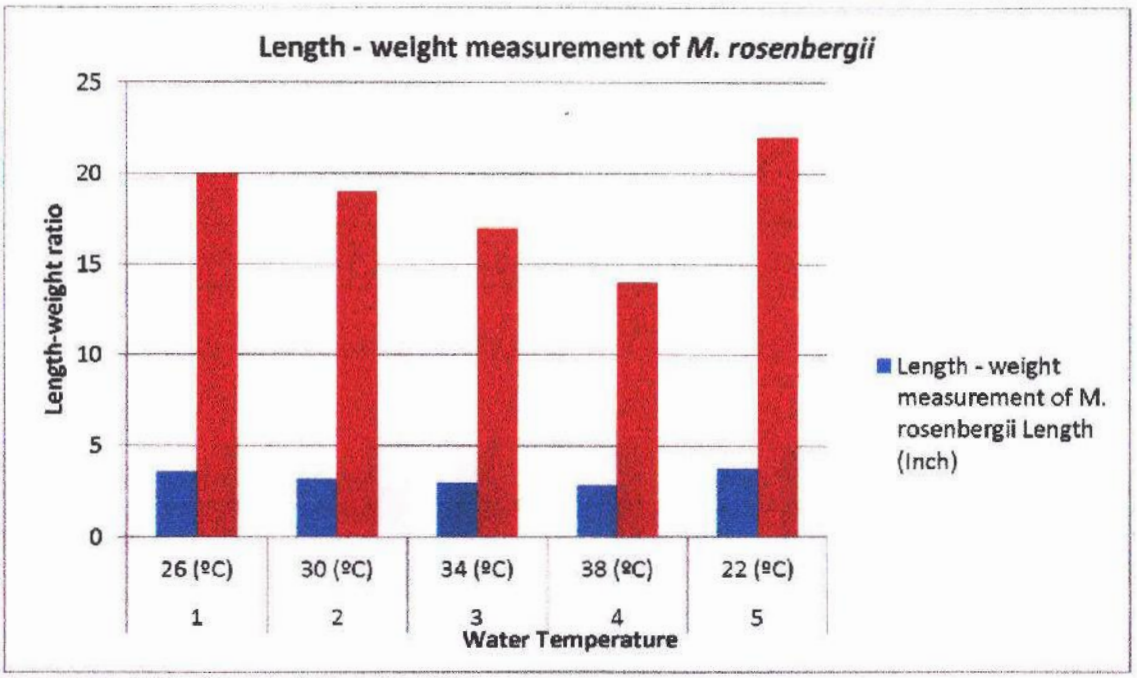


Fig-05: Length -weight measurement of of *M. rosenbergii* from experimental groups. Each bar indicate the ratio of length -weight per each experimental treatmented species. Bars with different numbers indicate the variations among the treatments.

4.3 Temperature effects on Prawns (*M. rosenbergii*) survival & mortality rate through the experiment:

The relationship between the temperature requirements of some fish species, using published data for growth optima, final preferences and lethal limits were examined (Lemaire et al.,2002; Vernberg & Silverthon, 1979.). A good correlation was found and it is suggested that the data established gives a good estimate of the temperature promoting maximum growth (Jobling, 1981). In another study, critical thermal minima (CTmin) and lower incipient lethal temperature (LILT) were determined for *Penaeus semisulcatus* juveniles at combinations of four acclimation temperatures (18, 22, 26 and 30°C) and four salinity levels (20, 25, 30 and 35 ppt)

Before the experiment, THC was found in Prawns body at the highest level $3.12x10^5$cells/ml and the lowest $2.77x10^5$ cells/ml. DHC for large granular, small granular, semi granular, non granular was recorded at high level accordingly; 14.45, 13.38, 31.3, 53.18 respectively.

4.4.2 Amount of THC during Experiment:

During the experiment through temperature treatment, the amount of THC recorded at high level $3.13x10^5$cells/ml at 30 (°C) than other variant temperatures, and for the control group was $3.12x10^5$cells/ml. THC data revealed that there was no significant difference ($P<0.05$) between treatment groups and control groups species.

4.4.3Amount of DHC during Experiment:

DHC data revealed that there was significant difference ($P<0.05$) of semi granular, non granular cell among the control groups and treatment groups of experimental prawn species .Large granular cells were found to be higher in control group (11.83%)than temperature treated group species of prawn.Semi granular, Non granular cells are compared to control and treatment groups in experimental species. Non granular, Semi granularhaemocyte was found to be lower at treatment group compared to control groups (45.68% and 10.23 %).

Here, Hemocyte (THC & DHC) of treatmented groups Prawns are given below through this table.

Temperature (°C) (Controlled)	Species (Treatmented)	THC(cell/ml)	DHC (%)			
			Large Granular	Semi Granular	Smaller Granular	Non Granular
26(°C)	T_1	$3.0x10^5$	10.74	10.6	26.37	48.74
30(°C)	T_2	$2.96x10^5$	11.52	10.25	10.65	50.20
34(°C)	T_3	$2.84x10^5$	10.62	10.45	27.12	48.31
38(°C)	T_4	$2.67x10^5$	11.24	10.78	29.50	46.06
22 (°C)	C	$2.93x10^5$	12.46	12.014	29.62	51.44

Table-04: THC & DHC of treatmented and controlled groups of Prawn.

Hernández-Sandoval (2008) observed that in *M. occidentale* (Holthuis, 1950) growth increased with a temperature increase of 25 °C to 28 °C with proper haemocytes, but beyond 31 °C both growth and survival decreased.

