GROWTH, SURVIVAL AND NUTRITIONAL PROFILE OF MARINE LARVAE (*Penaeus monodon*) FED WITH SELECTED MICROALGAE

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Roll No: 0119/01

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JUNE 2020

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Mohammad Jabedul Islam

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Dedicated

To my

Beloved parents

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Mohammad Jabedul Islam

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LIST OF ABBREVIATIONS

CF	Control Feed
CH25	25% *Chlorella vulgaris*
CH50	50% *Chlorella vulgaris*
CH75	75% *Chlorella vulgaris*
CL	Carapace length
C. vulgaris	*Chlorella vulgaris*
CMC	Carboxymethylcellulose
$CuSO_4$, $5H_2O$	Cupric sulphate
DW	Dry weight
DO	Dissolved oxygen
DoF	Department of Fisheries
EDTA[(b)], di-sodium salt	Ethylenediaminetetraacetic acid disodium salt
FAO	Food and Agriculture Organization
$FeCl_3$	Ferric chloride
FM	Fish meal
gm	gram
HCL	Hydrochloric acid
H^+	Hydrogen ion
H_3BO_3	Boric acid
H2O	Water
L	Liter
$MnCl_2$, $4H_2O$	Manganous chloride
NaOH	Sodium hydroxide
NaH_2PO_4, $2H_2O$	Sodium di-hydrogen orthophosphate
$(NH_4)6Mo_7O_{24}$, $4H_2O$	Ammonium molybdate
mg	milligram
min	Minute
ml	Milliliter
mm	Millimeter
MilliQH_2O	Highly purified water from MilliQ Water Purification System (Milllipore, Milford, MA)
mg L^{-1}	Milligram per liter

MT	Metric Ton
NO_2-N	Nitrite nitrogen
PL	Postlarvae
PLs	Postlarvaes
P. monodon	*Penaeus monodon*
rpm	rotation per minute
ROS	Reactive oxygen species
SE	Standard error
PO_4-P	Phosphate phosphorus
ppt	part per thousand
TAN	Total ammonia nitrogen
T25	25 % *Tetraselmis chuii*
T50	50 % *Tetraselmis chuii*
T75	75 % *Tetraselmis chuii*
$ZnCl_2$	Zinc cloride
%	Percentage
β	Beta
µL	Microlitre
µm	Micrometer
^oC	Degree celcius
SGR	Specific growth rate

ABSTRACT

Shrimp (*Penaeus monodon*) aquaculture is expanding in Bangladesh and plays an important role in socio-economic development. The growing demand for fishmeal in aquaculture diets has prompted the hunt for cheaper and sustainable protein ingredients. In this analysis, a PL shrimp *Penaeus monodon* feeding trial was performed in which two dried microalgae, *Tetraselmis chuii* and *Chlorella* sp., were used to substitute fish meal protein. 25% (T25), 50% (T50), 75% (T75) of *T. chuii* and 25% (CH25), 50% (CH50), 75% (CH75) of *Chlorella* sp. were supplemented to PLs in triplicates for 17 days for each treatment and fed to shrimp PL to determine the survival rate, growth efficiency and nutritional composition of the experimental diets. Fish meal (0%) feed replacement without microalgae treated as control. The findings demonstrated the significant ($p < 0.05$) high weight growth of two experimental diets: T25 % and CH25 % as compared to formulated feeds of 100% fish meal. In addition, the survival rates ($p < 0.05$) of PLs filled with T25 and CH25 % were significantly greater than those of the control. In the tanks for shrimp culture fed with *Tetraselmis chuii* and *Chlorella* sp., total ammonium nitrogen (TAN), nitrite nitrogen (NO_2-N) and phosphate phosphorus (PO_4-P) were significantly lower, $p < 0.05$). This study has shown that microalgae can potentially optimize water quality and reduce costs for sustainable shrimp cultivation.

Keywords: *Penaeus monodon*, Fish Meal, Microalgae, Water quality.

CHAPTER-1

INTRODUCTION

CHAPTER-1

INTRODUCTION

Aquaculture (farming of aquatic animals and plants) continues to be the world's leading food production industry for water, and in Asia and around the world has expanded rapidly (FAO, 2020). Bangladesh ranked 3rd in inland fish production worldwide, 5th in aquaculture production and 11th in 2018 in marine fish production (FAO, 2020). Bangladesh's all-out fishery production in 2018-2019 was 43.84 lakh metric tons, while inland open water (capture) accounts for 28.19% (12.36 lakh metric tons) and inland closed water (culture) accounts for 56.76% (24.89 lakh metric tons).Then again, marine fish production at the level of 6.599 lakh MT is 15.05 % (DoF, 2020). In 2018-19, fisheries sector contributes 3.50% to the national GDP and more than one-fourth (25.72%) to the agricultural GDP (DoF, 2020). Shrimp is one of Bangladesh's most valuable export commodities. In 2002-03, total production of shrimp and prawn, including caught shrimp, increased, from 1.60 lakh MT to 2.58 lakh MT in 2018-2019 and its growth rate was 1.44% (DoF, 2020). As the second biggest supporter of export earnings of Bangladesh, the fishery and aquaculture sectors emerged. By exporting nearly 73171.31 MT of fish and fishery products in 2018-19, the country earns about 630.29 million USD, where traded frozen shrimp/ prawn has a great contribution (DoF, 2020). Bangladesh, becoming possibly the world's most suitable countries in prawn and shrimp cultivation, with its large resources of shallow water bodies, gives a kind of chance of creeks and shrimp production (Ahmed et al., 2008; Islam, 2008). After garments clothes, frozen seafood is the second largest export product in Bangladesh (Rahman and Hossain, 2009). According to Bangladesh Frozen Food Exporter Association (BFFEA) 85% of shrimp exported from Bangladesh were sent to European countries while 15% were destined to the USA, Japan and other countries. Bangladesh earned 124.000 million USD by exporting frozen shrimp in the fiscal year 2018-19. In Bangladesh, frozen shrimp exports amounted to 36864 tons in 2002-03, around 33362.52 tons in 2018-19, (DoF, 2020). Similarly, numerous initiatives and enhancement projects are being revised for the increased production and promotion of shrimp aquaculture. The community cultivation approach is encouraged to boost the improvement of shrimp production and advance a business-accommodating supply chain by implementing good aquaculture practices.

In terms of production value, marine shrimp cultivation is one of the major and important commercial aquaculture activities because shrimps are considered a highly valued seafood commodity (Anand et al., 2014). For decades, shrimp aquaculture has been rising rapidly with aquaculture growth (Kim et al., 2015). Due to sufficient agro-climate conditions, adequate water supplies, cheap labor, foreign donor agencies and the involvement of multinational corporations, shrimp farming is increasing in Bangladesh (Paul and Vogl, 2011). *Penaeus monodon*, the largest Penaeid Indian tiger prawn, is widely distributed in Indian waters and forms an important fishery with strong export market demand. The giant *Penaeus monodon* tiger prawn is only one of approximately 100 species of commercially important penaeid prawns and shrimp in catching fisheries and/or aquaculture. It is the predominant cultural species in the Indo-Pacific region because of its large size, rapid growth rate, high survival, resistance to handling and good breeding in captivity (Motoh H, 1985).

The rapid expansion of aquaculture for commercial fish and crustacean production has led to a rising demand for improved and cost-effective feed production. In addition, the expansion of aquaculture is also limited because of its heavy dependency on fishmeal (Browdy et al., 2001). The cost of shrimp farming is largely dependent on feed, which accounts for 40-60% of operating costs (Tan et al., 2005). This is primarily due to the price in commercial diets of protein components (Bender et al., 2004).The 30 % digestible protein in feed is typically needed in the commercial culture process and is the most costly component of the diet (Ayisi et al., 2017). Protein is the costliest ingredient in realistic shrimp culture diets, with fish meal (FM) being the most widely used source of protein in commercial feeds (Oujifard et al., 2012).

Owing to its balanced content of amino acids, fatty acids, vitamins, and minerals, fishmeal is an important ingredient in marine shrimp diets (Suárez et al., 2009). Because of their high-quality amino acids and long-chain unsaturated fatty acids (UFAs), fish meal and fish oil are the key contributors to protein and oil (Tacon and Metian, 2008). Around 40% of all aquaculture production now relies heavily on commercial feed. In particular, this refers to high-value carnivorous animals, such as shrimp, salmon and trout, whose feed includes significant quantities of marine input in the form of fish meal (Alvarez et al., 2007). Commercial formulations of shrimp typically contain 25 to 50% FM (Amaya et al., 2007). The quantity of fresh fish used

for fish meals and fish oil has achieved or approached its highest sustainable production in recent years (Cashion et al., 2017; Kris-Etherton et al., 2009; Sarker et al., 2016). In addition, the consumption of fish competes with its use in human food, and fish oil and fish meal prices are increasing. These factors have a detrimental impact on the development of the demand for aquafeeds. The quest for alternative sources of protein and lipids to substitute or minimize the use of fish meal and fish oil has thus become one of the main recommendations for aquaculture research (Hossain and Chakraborty, 2017; Lee et al., 2018).

Limited supply and high demand, however, make the fish meal a costly ingredient and unprofitable for aquaculture operations. The increasing demand for fishmeal in aquaculture diets has prompted the requirement for cheaper and much more sustainable protein ingredients (Salze et al., 2010). While alternative plant proteins are initially selected to be less costly and more accessible than FM, plant proteins with an acceptable balance of amino acid profile, good digestibility, high protein content and palatability should also be considered (Sánchez-Lozano et al., 2009). Plant protein sources are both economically and nutritionally useful alternatives to fishmeal due to their low price and stable nutrient composition and availability. Due to its balanced amino acid profile, predictable structure, and worldwide availability, plant protein sources have gained significant attention when replacing fish meal in aquatic animal feed.