Graphical view on amount of hemocyte (DHC) in treatmented and controlled groups of prawn in our study:

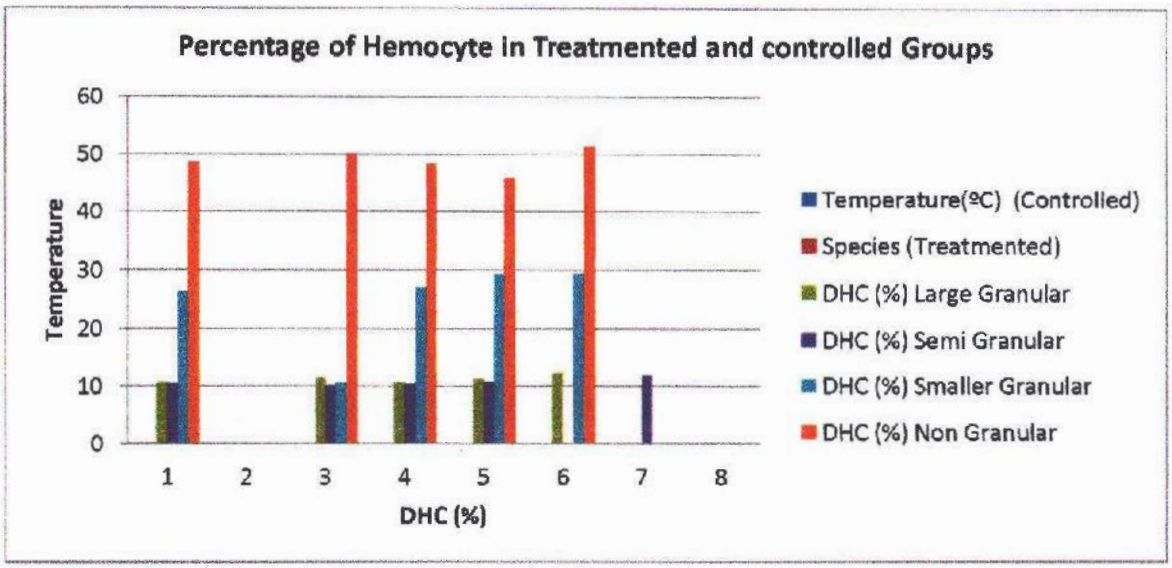


FIG-07: Percentage of differential haemocyte count (DHC) with the effects of temperature from the haemolymph of *Macrobrachium rosenbergii* from experimental groups. Each bars indicate the Percentage of DHC with the effect of temperature of replicates per each experimental treatment. Bars with different colors indicate the variations among the treatments ($p < 0.05$).

4.4.4 Difference of Hemocyte between controlled and treatmented groups Prawn:

After statistical data analysis, there was significant level ($P<0.05$) changes observed in control and treatmented groups of prawns hemocyte. In this table, here significant levels showed for semi granular, non granular and small granular hemocyte. But, there is no significant changes has observed for Total hemocyte ($P= 0.263$, $F= 1.54$) and large granular hemocyte.
Difference between DHC before and after challenge test in our study:

		Sum of Squares	df	Mean Square	F	Sig.
Large	Between Groups	9.074	4	2.269	3.377	.054
	Within Groups	6.718	10	.672		
	Total	15.792	14			
Semi	Between Groups	11.940	4	2.985	3.537	.048
	Within Groups	8.439	10	.844		
	Total	20.379	14			
Small	Between Groups	33.397	4	8.349	7.639	.004
	Within Groups	10.929	10	1.093		
	Total	44.326	14			
Non	Between Groups	61.360	4	15.340	13.846	.000
	Within Groups	11.079	10	1.108		
	Total	72.440	14			

Table-05: Comparison of DHC between control and treatment group.

Each value is mean ± SD of individual observations. The mean differences are significant at ($P<0.05$). Total groups, n=5 and total number of cases=15. So df (degree of freedom) for between group is (n-1) and df for within groups = total number of cases-n. Mean square=sum of square/degree of freedom. Each number indicate the comperative percentage of replicates per each experimental treatment. lines with Different letters indicate the significant variations among the treatments ($p < 0.05$) with F-value and Mean square.

De los Santos Romero et al. (2017) reported that 22.8 °C could be optimal in terms of survival, hence, both temperatures used here are close to a range in which an inverse relationship of survival with growth is observed, and that survival is temperature dependent, but other factors may affect the results depending on experimental conditions, among which feeding and physiological conditions are important.

Here, effects of temperature have showed through ONE-WAY-ANOVA with mean difference, standard error, significance level, 95% confidence interval (Lower bound and upper bound).

Dependent Variable	(I) Temperature	(J) Temperature	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Large	26 (°C)	30 (°C)	-.77333	.66921	.275	-2.2644	.7177
		34 (°C)	.12667	.66921	.854	-1.3644	1.6177
		38 (°C)	-.49333	.66921	.478	-1.9844	.9977
		control	-2.05000*	.66921	.012	-3.5411	-.5589
	30 (°C)	26 (°C)	.77333	.66921	.275	-.7177	2.2644
		34 (°C)	.90000	.66921	.208	-.5911	2.3911
		38 (°C)	.28000	.66921	.684	-1.2111	1.7711
		control	-1.27667	.66921	.086	-2.7677	.2144