Microalgal products have been reported (Boonyaratpalin, 2001; Ju et al., 2009; Supamattaya et al., 2005), macroalgae (Yeh et al., 2006), probiotics (Wang, 2007; Yang et al., 2010; Ziaei-Nejad et al., 2006), prebiotics (Zhang et al., 2012) and periphyton (Anand et al., 2013) and many are used as dietary supplements in shrimp farming to improve development, immune response and digestive enzyme activities. Using microalgae for animal nutrition provides an economically viable (Chen et al., 2020). In the animal nutrition and agricultural industries, microalgae have high potential application values as sources of proteins, lipids, vitamins, minerals and pigments, plus the carbon-neutral advantages of CO_2 fixation to mitigate the discharge of greenhouse gas (GHG) (Brune et al., 2009; Shah et al., 2018). There was no adverse impact on the growth and biochemical composition of aquatic animals when adding algal at a certain ratio in the feed (Dallaire et al., 2007). The nutritional values of algal biochemical components, including amino acids, fatty acids, carotenoids and

components, have been evaluated in several studies and the findings indicate that microalgae are promising for their economically viable incorporation into feed (Tibbetts, Bjornsson, et al., 2015). Currently, more than 40 species of microalgae have been used as live or dead cell food sources to enhance the characteristics of aquatic animals, such as fish, mussels and shrimps (Pulz and Gross, 2004; Rahman et al., 2018). The production of environmentally friendly feed additives as immunostimulants is promoted by legal limitations or bans on antibiotic administration in aquaculture (Hoseinifar et al., 2016). Microalgae antimicrobial metabolites have gained more and more interest in recent years (Cavallo et al., 2013). Algal products such as proteins and lipids are considered to be some of the most promising sources of feed in aquaculture, but their use in modern aquaculture is just beginning and needs further assessment (Tibbetts et al., 2015). For aquatic animals such as shrimp, the requirement of an EAA or EFA (essential fatty acid) is not as easily integrated into feed as its corresponding amino acid or fatty acid, as crystalline amino acids or fatty acids may often not be effectively used (Chen et al., 1992).

Research is therefore required to decide if less costly feed ingredients will substitute shrimp feed with fish meal. The aim of this research is to assess the potential of two commercial marine microalgae, *T. chuii* and *C. Vulgaris*, which is used as a source of alternate sources of protein for *P. monodon* diets.

1.1 Aims and objectives of the study:

The aim of this study is to observe the effects of inclusion of selected dried microalgae on growth rate, survival, and nutritional profile in *Penaeus monodon*. The objectives of this study were as follows:

- To conduct the mass culture, biomass harvest of microalgae and formulate diets for shrimp PL using microalgae
- To determine the water quality parameters from shrimp PLs culture tanks fed with microalgae
- To determine the nutritional composition of two different experimental microalgal strains

CHAPTER-2

REVIEW OF LITERATURE

CHAPTER-2

REVIEW OF LITERATURE

2.1 General description of microalgae:

Algae are a diverse aquatic species, typically classified as either macroalgae (i.e., seaweed) or microalgae, photosynthetic organisms (unicellular). Microalgae grow as aquatic plant families in aerated, liquid cultures where the cells have adequate access to light, carbon dioxide and other nutrients (Rosenberg et al., 2008). Algal habitats range from open oceans (occupied by planktonic microalgal species), to rocky shores (occupied by marine macroalgae or seaweeds and benthic microalgae), to freshwater habitats (often inhabited by noticeable filamentous algae), including rivers, lakes, ditches and ponds (Stengel et al., 2011). Algae are generally grown autotrophically; however, by degrading organic substances, some species can survive heterotrophically (Chen and Chen, 2006). Most algae are photosynthetic and have biological and ecological roles that are similar to those of plants (Stengel et al., 2011).

Microalgae are a diverse group of microorganisms that undergo photosynthesis in order to transform solar energy into chemical energy (Shanab et al., 2012). Algae are ecologically important as a primary producer and have been used by humans as food and medicine for centuries. Diversity of microalgae includes, prokaryotic microalgae such as cyanobacteria (Chloroxybacteria) and eukaryotic microalgae such as diatom (Bacilllariophyta), green algae (Chlorophyta) and red algae (Rhodophyta) (Michaud, 2013).While there were approximately 200,000 species before several million microalgae were present, there has been a lack of research on the biochemical properties of microalgae and only a limited number of species are currently commonly used globally (Richmond, 2006). Because of its high productivity, minimal seasonal variation, easier extraction and diversification, microalgae are considered superior to conventional plants (Christaki et al., 2012). In addition, microalgae can also grow rapidly, withstand tough environments and environmental stressors, such as heat, cold, salinity gradient and ultraviolet radiation exposure (Christaki et al., 2012). The worldwide market for food and feed supplements / nutraceuticals based on micro-algae is well established. Microalgae as a possible source of bioactive compounds such as phenol, tocopherols and carotenoids, which are safer and more sustainable, have been gradually taken into account (Safafar et al., 2015).

2.2 Application of microalgae in aquaculture

In recent years the use of microalgae based on its bioactive chemical component has developed rapidly in areas such as fruit, pharmaceutical, cosmetics, aquaculture and horticulture (Wang et al., 2015). This is due to the chemical composition of microalgae which are known to be rich in bioactive compounds such as omega-3-fatty acids, protein, lipid and also antioxidants (Ozkan and Berberoglu, 2013). All algae contain proteins, carbohydrates, lipids and nucleic acids in varying proportions (Koru and Delice, 2014). Microalgae act as the primary producer for the entire aquatic food chain which makes them a very important food source in aquaculture, especially during the culture of filter feeder shrimp, herbivorous and omnivorous fish and planktonic mollusks (Muller-Feuga, 2000). For many aquaculture species, especially marine finfish and invertebrates, the key function of microalgae as a preferred or compulsory source of feed is (Tredici et al., 2009). Microalgae are used in aquaculture to provide essential nutrients or as a color enhancer for the flesh and skin of fishes (Hemaiswarya et al., 2011). In intensive aquaculture, hatcheries cultivate and periodically administer individual strains of microalgae in separate reactors to the farmed species (Shields and Lupatsch, 2012). In addition, they are often used extensively during the larval rearing of mollusks, shrimps and some fish species (Catarina and Xavier, 2012). Several studies have shown that microalgae are suitable for aquaculture live feed due to their high sterols and essential fatty acids. For crustaceans, the sterols naturally available in microalgae are important as they cannot synthesize this (Teshima et al., 1982). In addition to lipids, vitamins or pigments, under optimized culture conditions, microalgae may also contain high protein amounts of up to 70% of dry weight (Richmond, 2006).

The nutritional value of microalgae varies between species and culture conditions and according to Fujii et al. (2010), microalgae species can contain between 30-40% protein, 10-20% lipid and 5-15% of carbohydrate under normal nutrient condition. The proximate composition of microalgae is influenced by culture parameters such as light (intensity and photoperiod), temperature and pH (Khatoon et al., 2014). In addition to their nutritional content, other essential requirements for the selection of microalgae species for aquaculture use are their simple cultivation method, non-toxic, cell size and shape, and the digestible cell wall (Hemaiswarya et al., 2011; Patil et al., 2007). The development of Hatchery rotary plants originally was based on live

microalgae and/or bakery yeast feed (Conceição et al., 2010). Genera and species of major microalgae strain used in aquaculture include *T. suecica, T. chuii, C. vulgaris, D. salina, H. pluvialis, C. calcitrans, N. oculata, Navicula sp.,* and *Isochrysis galbana* (Shields and Lupatsch, 2012). In addition to their applications in hatcheries, microalgae also attracted attention as animal feed supplements because of their bioactive constituents, which have contributed to their functional properties, according to Spolaore et al. (2006). Algae have been credited with enhancing the immune system even when used in limited amounts in livestock and aquaculture feeds (Turner et al., 2002), lipid metabolism (Güroy et al., 2011; Nakagawa, 1997), antiviral and antibacterial action, enhanced gut function (Michiels et al., 2011), stress tolerance (Nath et al., 2012; Sheikhzadeh et al., 2012) besides providing a protein source, amino acid (Gouveia et al., 2008). Aqueous microalgae extract has also been reported to exhibit antioxidants, anticancer and antiviral activities (Santoyo et al., 2012; Shanab et al., 2012). The extract of *Chorella autotrophica, Porphyridium cruentum* and *Ellipsoidon* sp., in a report by Fabregas et al. (1999), were found to be very effective in inhibiting the replication of haemorrhagic septicemia virus and African swine fever virus. In addition to its ability to produce antioxidants and other economically valuable bioactive compounds, microalgae also has commercial application because of the rapid growth rate and the lower amount of nutrients of nutrients required during culture (Lee et al., 2006).

2.3 Taxonomic Classification and Characterization of the Microalgae Species

2.3.1 *Tetraselmis chuii*

Domain: Eukaryote

Kingdom: Viridiplantae

Phylum: Chlorophyta

Class: Prasinophyceae

Order: Chlorodendrales

Family: Chlorodendraceae

Genus: *Tetraselmis*

According to Borowitzka (2018) *Tetraselmis* sp. are unicellular flagellates with an elapsed ovoid cell shape, with motile cells slightly flattened, and typically 10 µm long x 14 µm broad. It has four flagels equal in length, a medium to thick cell wall, and when swimming with the body rotating and the flagella at the front end, it moves in a straight line. This species reproduces by binary fusions and sexual reproduction has never been observed; the motile cells become non-motile when cell divisions occur, or the non-motile cells of some species are attached to the surface. During the dark time, cell divisions occur with the energy gathered during the day in the form of carbohydrate storage, while the separation of cells is caused by the onset of light. *Tetraselmis* is easy to grow, and a number of species are widely used as aquacultural feed, such as *Tetraselmis chui*, *Tetraselmiss uecica* and *Tetraselmis tetrahele*. Most recently, *Tetraselmis* euryhaline strains that can expand over a very large salinity range have gained attention as potential sustainable biofuel lipid sources (Fon-Sing and Borowitzka, 2016).