		26 (°C)	-.12667	.66921	.854	-1.6177	1.3644
		30 (°C)	-.90000	.66921	.208	-2.3911	.5911
	34 (°C)	38 (°C)	-.62000	.66921	.376	-2.1111	.8711
		control	-2.17667*	.66921	.009	-3.6677	-.6856
		26 (°C)	.49333	.66921	.478	-.9977	1.9844
	38 (°C)	30 (°C)	-.28000	.66921	.684	-1.7711	1.2111
		34 (°C)	.62000	.66921	.376	-.8711	2.1111
		control	-1.55667*	.66921	.042	-3.0477	-.0656
		26 (°C)	2.05000*	.66921	.012	.5589	3.5411
	control	30 (°C)	1.27667	.66921	.086	-.2144	2.7677
		34 (°C)	2.17667*	.66921	.009	.6856	3.6677
		38 (°C)	1.55667*	.66921	.042	.0656	3.0477
		30 (°C)	-.05333	.75008	.945	-1.7246	1.6180
	26 (°C)	34 (°C)	.14667	.75008	.849	-1.5246	1.8180
		38 (°C)	-.18000	.75008	.815	-1.8513	1.4913
		control	-2.23667*	.75008	.014	-3.9080	-.5654
		26 (°C)	.05333	.75008	.945	-1.6180	1.7246
	30 (°C)	34 (°C)	.20000	.75008	.795	-1.4713	1.8713
		38 (°C)	-.12667	.75008	.869	-1.7980	1.5446
		control	-2.18333*	.75008	.016	-3.8546	-.5120
		26 (°C)	-.14667	.75008	.849	-1.8180	1.5246
Semi	34 (°C)	30 (°C)	-.20000	.75008	.795	-1.8713	1.4713
		38 (°C)	-.32667	.75008	.672	-1.9980	1.3446
		control	-2.38333*	.75008	.010	-4.0546	-.7120
		26 (°C)	.18000	.75008	.815	-1.4913	1.8513
	38 (°C)	30 (°C)	.12667	.75008	.869	-1.5446	1.7980
		34 (°C)	.32667	.75008	.672	-1.3446	1.9980
		control	-2.05667*	.75008	.021	-3.7280	-.3854
		26 (°C)	2.23667*	.75008	.014	.5654	3.9080
	control	30 (°C)	2.18333*	.75008	.016	.5120	3.8546
		34 (°C)	2.38333*	.75008	.010	.7120	4.0546
		38 (°C)	2.05667*	.75008	.021	.3854	3.7280
		30 (°C)	-2.13333*	.85359	.031	-4.0352	-.2314
	26 (°C)	34 (°C)	-.75333	.85359	.398	-2.6552	1.1486
		38 (°C)	-3.13000*	.85359	.004	-5.0319	-1.2281
		control	-4.07000*	.85359	.001	-5.9719	-2.1681
		26 (°C)	2.13333*	.85359	.031	.2314	4.0352
	30 (°C)	34 (°C)	1.38000	.85359	.137	-.5219	3.2819
		38 (°C)	-.99667	.85359	.270	-2.8986	.9052
		control	-1.93667*	.85359	.047	-3.8386	-.0348
		26 (°C)	.75333	.85359	.398	-1.1486	2.6552
	34 (°C)	30 (°C)	-1.38000	.85359	.137	-3.2819	.5219
Small		38 (°C)	-2.37667*	.85359	.019	-4.2786	-.4748
		control	-3.31667*	.85359	.003	-5.2186	-1.4148
		26 (°C)	3.13000*	.85359	.004	1.2281	5.0319
	38 (°C)	30 (°C)	.99667	.85359	.270	-.9052	2.8986
		34 (°C)	2.37667*	.85359	.019	.4748	4.2786
		control	-.94000	.85359	.297	-2.8419	.9619
		26 (°C)	4.07000*	.85359	.001	2.1681	5.9719
	control	30 (°C)	1.93667*	.85359	.047	.0348	3.8386
		34 (°C)	3.31667*	.85359	.003	1.4148	5.2186
		38 (°C)	.94000	.85359	.297	-.9619	2.8419
		30 (°C)	-1.50333	.85943	.111	-3.4183	.4116
	26 (°C)	34 (°C)	.43667	.85943	.622	-1.4783	2.3516
		38 (°C)	2.68333*	.85943	.011	.7684	4.5983
		control	-3.38000*	.85943	.005	-5.2949	-1.4651
		26 (°C)	1.50333	.85943	.111	-.4116	3.4183
	30 (°C)	34 (°C)	1.94000*	.85943	.048	.0251	3.8549
		38 (°C)	4.18667*	.85943	.001	2.2717	6.1016
		control	-1.87667	.85943	.054	-3.7916	.0383
		26 (°C)	-.43667	.85943	.622	-2.3516	1.4783
	34 (°C)	30 (°C)	-1.94000*	.85943	.048	-3.8549	-.0251
Non		38 (°C)	2.24667*	.85943	.026	.3317	4.1616
		control	-5.81667*	.85943	.001	-5.7316	-1.9017
		26 (°C)	-2.68333*	.85943	.011	-4.5983	-.7684
	38 (°C)	30 (°C)	-4.18667*	.85943	.001	-6.1016	-2.2717
		34 (°C)	-2.24667*	.85943	.026	-4.1616	-.3317
		control	-6.06333*	.85943	.000	-7.9783	-4.1484
		26 (°C)	3.38000*	.85943	.003	1.4651	5.2949
	control	30 (°C)	1.87667	.85943	.054	-.0383	3.7916
		34 (°C)	3.81667*	.85943	.001	1.9017	5.7316
		38 (°C)	6.06333*	.85943	.000	4.1484	7.9783

Table-6: Effects of temperature on DHC before and after test with 95% confidence level

4.6 Biochemical analysis (Proximate Composition) of Prawn before and after test and its difference

4.6.1 Biochemical analysis (Proximate Composition) of Prawn before and after test

In another study, results show that food directly affects development of individuals when temperature and photoperiod are manipulated. This effect can be reflected in enzymatic activity during digestion, which in turn affects survival and growth rate (Bates et al., 2016). The interaction between the highest temperature and the longest photoperiod resulted in optimal growth, which agrees with previous research with other species of *Macrobrachium*. Most of the previous research nevertheless analyzed these effects separately, so that the effect of the interaction remained unclear. Prawns were reared at 4 different water temperatures (26, 30, 34, 38°C) for a certain period and the lipid, protein, ash, moisture concentrations were measured at the end of that period. Different letters indicate statistically significant differences among treatments for each experiment ($P< 0.05$). In experiments, the protein (17.89%) and moisture (75.95%) concentrations were higher among control groups compared to the treatments, while the lipid (2.14%) and ash (2.1%) concentrations are highest in Prawns at treatments group compared to control groups.

Madlen (2010) showed that growth in *M. rosenbergii* (De Man, 1879) declined above 34 °C, perhaps because of failure of enzymatic function. New (1995), Madlen (2010), and Anger (2013) reported that the growth rate of *M. rosenbergii* increased from 24 °C to 29 °C. Trinidad et al. (2010) indicated that higher average sizes (1.72 g and 5.55 cm) in *Macrobrachium jelskii* (Miers, 1877) were obtained at 29.97 ± 0.81 °C. The same trend is observed even considering differences in species and size of individuals. Other non-tropical species of *Macrobrachium* might respond similarly. Yue et al. (2009) reported that growth and survival of the crayfish *Procambarusclarkii* (Girard, 1852) decrease beyond 34 °C, mostly because of a combination of high oxygen and energy demand. Our research suggests that growth could be improved by manipulating temperature and photoperiod together by having an effect on the molt cycle and, therefore, on growth, an observation previously made by Aiken & Waddy (1992) .

Here, percentage of Biochemical analysis (Proximate Composition) of Prawn, treatmented and controlled groups are given below through this table:

Temperature(°C) (Controlled)	Species (Treatmented)	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)
26 (°C)	T_1	15.47	2.39	72.18	2.74
30 (°C)	T_2	13.03	2.12	69.87	2.55
34 (°C)	T_3	15.28	2.40	72.40	2.02
38 (°C)	T_4	14.59	2.12	72.29	2.37
22 (°C)	Controlled Species	17.59	1.58	76.79	1.76

Table-7: Percentage of Biochemical analysis (Proximate Composition) of Prawn; treatmented and controlled groups.

In a study, the general opinion is that temperature has important effect on the body mechanism of shrimps, It is suggested that this effect on the efficiency of digestion is due to the similarities in the digestive system of crustaceans, having the same general enzymatic activity and with the same origin, but with subtle differences due to adaptations of species to particular conditions (Mc Gaw & Curtis, 2013). Because photoperiod could have an effect on enzymatic activity, photoperiodicity might enhance growth depending on circumstances, as previously stated for most tropical decapods (Mazlum et al., 2011). Growth rate was also affected by water temperature, and high growth rate produced high FCR as previously observed in *M. rosenbergii* (Chavez et al., 1991). Feeding in this species can also increase when temperatures increased from 23 °C to 33 °C, increasing growth (Niu et al., 2003). *Macrobrachium tenellum* was reported to grow well from 25 °C to 32 °C (Espinosa-Chaurand et al., 2012), but our research shows that optimum growths is between 29 °C and 32 °C. Digestion, feeding, and food absorption is therefore optimal within this temperature range. In terms of survival, significant differences were observed between the control and all treatments, which suggests that even if the growth rate is affected by the treatments, none of the treatments were outside the range for the species.

Here, Graphical view on percentages of Biochemical analysis (Proximate Composition) of Prawn; treatmented and controlled groups are given bellow:

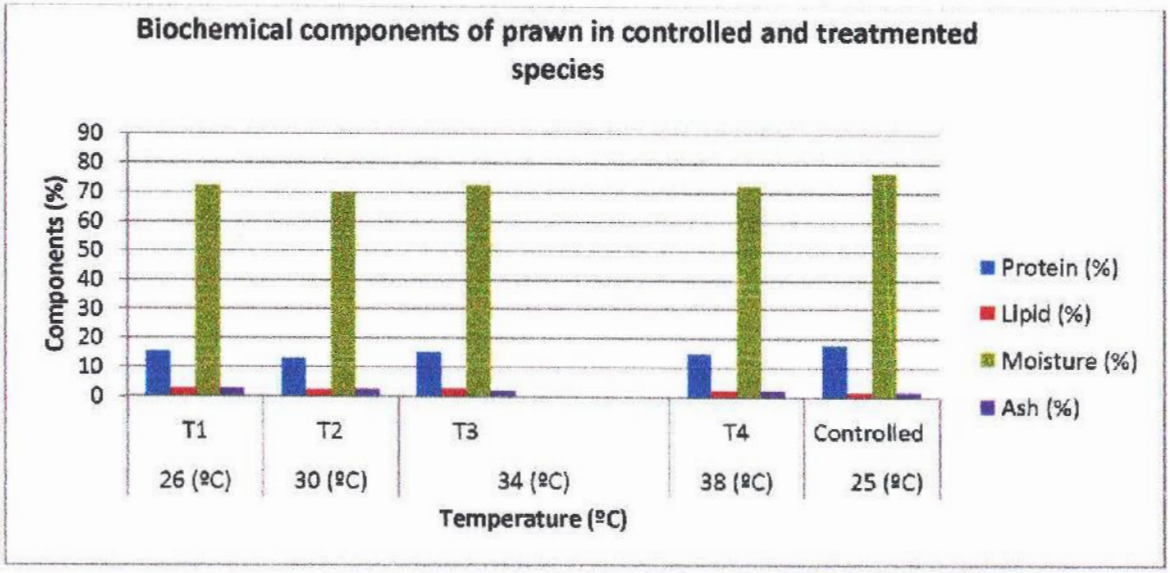


Fig-08: Temperatures effect on Prawns Biochemical composition components. Biochemical analysis of Prawn *(M. rosenbergii)* with effects of temperature with the percentage of protein, lipid , ash and Moisture. These bar represent the percentage of each replicates per each experimental treatmented groups and controlled groups.

4.6.2 Difference on percentage of Biochemical analysis (Proximate Composition) of Prawn before and after test:

Total protein, lipid and carbohydrate variations for the whole embryonic development were measured every 48 hours in samples of eggs of *Macrobrachium occidentale* (Holthuis), 1950, a small freshwater caridean. Proteins are the main component followed by lipids and carbohydrate only as minor constituent. The influence of temperature on biochemical composition, survival and duration of development of *Cherax quadricarinatus* from egg extrusion to juvenile was analyzed. Berried females were individually subjected to each of 22, 25, 28 and 31 °C (n=5 per temperature). Egg samples were obtained every 3 days from egg extrusion to juvenile stage for biochemical analysis. Duration of development and survival decreased with increasing temperature. At 22 and 25 °C half of the initial lipid content was consumed during development. At 28 and 31 °C, 80% of the initial amount of lipids was consumed. For proteins, depletion rate was significantly lower at 25 °C (36% of the initial amount) than at 22, 28 and 31 °C (61–65% of the initial amount). For carbohydrates, a significant consumption was observed only at 22 °C. Total energy consumption was lower at 22 and 25 °C than at 28 and 31 °C. We conclude that 22–25 °C is the optimal temperature range for *C. quadricarinatus* egg incubation, although 25

°C might be better in terms of development duration in terms of survival, energy cost and protein consumption (Guerrero,2012; Wang, 2007; Floch et al., 1957).

During the experiment through temperature treatment, the Biochemical analysis (Proximate Composition) of Prawn recorded at high level protein (16.2%) at 30 (°C) than other variant temperatures. Biochemical analysis (Proximate Composition)data revealed that there was significant difference (P<0.05) among protein, moisture, ash among the control groups and treatment groups of experimental prawn species. And there was no significant difference (P<0.05) for lipid between treatment groups and control groups species.

Difference of percentage of Biochemical analysis (Proximate Composition) of Prawn before and after challenge test:

		Sum of Squares	df	Mean Square	F	Sig.
Protein	Between Groups	72.056	4	18.014	12.570	.001
	Within Groups	14.331	10	1.433		
	Total	86.387	14			
Lipid	Between Groups	.982	4	.246	3.208	.061
	Within Groups	.766	10	.077		
	Total	1.748	14			
Moisture	Between Groups	233.644	4	58.411	80.671	.000
	Within Groups	7.241	10	.724		
	Total	240.885	14			
Ash	Between Groups	4.245	4	1.061	10.416	.001
	Within Groups	1.019	10	.102		
	Total	5.264	14			

Table-08: Difference of percentage of Biochemical analysis (Proximate Composition) of Prawn between control and treatment group.