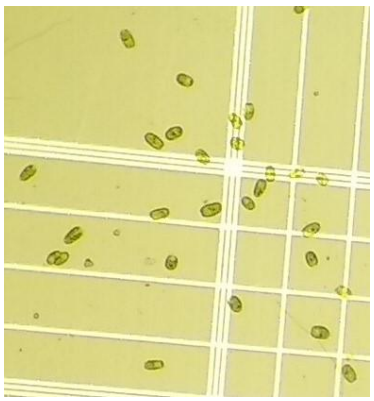


Figure 1: *Tetraselmis chuii* under optika microscope

2.3.2 *Chlorella vulgaris*

Domain: Eukaryota

Phylum: Chlorophyta

Class: Trebouxiophyceae

Order: Chlorellales

Family: Chlorellaceae

Genus: *Chlorella*

The first algae with small globular cells to be isolated by Beijerinck (1890) in culture is *Chlorella* (green algae; Chlorophyta). *Chlorella* was also one of the first considered microalgae developed for mass cultivation. The *Chlorella* type is a single, spherical to ovoid microalgae of 2.0 – 10.0 μm diameter with a cup-like chloroplast (Bock et al., 2011; Phukan et al., 2011). The cells are covered by a thin cellulose wall. *Chlorella* was initially used as a food source rich in protein, but was also proposed as a source of biofuel. *Chlorella* predominantly freshwater but a few marine species are known and especially abundant in very nutrient-rich. Currently, 44 *Chlorella* species are recognized (Guiry and Guiry, 2017), but with further research, this number is likely to change. This involves high-temperature tolerance strains, as some strains can grow between 15ºC and 40ºC (Masojídek and Torzillo, 2008). The strains of *Chlorella* grow autotrophically in both inorganic and mixotrophic and heterotrophic environments (e.g., with the addition of acetic acid and glucose). Autospores which reproduce asexually by mitosis are the *Chlorella* cells (Yamamoto et al., 2004). As it is commonly used as a health food and feed supplement, as well as in the pharmaceutical and cosmetics industry, *Chlorella* is widely used as a healthy food and feed supplement, as well as in the pharmaceutical and cosmetics industry. It contains proteins, carotenoids, some immunostimulators, polysaccharides, vitamins, and minerals. *Chlorella* strains may be suitable for diesel replacements (Xu et al., 2006).

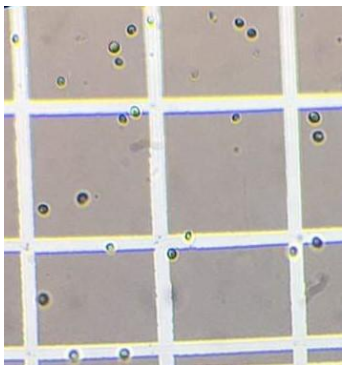


Figure 2: *Chlorella vulgaris* under optika microscope

2.4 Biology and Ecology of *Penaeus monodon*

2.4.1 *Penaeus monodon*

Penaeus monodon is known in Asian countries as the tiger shrimp, particularly in the waters of the Bay of Bengal. A variety of environmental factors affect the growth and survival of *Penaeus monodon* (Fabricius) (Chakraborti, 1986).

Figure 3: Black tiger shrimp (*Penaeus monodon*) (Courtesy: Anglingforfun)

2.4.2 Taxonomic Characteristics and classification:

John Christ Fabricius published the initial description of *Penaeus monodon* in 1798. (Mohamed, 1967). The giant tiger prawn's taxonomic description is as follows:

Phylum: Arthropoda

Class: Crustacea

Subclass: Malacostraca

Order: Decapoda

Family: Penaeidae

Genus: *Penaeus*

Specie: *P. monodon*

2.4.3 Morphology

The morphological features of *P.monodon* have been described in detail by many scientists from various countries (Motoh, 1981; Motoh and Buri, 1981; Motoh H, 1985; Racek and Yaldwyn, 1970; Feng et al., 2018). The following description includes important features sufficient for the identification of this species.

The shell is smooth, shiny and glamorous. The rostrum reaches beyond the tip of the antennular peduncle, has a sigmoidal form and has 6-8 dorsal and 2-4 ventral teeth, respectively, mostly 7 and 3. The carapace is carinated, nearly touching the posterior margin of the carapace with the rostral carina. The posterior one-third to one-half distance between the carapace's post-orbital edge and the hepatic spine is filled by the gastro-orbital carina. A prominent and almost horizontal hepatic carina is included. The antennular flagellum is sub-equal to or slightly longer than the peduncle. Exopods are present in the first four pereopods, but they are absent in the fifth. The abdomen is carinated dorsally from the anterior one-third of the fourth to the posterior end of the sixth. The telson has a median groove, but without a dorsolateral spine.

2.4.4 Color in life:

The abdomen and the carapace are transversely banded in red and white. The antennas are brown greyish. Pereopods and pleopods are brown and fringing setae red. When in shallow brackish water or in pools, the color becomes dark brown and sometimes blackish (Motoh and Buri, 1981).

2.4.5 Distribution

Giant tiger shrimp is widely distributed in most of the Indo-Pacific region, including Indonesia, Thailand, Malaysia, Hungary, Singapore, Hungary, South Africa, Somalia, Madagascar, Saudi Arabia, Oman, Pakistan, Indian, Bangladesh, Sri Lanka and Papua New Guinea (Motoh, 1985; Altuve et al. 2008; Gómez-Lemos and Campos 2008; Cintra et al. 2011). In general, *P. monodon* is distributed at longitude between 30 °E and 155 °E and at latitude between 35 °N and 35 °S, with the main fishing grounds located in tropical countries, in particular Indonesia, Malaysia and the Philippines. The fry, juvenile, and adolescent inhabit shore areas and mangrove estuaries, while most of the adults inhabit deeper waters down to 162 m (Motoh, 1985).

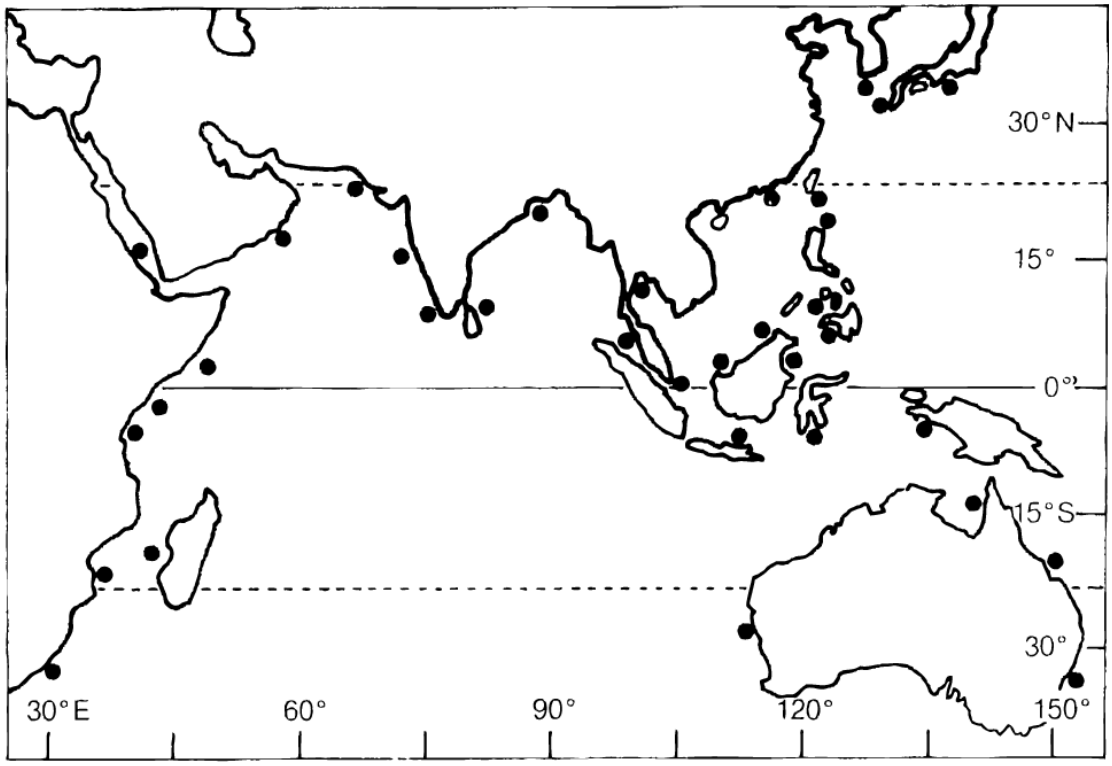


Figure 4: Geographic distribution of *Penaeus monodon* (Motoh 1981)

2.4.6 Life history

2.4.6.1 Reproduction and Sexuality

P. monodon has a heterosexual nature. The female is comparatively bigger than the male. A combined petasma, two masculine appendixes on exopods of the second pleopods, and a genital opening at the coxa of one fifth pereopod of the male can discern the sexually mature crevice. The external genital organs are the mixture. In females thelycum is found on the coxa of the third pereopod from the fourth and fifth pereopod, with a genital opening (Motoh, 1985). Age and size of *P.monodon* (male) have a major effect on spermatophore development, growth and maturation, sperm count and normal sperm percentage (Jiang et al., 2009)

2.4.6.2 Morphological Development

2.4.6.2.1 Embryo:

Eggs, with a diameter ranging from 0.27 to 0.31 mm and an average of 0.29 mm, are spherical, yellowish-green, and minute. In calm waters, eggs begin to sink slowly. The 2-celled, 4-celled, morula and embryonic nauplius cleavage stages occur

approximately 0.5, 1, 1.8 and 11 hours after spawning, respectively. It is noted that the nauplius shifts intermittently in each egg before hatching (Motoh, 1985).

2.4.6.2.2 Larva:

The larval stage is made up of 6 nauplius, 3 protozoa, 3 mysis, and 3 or 4 megalopa substages, requiring approximately 1.5 days, 5 days, 4-5 days, and 6-15 days for growth, respectively (Motoh, 1979, 1981, 1985). Larvae demonstrate offshore planktonic behavior at offshore with antennal propulsion for swimming in mysis nauplius, antennal and thoracic propulsion in mysis, and megalopa abdominal propulsion. Since the nauplii use yolk granules inside their body, in protozoa and mysis (collectively called zoea) substages, feeding begin. According to Motoh (1985), the earlier juvenile stage megalopa (typically known as postlarva or 'fry' for commercial purposes) is translucent with a dark brown stripe at the tip of the teleson on the ventral side of the antennular flagellum. Under laboratory conditions, on the sixth day of the post-larval period, post-larvae become benthic. The megalopa reaches the nursery soil under normal conditions. Megalopa carapace lengths range between 1.2 mm and 2.3 mm. Even during the postlarval stage, shrimp undergo gut morphological changes during the ontogeny (Cicala et al., 2020).

2.4.6.2.3 Juvenile:

As in the megalopa, the earlier juvenile stage has a translucent body on the ventral side with a dark brown streak. The earlier juvenile stages were described by Motoh (1985) as follows: (1) relatively shorter sixth abdominal segment compared to the length of the carapace, (2) greater body size, (3) full rostral spine formula, (4) complete gill system, and (5) benthic behavior.

The body becomes blackish in color and voluminous in the later stage, and the rostrum has 7 dorsal and 3 ventral spines. As in adults, the juvenile crawls are using the pereopods and swims using the pleopods. The carapace length ranges from 2.2 to 11.0 mm. The early postmysis phases of the giant tiger prawn have been described (Motoh, 1985; Motoh and Buri, 1980, 1981).

2.4.6.2.4 Adolescent:

This stage resembles the adult prawn. Sexes are now distinct beginning at 11 mm Carapace Length (CL). The minimum size of males with a joined petasma is

approximately 30 mm CL and the minimum size of females with adult-like thelycum is approximately 37 mm CL. The adolescent's carapace length ranges between 11 and 34 mm.(Motoh , 1985)

2.4.6.2.5 Subadult:

This stage is the onset of sexual maturity. In its terminal ampoule, the male has spermatozoa. The thelycum of the female now contains spermatozoa. Females grow

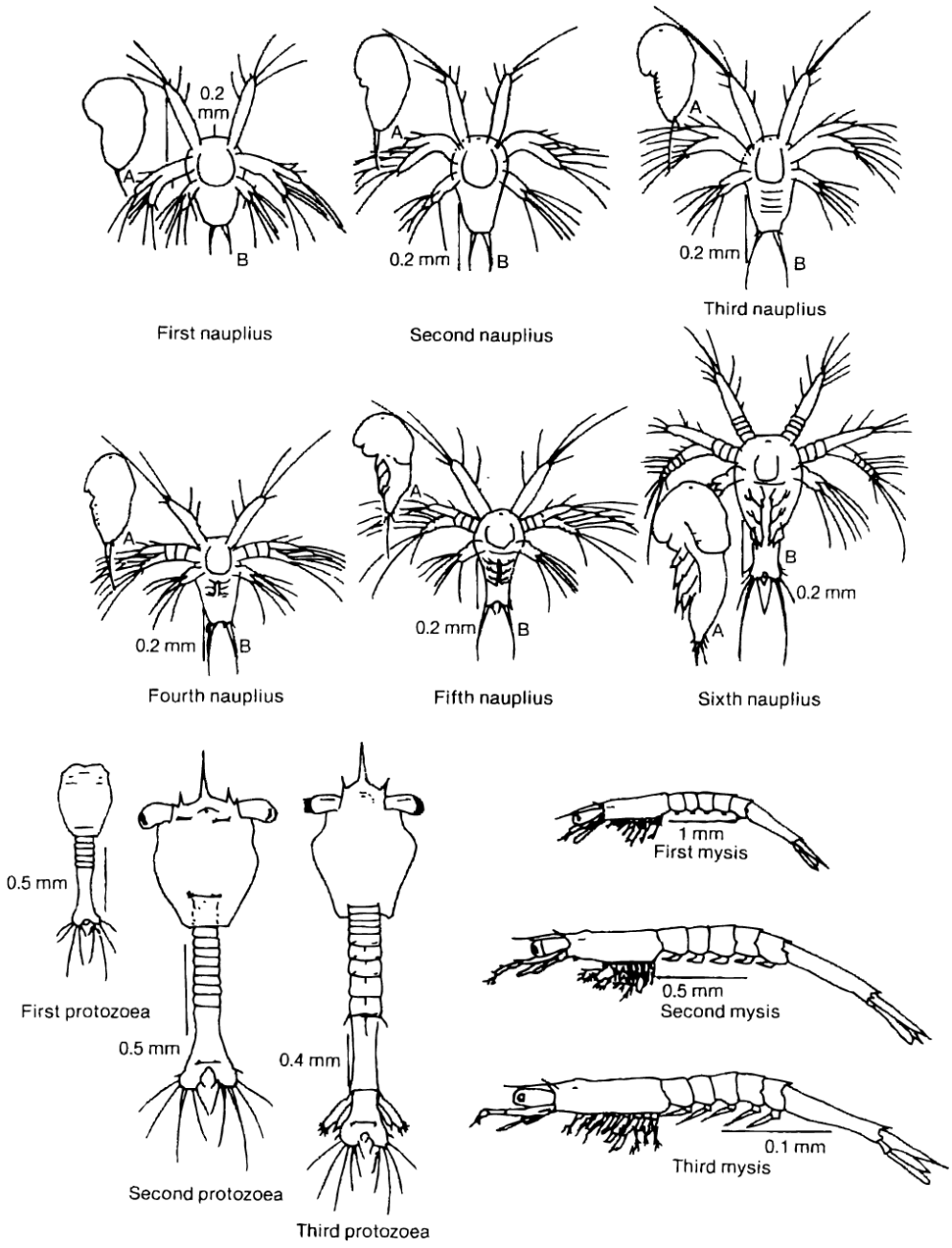


Figure 5: Larval stages of *P. monodon* (Motoh, 1981, 1985)

faster at this stage (30 mm CL) and migration from nursery to spawning grounds starts. The first copulation occurs between males and females with a minimum of 37 mm and 47 mm CL, respectively, during migration.

2.4.6.2.6 Adult:

This stage has very similar appendages to the subadult and is marked by the completion of sexual maturity. This varies only with the size and habitat of the subadult. Males possess spermatozoa, and females start to spawn offshore although a few spawns in shallow water. In most species, a second or more copulation can occur.

The maximum total length recorded was 336 mm (Holthuis 1980), while a mature female of 307 mm according to Mohamed (1967). The largest male ever discovered in the Philippines was 71 mm CL, while the female was 81 mm CL with a body length of 270 mm or 240 g. (Motoh and Buri, 1980). Adult carapace lengths range between 37 and 71 mm for males and 47 and 81 mm for females. Ecologically, in order to complete their life cycle, penaeid prawns need to go through two main ecosystems: the offshore and the coastal inshore habitats (Rönnbäck et al., 2002).

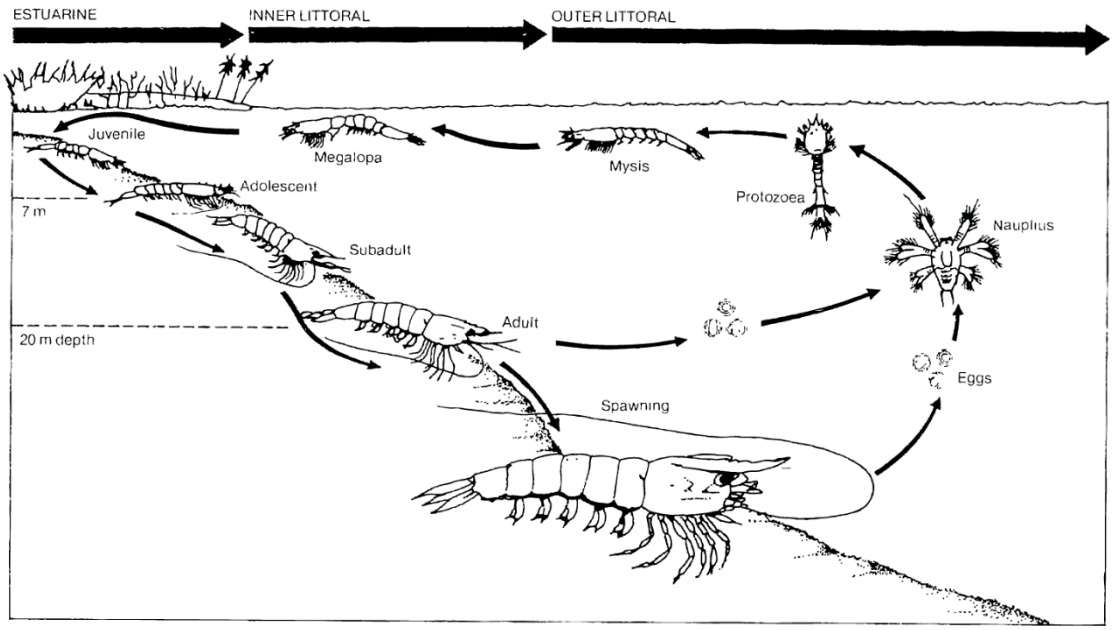


Figure 6: Diagram of the life history of the giant tiger prawn, *P. monodon* (Motoh, 1981)

2.4.7 Salinity tolerance:

Previous studies have demonstrated that the salinity tolerance of *Penaeus monodon* (Fabricius 1798) ranged from 1 to 57 ‰, and the most suitable salinity range for growth was from 10 to 35 ‰ (Chen et al., 2020).Salinity especially affects the distribution of shrimp during reproduction cycle (Mosha and Gallardo, 2013) .

2.4.8 Longevity:

There is no reliable method developed to determine the age of an individual prawn. Villaluz et al., (1969) believed that the life span of *P. monodon* is one to two years; Motoh (1981) estimated it to be about one and a half years for males and about two years for females.