Total groups, n=5 and total number of cases=15. So df (degree of freedom) for between group is (n-1) and df for within groups = total number of cases-n. Mean square=sum of square/degree of freedom.

In this test, here Higher F value is recorded for Moisture in between treatmented and controlled groups is 80.671, which indicates the variation between sample means, the higher the variation between sample means relative to the variation within the samples. The higher the F value, the lower the corresponding P –value.

Here, Graphical view Difference of percentage of Biochemical analysis (Proximate Composition) of Prawn between control and treatment groups are given bellow:

Each number indicate the comperative percentage of replicates per each experimental treatment, lines with different letters indicate the significant variations among the treatments ($p < 0.05$) with F-value and Mean square.

Here, effects of temperature have showed through ONE-WAY-ANOVA with mean difference, standard error, significance level, 95% confidence interval (Lower bound and upper bound).

	(I) Temperature	(J) Temperature	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Protein	26 (°C)	30 (°C)	-.64333	.97745	.525	-1.5346	2.8212
		34 (°C)	-1.85333	.97745	.087	-.3246	4.0312
		38 (°C)	-4.22000*	.97745	.002	2.0421	6.3979
		control	2.45000*	.97745	.031	.4621	4.6279
	30 (°C)	26 (°C)	.64333	.97745	.525	-2.8212	1.5346
		34 (°C)	-1.21000	.97745	.244	-.9679	3.3879
		38 (°C)	-3.57667*	.97745	.004	1.3988	5.7546
		control	-3.09333*	.97745	.010	-5.2712	-.9154
	34 (°C)	26 (°C)	1.85333	.97745	.087	-4.0312	.3246
		30 (°C)	1.21000	.97745	.244	-3.3879	.9679
		38 (°C)	-2.36667*	.97745	.036	.1888	4.5446
		control	-4.30333*	.97745	.001	-6.4812	-2.1254
	38 (°C)	26 (°C)	4.22000*	.97745	.002	-6.3979	-2.0421
		30 (°C)	3.57667*	.97745	.004	-5.7546	-1.3988
		34 (°C)	2.36667*	.97745	.036	-4.5446	-.1888
		control	-6.67000*	.97745	.000	-8.8479	-4.4921
	control	26 (°C)	2.45000*	.97745	.031	.2721	4.6279
		30 (°C)	3.09333*	.97745	.010	.9154	5.2712
		34 (°C)	4.30333*	.97745	.001	2.1254	6.4812
		38 (°C)	6.67000*	.97745	.000	4.4921	8.8479
Lipid	26 (°C)	30 (°C)	.27000	.22591	.260	-.2334	.7734
		34 (°C)	-.01667	.22591	.943	-.5200	.4867
		38 (°C)	.26333	.22591	.271	-.2400	.7667
		control	.69000*	.22591	.012	.1866	1.1934
	30 (°C)	26 (°C)	-.27000	.22591	.260	-.7734	.2334
		34 (°C)	-.28667	.22591	.233	-.7900	.2167
		38 (°C)	-.00667	.22591	.977	-.5100	.4967
		control	.42000	.22591	.093	-.0834	.9234
	34 (°C)	26 (°C)	.01667	.22591	.943	-.4867	.5200
		30 (°C)	.28667	.22591	.233	-.2167	.7900
		38 (°C)	.28000	.22591	.243	-.2234	.7834
		control	.70667*	.22591	.011	.2033	1.2100
	38 (°C)	26 (°C)	-.26333	.22591	.271	-.7667	.2400
		30 (°C)	.00667	.22591	.977	-.4967	.5100
		34 (°C)	-.28000	.22591	.243	-.7834	.2234
		control	.42667	.22591	.088	-.0767	.9300
	control	26 (°C)	-.69000*	.22591	.012	-1.1934	-.1866
		30 (°C)	-.42000	.22591	.093	-.9234	.0834
		34 (°C)	-.70667*	.22591	.011	-1.2100	-.2033
		38 (°C)	-.42667	.22591	.088	-.9300	.0767
Moisture	26 (°C)	30 (°C)	2.81333*	.69477	.008	.7653	4.8614
		34 (°C)	4.44333*	.69477	.000	2.8953	5.9914
		38 (°C)	5.89333*	.69477	.000	4.3453	7.4414
		control	-5.85667*	.69477	.000	-6.9047	-3.8086
	30 (°C)	26 (°C)	-2.81333*	.69477	.008	-4.8614	-.7653
		34 (°C)	2.13000*	.69477	.012	.5819	3.6781
		38 (°C)	3.58000*	.69477	.000	2.0319	5.1281
		control	-7.67000*	.69477	.000	-9.2181	-6.1219
	34 (°C)	26 (°C)	-4.44333*	.69477	.000	-5.9914	-2.8953
		30 (°C)	-2.13000*	.69477	.012	-3.6781	-.5819
		38 (°C)	1.45000	.69477	.063	-.0981	2.9981
		control	-9.80000*	.69477	.000	-11.3481	-8.2519
	38 (°C)	26 (°C)	-5.89333*	.69477	.000	-7.4414	-4.3453
		30 (°C)	-3.58000*	.69477	.000	-5.1281	-2.0319
		34 (°C)	-1.45000	.69477	.063	-2.9981	.0981
		control	-11.25000*	.69477	.000	-12.7981	-9.7019
	control	26 (°C)	5.85667*	.69477	.000	3.8086	6.9047
		30 (°C)	7.67000*	.69477	.000	6.1219	9.2181
		34 (°C)	9.80000*	.69477	.000	8.2519	11.3481
		38 (°C)	11.25000*	.69477	.000	9.7019	12.7981