2.4.9 Feeding behavior

Penaeids are generally considered to be *P. monodon* omnivores, especially prefer crustaceans, plants, polychaetes, molluscs, fish, and insects. This result was confirmed by Thomas (1972) and he clarified that mud and sand found in the gut were unintentionally swallowed. A study recorded that the sugpo fry stage relishes food with plankton (lablab). *P. monodon* food also consisted of crustacea (small crabs and shrimps) and mollusks, which accounted for 85% of the food eaten, according to Marte (1980). Fish, polychaetes, ophiuroids, debris, sand, and silt made up the remaining 15%. This suggests that, instead of a scavenger or detritus feeder, the giant tiger prawn is more of a hunter of slow-moving benthic macro-invertebrates. Kuttyamma (1974) noted that debris composed of mud and organic matter was the main portion of the stomach material, while crustaceans ranked next in number. All these findings indicate that *P. monodon*, particularly in the natural environment, is more of a carnivore with a preference for crustaceans, but it also feeds on other organisms available, including algae.

During the ebb tide (Marte, 1980), *P. monodon* seems to have increased feeding behavior and displays certain food preferences during seasonal food variations (Kuttyamma, 1974). By seizing the food with its pinchers and moving food to the mouth to nibble, this species eats. Four hours after ingestion, undigested food defecated (Marte, 1980). According to Uddin et al. (2020), from November to February, *P. monodon* demonstrated vigorous feeding.

2.4.10 Nutritional Requirement for Shrimp post larvae

For normal growth and development, shrimp larvae require sufficient amounts of proteins, lipids, carbohydrates, minerals, and vitamins. According to Wyk (1999) The recommended protein requirement for shrimps of 0.002 to 0.025 g to 1 g, 1 to 3 g and > 3 g is: 50%, 45%, 40% and 35% of the total protein, respectively. Meanwhile, the 0.002 to 0.25 g, 0.25 to 1 g, 1 to 3 g and > 3 g recommended lipid requirements for shrimp are 15%, 9%, 7.5% and 6.5%, respectively. Shrimp larvae typically need 50-57 % protein, 12-15 % lipid and 20 % carbohydrate (Iba et al., 2014).Microalgae can be considered a good candidate for shrimp aquaculture if they contain a protein above 25% of dry weight, 8-30% carbohydrate and around 10% fat (Nuñez et al., 2002). With *Tetraselmis* sp. when cultured in 30 ppt salinity, it was found to have 55.2% protein, 21.2% lipid and 22.5% carbohydrate (Khatoon et al., 2014). *Chlorella*, meanwhile, was found to have 47.82% protein, 13.32% and 8.08% carbohydrate (Tokuşoglu and Ünal, 2003). According to Kay and Barton (1991), it's about 20% fat, 45% protein, 20% starch, 10% different minerals and vitamins.

CHAPTER-3

METHODOLOGY

CHAPTER-3

METHODOLOGY

This chapter deals with the methods that are followed and materials that are used to achieve the objectives of the study. Methodology is an essential and integral part of any research. In this study, a scientific and logical methodology has been followed. Experiments were conducted in two phases. During the first phase, two selected marine microalgae species were cultured. Dried biomass of selected microalgae was kept in the refrigerator for further use during feed preparation. In the second phase, growth, survival rate and nutritional profile of marine larvae *Penaeus monodon* were studied through feeding with the two algal diets.

3.1 Study area and Collection of microalgae species:

In this study selected microalgae *Tetraselmis chuii*, and *Chlorella vulgaris* were collected from the laboratory of Live Feed Research Corner, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Bangladesh. Using Conway culture medium, the pure *T. chuii*, and *C. vulgaris* stock cultures were maintained at 28 ppt salinity (Khatoon et al., 2018). The subculture of selected microalgae was carried out every two weeks to maintain a pure and stable stock culture. Pure seed stock of all microalgae species were maintained at 28 ppt salinity in Conway medium with a 24 hours photoperiod.

3.2 Seawater collection:

Water was collected from the nearest seabeach (Sagorika seabeach, Kattoli, Chattogram) during the high tide. Collected seawater brought in the laboratory and kept overnight to settle soluble particle in water. Water was then filtered using a Whatman GMF Circles 4.7cm paper by a vacuum pump. Filtered water was autoclaved at 115ºC for 15 minutes. Filtered and sterilized water was used to prepare media for microalgae culture.

3.3 Media Preparation

Three stock solutions (macronutrients, trace metal solutions and vitamins) were prepared by dilution of the chemicals in water as shown in Table 1. The Conway medium was prepared by adding Solution A, Solution B and Solution C respectively 1 ml, 0.5 ml and 0.1 ml into 1 L of filtered and sterilized seawater at 28 ppt salinity.

Table 1: Chemical composition of Conway medium (Tompkins et al., 1995)

Constituents	Quantities
Solution A- Macronutrients	
Sodium nitrate ($NaNO_3$)	20g
Ferric chloride ($FeCl_3$)	1.3g
Boric acid (H_3BO_3)	33.4g
EDTA(b), di-sodium salt	45g
Manganous chloride ($MnCl_2$, $4H_2O$)	0.36g
Sodium di-hydrogen orthophosphate (NaH_2PO_4, $2H_2O$)	20g
Distilled water	1000ml
Solution B- trace metal	
Zinc chloride ($ZnCl_2$)	4.2g
Cobaltous chloride ($CoCl_2$, 6 H_2O)	4.0g
Ammonium molybdate $(NH_4)6Mo_7O_{24}$, $4H_2O$	1.8g
Cupric sulphate ($CuSO_4$, $5H_2O$)	4.0g
Distilled water	1000ml
Acidify with HCl to obtain a clear solution	
Solution-C Vitamins	
Vitamin B1	200mg
Vitamin B12	10mg
Distilled water	1000ml

3.4 Culture of Microalgae:

Microalgae were cultured at 28 ppt salinity, 23°C, and 1600 lux with 24 hours of photoperiod. Continuous aeration was provided throughout the culture period. The initial cell density was 1 x 10^5 cells ml^{-1} for each treatment. Growth experiments for each microalgal species were carried out in three replicates until the death phase. During the growth curve experiment, growth parameters in terms of cell density and optical density were measured daily. All experimental cultures for biomass collection were harvested in the stationary phase as determined by the growth experiment previously. *T. chuii* and *C. vulgaris* on Day 8, were harvested by centrifugation using

HERMLE Z 206A High-Speed Refrigerated Centrifuge at 5000 rpm for 5 minutes and washed twice with sterilized distilled water. Samples were then freeze-dried and kept at -20 °C before further analysis.

3.5 Mass Culture of Microalgae:

Mass cultures of selected potential species were done on large scale in a 20 L vessel using Conway medium. From an initial starter culture volume of 20 ml to 100 ml, the culture was eventually enlarged. Initially, 20 ml of microalgae stock cultures were combined as concentrated stock with 30 ml of medium in each flask (total culture volume 50 ml), with batch cultures rising in volume (250ml, 500 ml and 1 L) before being moved to a larger container with a culture medium of 20 L. In their exponential growth process, the culture has been transferred. Once the culture entered its stationary level, all cells were harvested for 5 minutes by centrifugation at 5000 rpm using a continuous tubular centrifuge J-025 (HERMLE Z 206A) and subsequently washed with sterilized distilled water twice. Using a refrigerator, samples were then freeze-dried and held at -20°C until the next procedure. Mass culture was conducted continuously until the feeding experiment produced a sufficient amount of microalgal biomass.

3.6 Feed Formulation for *Penaeus monodon* Postlarvae

Seven diets were formulated replacing different percentages of microalgae replacement of fish meal inclusion at 0%, 25%, 50% and 75%. The freeze-dried microalgae biomass was ground into fine particles (diameter of 0.4 – 0.5 mm) by using a mortar and pestle and then mixed with the custom-made feed pellet by adding 1% carboxymethylcellulose (CMC) and distilled water to form a concentrated paste. The feed was then freeze-dried using a refrigerator and ground into fine particles. The finished feed was stored at -20°C until use.

3.7 Proximate Analysis of the Formulated Feed

3.7.1 Protein Analysis:

According to Lowry et al. (1951) protein content was analyzed. For each sample, 5-6 mg of freeze-dried microalgae was taken and mixed with distilled water into a 25 ml solution. 25 ml of the prepared sample, 0.5 ml for protein analysis was taken from each sample. Reactive 1 (1% potassium sodium tartarate tartrate) and Reactive 2 (2 g

sodium carbonate per 100 ml of 0.1 N NaOH) were prepared prior to this. By adding 1 ml Reactive 1 to 50 ml Reactive, a mixed reagent was prepared. Then 0.5 ml of the sample with 0.5 ml of 1 N sodium hydroxide was added and held for 5 minutes in a 100 °C water bath. It was then cooled in a water bath and 10 minutes after cooling 2.5 ml of the prepared mixture reagent was added. The mixed solution was applied with 0.5 ml of Folin reagent and kept for 30 minutes in dark places. A spectrophotometer (UV-1601, Shimadzu) at a wavelength of 750 nm was used for the reading of the mixed solution.

3.7.2 Lipid Analysis

On the basis of Marsh and Weinstein (1966), lipid analyses were performed using the sulphuric acid charring process, using tripalmitin as standard after lipid extracting using the Bligh and Dyer (1959) method. The samples were extracted is extracted from the 4.5 ml of methanol: chloroform (2:1 concentration), and centrifuged for 10 minutes at 10, 000 rpm. After the supernatant has been extracted from the bio-mass, the sample has been centrifuged again for the separation of two phases with 1.5 ml of chloroform and 1.5 ml of distilled water. The polar phase was extracted and evaporated with a water bath at 35 °C with the pipette following centrifugation. In 1 ml of chloroform the dry waste was solubilized. This solution was then taken from three aliquots of 200 μL each and then transferred to test tubes, and the solvent was again evaporated. If fully dried, 2 ml of sulfuric acid concentrate is added. The carbonization process took 15 minutes at 200°C, then the tubes were cooled and 3 ml of water were applied to every tube. The density of the optical density was 375 nm.

3.7.3 Carbohydrate Analysis

Based on the Dubois et al. (1956) process, carbohydrate analysis was carried out (1956). 5-6 mg was taken for each sample and then combined with distilled water to form a 25 ml solution. 5% phenol solution and concentrated sulphuric acid were prepared prior to analysis. By adding 1 ml of 5% phenolic solution and 5 ml of concentrated sulphuric acid, the samples were analyzed. The optical density in a spectrophotometer was measured at 488 nm (Shimadzu UV-1601, Japan).