Ash	26 (°C)	30 (°C)	-.81333*	.26064	.011	-1.3941	-.2326
		34 (°C)	-1.19000*	.26064	.001	-1.7707	-.6093
		38 (°C)	-1.29333*	.26064	.001	-1.8741	-.7126
		control	-.13333	.26064	.620	-.7141	.4474
	30 (°C)	26 (°C)	.81333*	.26064	.011	.2326	1.3941
		34 (°C)	-.37667	.26064	.179	-.9574	.2041
		38 (°C)	-.48000	.26064	.095	-1.0607	.1007
		control	.68000*	.26064	.026	.0993	1.2607
	34 (°C)	26 (°C)	1.19000*	.26064	.001	.6093	1.7707
		30 (°C)	.37667	.26064	.179	-.2041	.9574
		38 (°C)	-.10333	.26064	.700	-.6841	.4774
		control	1.05667*	.26064	.002	.4759	1.6374
	38 (°C)	26 (°C)	1.29333*	.26064	.001	.7126	1.8741
		30 (°C)	.48000	.26064	.095	-.1007	1.0607
		34 (°C)	.10333	.26064	.700	-.4774	.6841
		control	1.16000*	.26064	.001	.5793	1.7407
	control	26 (°C)	.13333	.26064	.620	-.4474	.7141
		30 (°C)	-.68000*	.26064	.026	-1.2607	-.0993
		34 (°C)	-1.05667*	.26064	.002	-1.6374	-.4759
		38 (°C)	-1.16000*	.26064	.001	-1.7407	-.5793

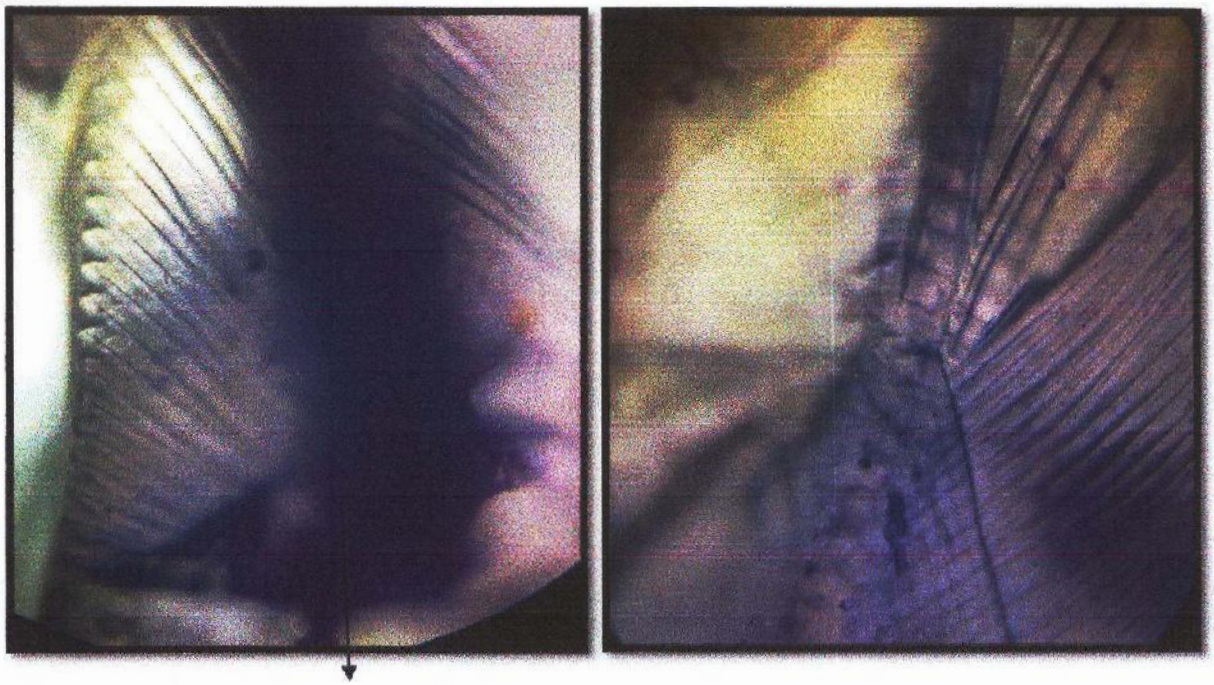
The mean difference is significant at the 0.05 level.

Table-09: Effects of different water temperatures on biochemical changes of muscle compositions in prawns with 95% confidence level. Value of Mean Diiference and Std. Error for different temperature for protein, ash, moisture and lipid is showed here.

4.8 Histological changes of Gills:

The results of the histological analysis of gill showed that the number of hemocytes in the anterior gills of the prawns increased proportionately with the increases in temperature. In aquatic organisms, the gills represent a vital organ, since they play an important role in the transport of respiratory gases and regulate the osmotic and ionic balance. Toxic substances may cause damage to gill tissues, thereby reducing the oxygen consumption and disrupting the osmoregulatory function of aquatic organisms (Cuzon et al., 2010). In the present study, exposure of prawns to higher temperature (38 °C) resulted in notable structural alterations of the gill lamellae including accumulation of hemocytes (HC) in the hemocoelic space, swelling (SL) and fusion (FL) of lamellae, lifting of lamellar epithelium (LLE), abnormal gill tips (AG), necrotic (NL), hyperplastic (HY) of a disorganized mass (DM) of disrupted gill lamellae. Inflammatory changes, such as swelling, lifting of lamellar epithelium and hyperplasia have also been noted in the gill lamellae. Thus, the changes observed in test prawns exposed to higher temperature in the present study were more likely to have represented a progressive loss of basic biological functions of the gills rather than protective mechanisms. There is a prevalence of regressive damage with high scores with the presence of many necroses and scalling of the

epithelium, besides lamellar fusion and thickening of the lamellar epithelium. However, what calls a lot of attention is the edemas found in th gill structure. In the treatment with room or medium temperature. The gills are evenly distributed, with no degeneration of the lamellar structures observed.



Damages of Gill

Fig-9: Gill structure of *M.rosenbergii* with temperature effect during the experiment.

The tissue damage induced by various organic pollutants in aquatic animals is well-documented, but there is a dearth of information relating to the histological alterations induced by pesticides in freshwater prawns of the genus *Macrobrachium* (Bhavan & Geraldine, 2000). In the present study the freshwater prawn *Macrobrachium rosenbergii* (total length, 2.5–4.0 cm; weight, 0.70–1.0 g) were exposed to different temperatures for a period. The tissue of gills of the prawns were then dissected out and processed for light microscopic studies. Exposure to different temperature was found to result in several alterations in the gills of *M. rosenbergii*. The alterations included: accumulation of hemocytes in the hemocoelic space, swelling and fusion of lamellae, abnormal gill tips, hyperplastic, necrotic, and clavate-globate lamellae of the gills. The results obtained suggest that the gills of prawns exposed to different temperatures were structurally altered.