3.8 Experimental Setup

Twenty-one black 10 L plastic buckets were used in this experiment. The tanks were washed thoroughly and soaked in 20 mg L^{-1} chlorine overnight to prevent any disease transfer through the rearing containers. Then the tanks were rinsed intensively with running tap water until the smell of chlorine was gone and they were placed to dry under the sun. Each tank was filled with 5 L of filtered dechlorinated seawater at 26 ppt prior to stocking. Tanks were not arranged randomly as the feeding trials were carried out in a closed hatchery with a uniformly distributed fluorescent light. *P. monodon* postlarvae at the stage of PL 2 were obtained from a commercial hatchery and remained in the laboratory for 2days conditioning. PL 5 was stocked at a density of 50 PL L^{-1} according to the study done by Khatoon et al. (2007). Every tank was given constant aeration and the hatchery tanks were maintained under a 12 h light: 12 h dark cycle. The shrimp were fed at 6:00 am, 12:00 pm, 6:00 pm, 12:00 am, four times a day. The shrimp PLs were fed with the formulated feed mixed with different algae at 0%, 25%, 50% and 75%. All treatment was administered in triplicates and for a total of 17 days the experiment was conducted. In order to maintain water quality, fecal matter and the remainder of the uneaten feed were siphoned out daily at less than 10% of culture volume. The survived shrimp from each tank were counted at the end of the feeding trials and weighed to estimate mean survival for the treatment and control groups.

3.9 Physical Analysis

Using pH meter (Hanna instrument), portable Refractometer and Dissolved Oxygen Meter (DO-5509), temperature, pH, salinity and dissolved oxygen in culture tanks were measured daily respectively.

3.10 Chemical Analysis

On alternate days, total ammonia nitrogen, nitrite, and phosphate phosphorous were calculated according to the Parsons et al. (1984). An alternate day, 30 ml of water sample was obtained from each tank and filtered using a 4.7cm Whatman GMF Circles before analysis.

3.10.1 Total ammonium nitrogen (TAN)

There were five ml of water samples placed in a test tube. 0.4 ml of phenol solution (20 g of analytical grade phenol dissolved in 200 ml of 95% v/v ethyl alcohol) and 0.4 ml of sodium nitroprusside were applied to the sample (1 g of sodium nitroprusside dissolved in 200 ml of MiliQH_2O). To begin the reaction, 1 ml of oxidizing solution was then added. By mixing 100 ml of alkaline reagents (100 g of sodium citrate and 5 g of sodium hydroxide dissolved in 500 ml of MiliQH_2O) and 25 ml of sodium hypochlorite solution, the oxidizing solution was prepared. The tubes were coated with parafilm and incubated for 1 hour at room temperature (20 - 27ºC) before being measured with a Shimadzu spectrophotometer at 640 nm (Shimadzu UV-1601, Japan)

3.10.2 Nitrite (NO_2-N)

In a test tube containing 10 ml of a water sample, sulfanilamide solution (0.2 ml) was applied. By dissolving 5 g of sulfanilamide in a mixture of 50 ml of concentrated hydrochloric acid, the sulfanilamide solution was prepared and diluted with MiliQH_2O to 500 ml. Then, after 8 minutes, 1 ml of NED reagent (0.5 g of dissolved N-(1-naphthyl)-ethylene diamine dihydrochloride in 500 ml of MiliQH_2O) was applied to the tube and immediately mixed. The Shimadzu spectrophotometer measured the extinction at 543 nm one hour later (Shimadzu UV-1601, Japan).

3.10.3 Determination of Phosphate phosphorus (PO_4-P)

One ml of mixed reagent was added to the test tube containing 10 ml of a water sample. A mixed reagent was prepared by mixing 100 ml of 0.02 M ammonium molybdate, 250 ml sulfuric acid, 100 ml of 0.31 M ascorbic acid and 100 ml of 0.002 M potassium antimonyl-tartrate. After 5 min and preferably within the first 2-3 hours, by using Shimadzu spectrophotometer (Shimadzu UV-1601, Japan) extinction was measured at 885 nm.

3.11 Survival and Growth Analysis of Shrimp Postlarvae:

The specific growth rate (SGR) was calculated from the bodyweight based on the formula of Ricker (1990): SGR = (ln wf -ln wi/Δt) x100where: ln wf is the natural logarithm of the weight at time (t) and ln wi is the natural logarithm of the initial weight of the shrimp postlarvae. The weight of the postlarvae was taken on the initial

day (PL5) and when the postlarvae reached the PL17 stage. Shrimp PLs survival was determined at the end of the experiment.

CHAPTER-4

RESULTS

CHAPTER-4

RESULTS

The results section presents the finding of a study in a systematic, logical, concise and comprehensive manner. This section contains detailed information about on the present research work.

4.1 Proximate composition of different microalgae:

The total protein, lipid, and carbohydrate content of microalgae on dry weight basis is illustrated in Figure 7. The content of proteins, lipids, and carbohydrates for *Chlorella* sp. Was (43 ± 2.9%), (12 ± 0.3%), (23 ± 1.6%), respectively. In *Tetraselmis* sp., the highest content of protein (57 ± 0.7%) was found. The lipid and carbohydrate content were (19 ± 1.3%) and (17 ± 0.9%) respectively.

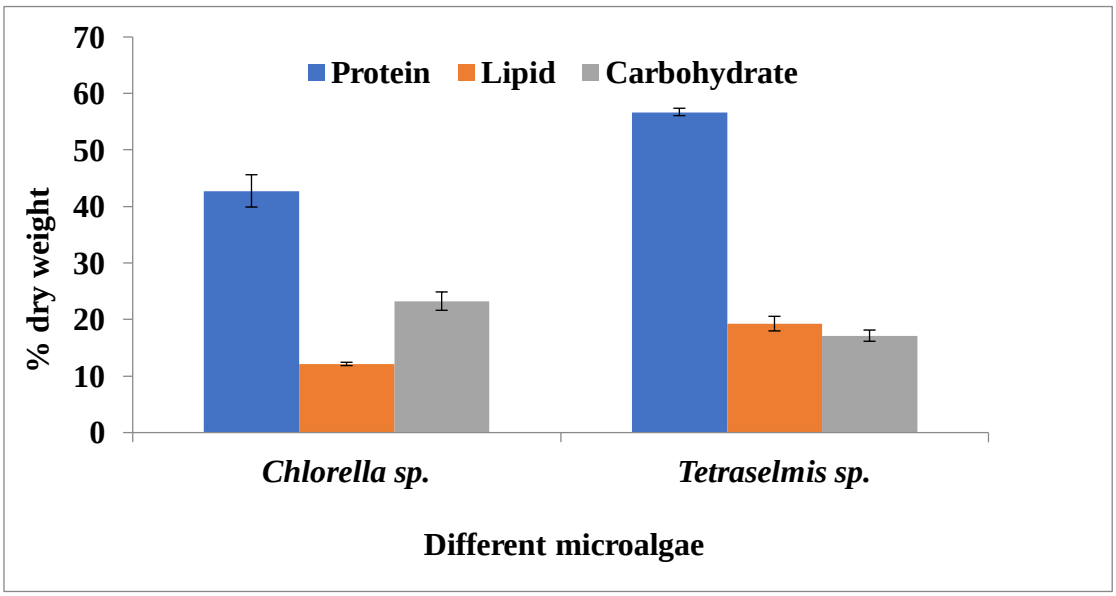


Figure 7: Proximate composition of dried algae (a) *Chlorella* sp. (b) *Tetraselmis chuii* used to replace fish meal in diets of *Penaeus monodon* PLs

4.2 Proximate Composition of the Formulated Feed

For each prepared formulated feed, Table 2 shows the total protein, lipid, and carbohydrate content. With a total of 40 ± 0.5%, the highest protein content was contained in commercial feed (CF), which is significantly higher in comparison to other treatments. In this analysis, as the addition of *T.chuii* increases, the total protein content decreases. The concentration of *C. vulgaris* in the prepared formulated feed is

followed by T25 (39.8 ± 0.17%), CH25 (39.6 ± 0.06%), T50 (39.4 ± 0.09%), CH50 (38.9 ± 0.23%), T75 (39.4 ± 0.09%) and CH75 (38.4 ± 0.17%) respectively.

Table 2: Proximate Composition of the Formulated Feed

Formulated Feed	Total Protein (% dw)	Total Lipid (%dw)	Total Carbohydrate (%dw)
CF	40.0± 0.29	12.66 ± 0.34	19.33 ± 0.23
T25	39.8 ± 0.17	10.81 ± 0.23	23.12 ± 0.28
T50	39.5 ± 0.28	10.73 ± 0.08	25.47 ± 0.39
T75	39.4 ± 0.09	10.90 ± 0.06	25.91 ± 0.22
CH25	39.6 ± 0.06	11.87 ± 0.21	24.66 ± 0.61
CH50	38.9 ± 0.23	11.90 ± 0.18	27.13 ± 0.24
CH75	38.4 ± 0.17	10.87 ± 0.19	26.42 ± 0.29

4.3 Proximate composition of Shrimp postlarvae:

Figure 8 illustrates the total protein, lipid, and carbohydrate content for each shrimp post larvae treated with formulated feed and replacement of microalgae in different percentage. In this research, the highest total protein content (39.0 ± 0.6%) was found in CH25 followed by T25 (34 ± 0.7%), T75 (34 ± 0.2%), CH75 (32 ± 0.4%), CF (30 ± 0.7%) and CH50 (29± 0.8%) respectively. The highest protein content was found in postlarvae of CH25 treated tank with a total of 39 ± 0.6%, which is significantly higher compared to other treatment. Figure 8 also illustrates the total lipid content for each of the formulated feed prepared. In this research, the highest total lipid content (18.0 ± 0.2%) was found in T25 treated shrimp postlarvae followed by CH25 (14 ± 0.3%), T50 (13 ± 0.7%), CH75 (12 ± 0.6%), CH50 (10 ± 0.2%) and CF (8 ± 0.1%) respectively. However, the total lipid content of T25 did not differ significantly ($p>0.05$) with T50, T75, and T75. Table 4 also illustrate the total carbohydrate content for each of the formulated feed prepared. In this feeding trial experiment, the highest total carbohydrate content (13.0 ± 0.5 %) was found in T25 treated shrimp postlarvae followed by CH25 (12 ± 0.6%), CH50 (12 ± 0.5%), T50 (11 ± 0.6%), CH75 (11 ± 0.7 %),T75 (10 ± 0.2%) and CF (7 ± 0.2%) respectively. However, the total lipid content of T25 did not differ significantly ($p>0.05$) with T50, T75, and T75.