CHAPTER 5

DISCUSSION

CHAPTER – 5

5. Discussion:

5.1 Survival rate of Prawn through experiment (Different temperatures):

Larvae of the freshwater prawn *Macrobrachium australiense* were reared to postlarvae at various combinations of temperature and development was highly dependent on temperature within the range of 20 to 30°C, being fastest at 30°C, slowest at 20°C (Lee and Fielder, 1981).The effect of water temperature on growth, survival, and thermal behavior was studied in the juveniles of the prawn *Macrobrachium occidentale* (Holthuis, 1950) by exposing them to a replicate experimental design that included the complete temperature range in its natural environment. The optimal growth was between 25 °C and 28 °C. Survival was 100% at 22 °C, 97% at 28 °C, and 92% at 25 °C and 31 °C (Sandoval et al., 2018). Temperature is a significant environmental factor in aquaculture. To investigate the physiological responses during temperature treatment (26-38°C), experimental Prawn species *(M.rosenbergii)* were treated with 4 variants of temperatures. The higher level of temperature (38 °C) increases the mortality of experimental species within few hours for low adaptation. Mortality also had observed at 34°C but most of the cases after collecting hemocyte. Prawns survival rate was observed normal at 26 to 30 °C respectively during the experiment.

5.2 Blood cell composition:

The blood of Prawns or hemolymph hasacellular component, which is the hemocytes, and a liquid components constituted plasma that contains different humoral factors. THC reduction, when water temperature decreased to an unconformable level. These results indicate that water temperature decrease can induce oxidative stress on Prawns hemocytes.

5.2.1 Total hemocyte count (THC) in the experiment

In this study, there was no significant difference ($P<0.05$) of THC between the control and treatment groups. The THCs of the Prawns were decreased significantly after collecting haemocyte exposure to higher temperature treatment at 38°C; similar observations were found in

the treatment three for abnormal adaptation for collecting blood. There observed reduction in the number of circulating haemocytes in the prawns which was maintained. For, Total hemocyte (P= 0.263, F= 1.54); so this indicates that non significant difference between two groups. High F value indicates the more significant difference and here the F value is not so high. As the P value is not significant for THC, so the present study, showing a non significant difference of THC just before and after the challenging test.

5.2.2 Differential haemocyte count (DHC) in the experiment

The circulatory haemocytes of crustacean comprises of non-granular cell (NGC), semigranular cell (SGC), Smaller granular cell (SmGC) and large granular cell (LGC). In this study, all those four types ofhaemocytes were observed in *M. rosenbergii* where the non granular cells comprise the highest proportion of THC that was very similar to the result of Zhou et al., (2017).Highest F value was found for non granular hemocyte cell (F= 7.63, P= .004) and small granular cell (P= .004). Percentage of DHC before test from haemocytes of *Macrobrachium rosenbergii* with 48% non granular, 11% semi granular, 30% smaller granular and 11% large granular. This pie chart represent the ratio (%) of replicates per each experimental treatment. Per unit (Fig:07) with different colors indicate the significant variations among the treatments ($p < 0.05$).
And percentage of DHC after test from haemocytes of *Macrobrachium rosenbergii* with 51% non granular, 9% semi granular, 29% smaller granular and 11% large granular.

5.3 Proximate Composition:

With respect to the biochemical composition of *M.rosenbergii* individuals, temperature was found to have no effect on the concentrations of lipid (P= 0.061) at the end of the rearing periods of both experiments. This finding may be explained by the fact that the type of diet, food availability and water quality, which are some of the factors influencing the storage-mobilization cycle of lipid concentration in crustaceans, were similar among treatments throughout that period. At the four temperatures evaluated, moisture, protein was the most abundant biochemical component, while lipid and ash represented a minor fraction of body composition. With respect to lipids, a tendency towards higher levels was found in prawns controlled group (1.89%) and for the treatment group was high (2.98%) at 26 °C in the experiments. This is in line with previous results that reported decreasing consumption rates of lipids with decreasing incubation

temperatures in embryos of the freshwater crayfish *Cheraxquadri carinatus* and the prawn *M. americanum* In the present study, the lower concentration of moisture was found in prawns at 30 and 38°C, with respect to that of shrimps at 38°C, may be a consequence of an increase in all the metabolic processes involved in growth. In this sense, Childress et al. (1990) showed for several species of benthic deep-sea decapod crustaceans that metabolic rates decline with decreasing temperature. Higher metabolic rates at higher temperatures are accompanied by an increase in energy demand, which is mainly supplied by lipids in aquatic organisms. The lowest ash concentration was found in control groups compared to treatment groups. In shrimps reared at 28°C may also be explained by the fact that reproduction was more pronounced at that temperature than at the other temperatures tested. In this sense, several studies have demonstrated that during periods of high energy demand, such as oogenesis, there is a pronounced degradation of lipids for yolk synthesis, which is the major source of nutrients for the developing embryo. In another study, average values of the proteins, carbohydrates, lipids, ash and moisture as in cultured and frozen prawns were recorded as 74.24 ± 0.49, 5.50 ± 0.34, 9.09 ± 0.09, 9.71 ± 0.19, 77.14 ± 0.19 and 60.55 ± 0.35, 8,23 ± 0.18, 7.98 ± 0.13, 21.61 ± 0.42, 74.93 ± 0.23, respectively. The higher amounts of proteins, lipids were identified in cultured prawn (Reddy, 2014) . As for body weight and GI, biochemical composition patterns were similar in both experiments, further indicating that the temperature experienced during embryonic development did not affect the subsequent growth performance of *N. heteropodaheteropoda* (Tropea et al.,2015*)*. The significant difference had observed among protein, moisture, ash between controlled and treatmented groups where F value is largely higher than its critical value and P value is lower than 0.05.

5.4 Histological changes of gill at different temperatures:

Gills are vulnerable to environmental changes because they are directly exposed to the external environmental conditions, such as water temperature. This is fundamentally important for understanding the response mechanisms and the adaptation of crabs to gill conditions under temperature stress (Enami,1951). Histological analysis can establish study of environmental stress in targeted tissues such as gills (Sun et al.,2015). Gills are among the most important tissues for the regulation of the body fluids of a hemolymph. It is also considered to be the first line of defense against any environmental disturbance (Sun et al., 2015) and it remains in close

contact with the external environment, being particularly sensitive to changes in the water, including temperature. With respect to the histological development of the gills of *M. rosenbergii* individuals, it was found that temperature had an effect on the number of hemocytes in the primary gill and secondary lamellae at the end of the Prawn's final instar stages. Gills are commonly used as baseline information for determining water conditions, especially environmental hypoxia, which is caused by the increasing temperatures of surface water. However, to the best of the author's knowledge, nothing is known of the long term effects of water temperature either for organs or other tissue in portunid –crabs (Enami, 1951). The results of this study show that increases in water temperature can increase the number of hemocytes at (26 -30°C). Little is known of the effects of long term-culture of water temperature on the gills of other brachyuran prawns, so it was not possible to compare with similar results in other species.

CHAPTER 6

CONCLUSION

CHAPTER-6

Conclusion:

Bangladesh possesses vast marine water resources. Despite the abundance of marine waters, only about 15.31 percent of country's total fish production is contributed by the marine sector. The government sets utmost priority regarding the protection, conservation and biodiversity of marine and coastal resources. As a result, the Saint Martin Island and the Sundarbans, the world-famous mangrove forest, have been declared as sanctuaries to develop and protect the fisheries resources as well as biodiversity of that area. Giant freshwater prawn *Macrobrachium rosenbergii* is commercially cultured in India and other south Asian countries. Although its culture is profitable, some constraints within the grow-out phase still remain. *M. rosenbergii* is territorial to some degree and exhibits aggressive and social behavior. Social suppression of growth in this species has demonstrated by laboratory studies. A differential individual growth phenomenon, territorial behaviour, and the cannibalistic nature (particularly when food becomes insufficient) of freshwater prawn are controlled most often by social interactions. These factors can be partially addressed by culture at low density, providing shelters and sufficient food, and employing poly culture practices (Boock et al., 2016). Growth variation within the male population is higher in high density culture environments. In high density monoculture of *M. rosenbergii* differential growth rate (heterogeneous individual growth) has been reported. Use of shelters in culture ponds has been reported to reduce the growth variation within a population of freshwater prawn. Provision of shelters also helps to increase the survival and overall production by decreasing cannibalism (Lee and Fielder, 1981). The culture of *Macrobrachium* spp. is less likely to have a detrimental impact because freshwater prawns cannot be reared at densities as high as those commonly used in marine shrimp farming. Productivity is generally lower, management is less labour intensive, and the potential for the abuse or waste of resources is minimal, and (unlike the inland culture of marine shrimp) the grow-out of *Macrobrachium* does not make agricultural land saline. Specific negative effects of *M. rosenbergii* culture on the environment have yet to be documented (Sandoval et al., 2018).

The present work was carried out to describe the histological changes during development of the freshwater prawn ***Macrobrachium rosenbergii*** based on some morphological and histological features. In addition the biochemical composition of muscle was investigated during the

Histological development of the studied species. *M. rosenbergii* showed a significantly higher tolerance to elevated temperature. These results revealed that Freshwater Prawn can adapt to a certain level of temperature (26-30°C) by self-regulation. In this study, protein and lipid were observed to be the main energy source of *Macrobrachium rosenbergii* during temperature treatment. In conclusion, higher temperature affected the survival rate of these prawns for different genders, the biochemical composition of the prawns, and the histological conditions of their gills. Future research that could bring a approach to bear is necessary to better understand the effects of water temperatures on haemocytes and histology of prawns with structure components.

CHAPTER 7

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CHAPTER 8

Appendix

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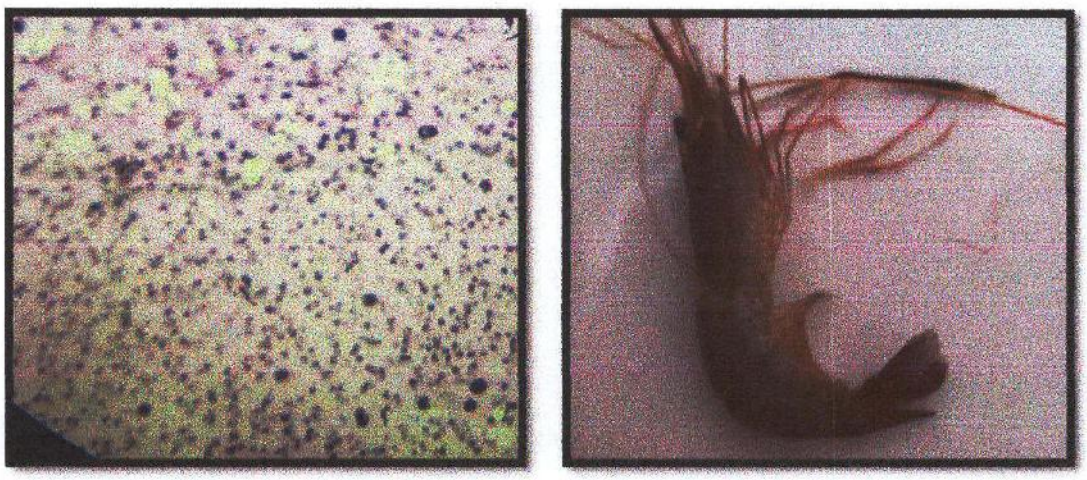
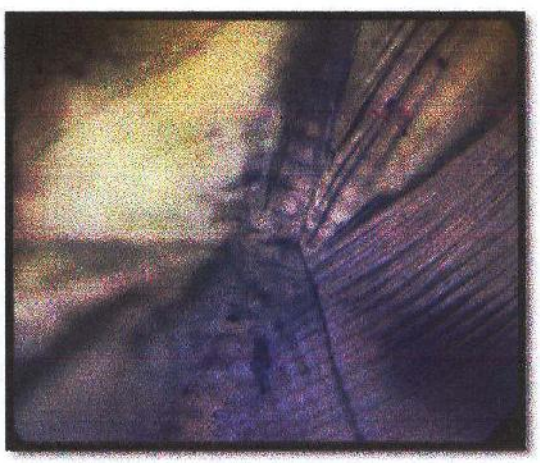
Appendix:



Pic: Blood cell of prawn (*M. rosenbergii*)



Pic: Gill lamellae (*M. rosenbergii*)