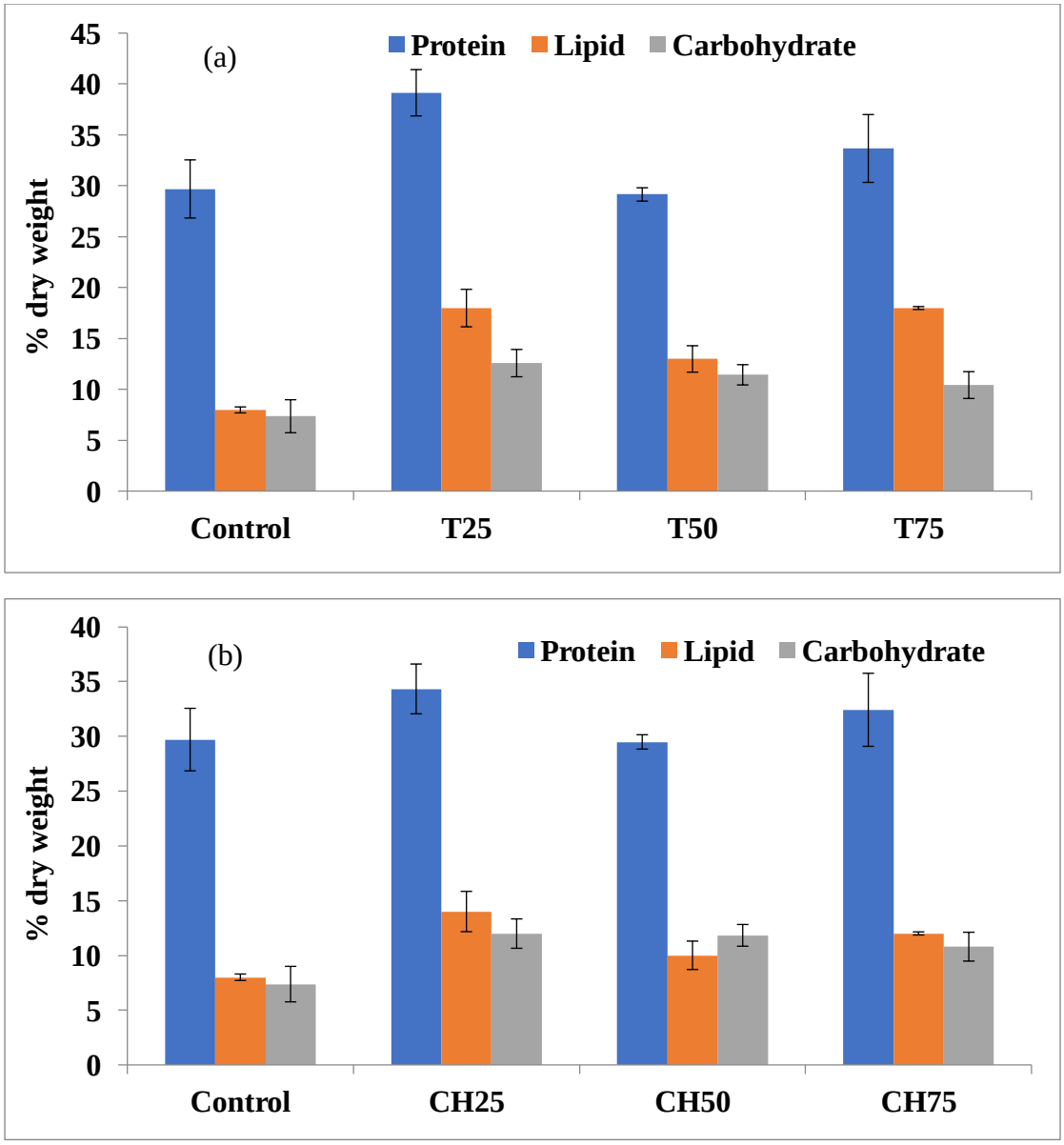


Figure 8: Proximate composition of *Penaeus monodon* fed with two different algae (a) *Tetraselmis chuii* (b) *Chlorella* sp. with the replacement of fish meal (25%, 50% and 75%) over the 17 days of period.

4.4 Water Quality in Shrimp Culture Tanks

4.4.1 Physical Analyses of Water Quality

There was no significant difference amongst all treatments with respect to the physical parameters (temperature, salinity, light and pH) of the water (Table 3) during the whole experimental period.

Table 3: Temperature (ºC), dissolved oxygen (mgL^{-1}), pH range and salinity (ppt) range in control and treated tanks

	Parameter			
Treatment	Temperature (ºC)	DO (mgL^{-1})	pH	Salinity (ppt)
CF	26.8 ± 0.08	5.8 ± 0.09	8.2± 0.05	26.4 ± 0.15
T25	26.5 ± 0.11	5.9 ± 0.05	8.2 ± 0.03	26.5 ± 0.13
T50	26.1 ± 0.13	5.9 ± 0.05	8.2 ± 0.03	26.2 ±0.09
T75	26.3 ± 0.13	5.7 ± 0.07	8.1 ± 0.04	26.4 ± 0.11
CH25	26.5 ± 0.09	5.9 ± 0.05	8.2 ± 0.04	26.4 ± 0.14
CH50	26.4 ± 0.15	5.8 ± 0.10	8.1 ± 0.03	26.3 ± 0.10
CH75	26.7 ± 0.06	5.9 ± 0.08	8.2 ± 0.02	26.5 ± 0.11

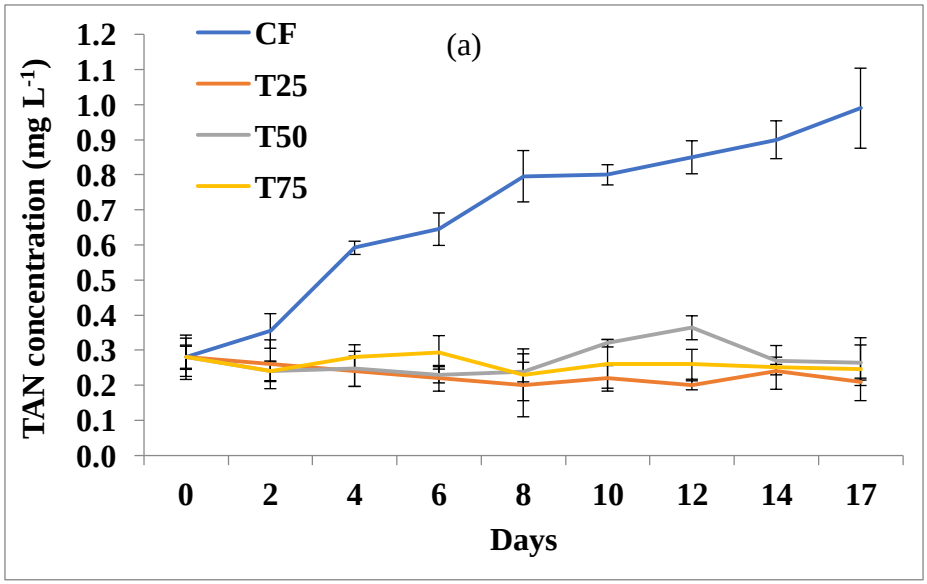
4.4.2 Chemical Analyses of Water Quality

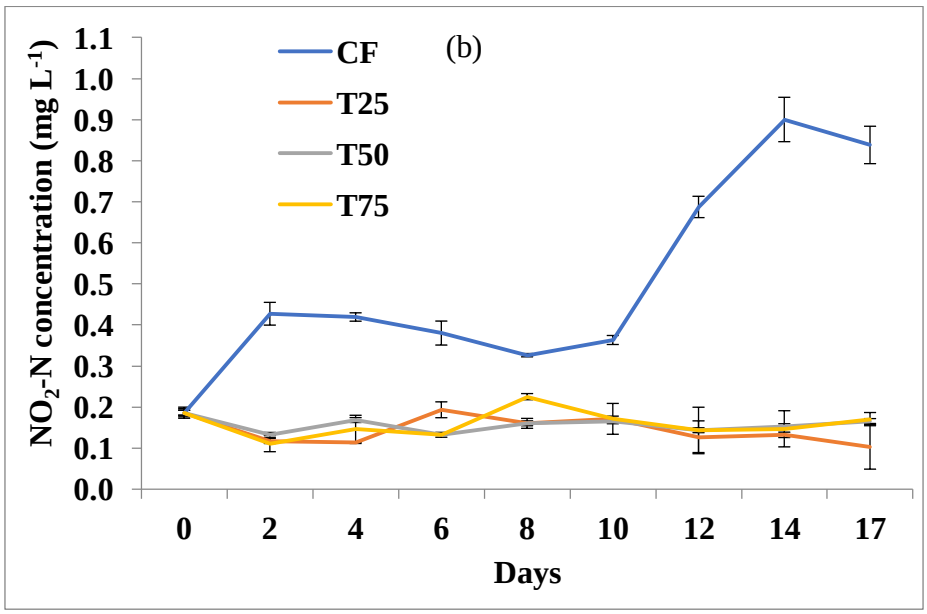
Figure 9(a) shows the total ammonia nitrogen (TAN) of the water sample from *P.monodon* postlarvae (PLs) tanks fed with different experimental diets. There was a significant increase ($p<0.05$) in the TAN of the control tank (CF) on Day2 (0.355 ± 0.05 mg L^{-1}) and the value continue to increase until the end of the experiment on Day17 (0.99 ± 0.01mg L^{-1}). On the other hand, the TAN of the water sample from the tank fed with the addition of different *T. chuii* percentage showed a significantly lower ($p<0.05$) TAN and a significant decrease ($p<0.05$) in the TAN towards the end of the experiment compared to control (CF). Although T25 showed a significantly lower ($p<0.05$) TAN compared to other treatments, both T50 and T75 also manage to maintain a low TAN throughout the experiment compared to control (Figure 9a).

Based on Figure 9(b), the NO_2-N of water sample from the tank fed with the addition of different *T. chuii* percentage showed a significantly lower ($p<0.05$) NO_2-N throughout the experiment and the NO_2-N was maintained at a relatively low level until Day17. On the other hand, control (CF) treatment showed significantly higher NO_2-N on day 14 (0.99± 0.05mg L^{-1}) compared to other treatment and the NO_2-N value increased significantly ($p<0.05$) existed in the NO_2-N between T75 (0.294 ± 0.05mg L^{-1}) and T50 (0.364 ± 0.05mg L^{-1}) (Figure 9b).

Figure 9(c) showed phosphate phosphorus (PO_4-P) (mg L^{-1}) of water sample from the tank fed with the addition of different *T. chuii* percentages. The PO_4-P of CF significantly raised ($p<0.05$) with the increase of the culture period. All treatments

with *T. chuii* addition showed an increase in the PO_4-P content of T75, CF and T25 from the initial day up to Day 4. However, on Day8, the PO_4-P T75 increased from 0.11 ± 0.00 mg L^{-1} to 0.197 ± 0.01 mg L^{-1} on Day10 and gradually decreased. Although T25 showed a significantly lower ($p<0.05$) TAN compared to other treatments (Figure 9c).





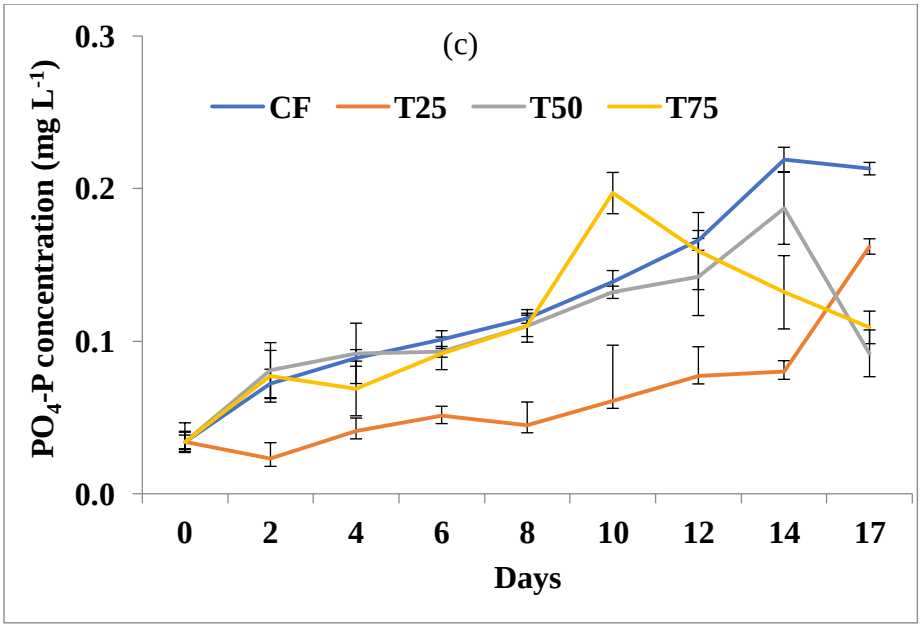


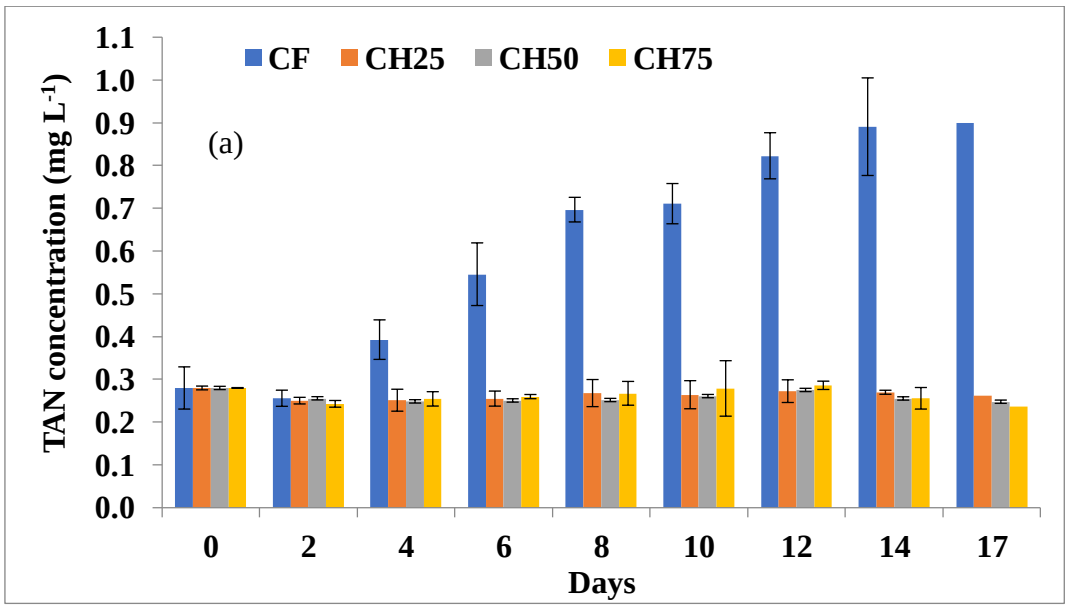
Figure 9: Total ammonia nitrogen (mgL^{-1}), NO_2-N concentration (mgL^{-1}) and PO_4-P concentration (mgL^{-1}) of all treatments at entire culture period fed with *Tetraselmis chuii* as experimental diet.

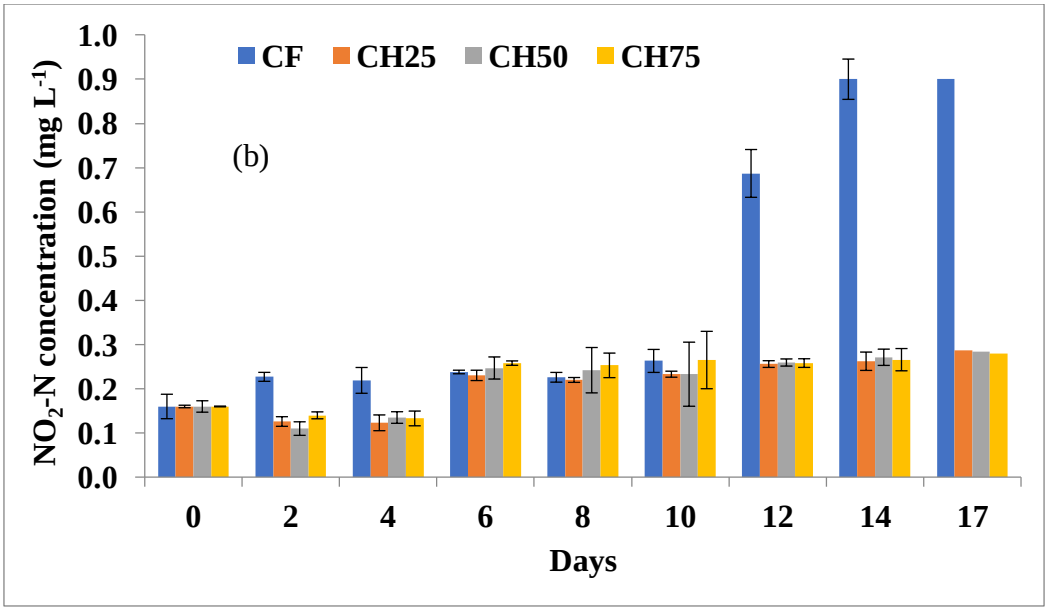
Based on Figure 10(a), the TAN of the water sample from the tank fed with the addition of different percentage *C.vulgaris* showed a significantly lower ($p<0.05$) TAN. A significant decrease ($p<0.05$) in the TAN towards the end of the experiment compared to control (CF) was observed. The TAN of CH25, CH50 and CH75 did not differ significantly ($p>0.05$) throughout the experiment and the NO_2-N was maintained at a relatively low level until Day17. On the other hand, control (CF) showed significantly higher TAN compared to other treatments and the TAN value increased significantly ($p<0.05$) until Day17 compared to treatments with different percentages of *C.vulgaris* addition (Figure 10a).

Based on Figure 10(b) the NO_2-N of water sample from the tank fed with the addition of different *C.vulgaris* percentage showed a significantly lower ($p<0.05$) NO_2-N. A significant decrease ($p<0.05$) in the NO_2-N towards the end of the experiment compared to control (CF) diet was observed. The NO_2-N of CH25, CH50 and CH75 did not differ significantly throughout the experiment and the NO_2-N was maintained at a relatively low level until Day17. On the other hand, control (CF) diet showed significantly higher NO_2-N compared to other treatment and the NO_2-N value increased significantly until Day14 compared to other treatments of *C.vulgaris*

inclusion. However, by Day12, no significant difference ($p>0.05$) existed in the NO2-N among CH25 (0.2559 ± 0.0 mgL-1), CH75 (0.2581 ± 0.0 mgL^{-1}) and T50 (0.2594 ± 0.0 mgL^{-1}) (Figure 10b).

Figure 10(c) showed phosphate phosphorus (PO_4-P) (mgL^{-1}) of water sample from the tank fed with the different *C.vulgaris* concentration. The PO_4-P of CF significantly increased ($p<0.05$) with the rise of the culture period. An increase in the PO_4-P content was noticed in CH75, CF and CH25 treatments from the initial day up to Day 6. However, on Day10, the PO_4-P of CH50 increased from 0.105 ± 0.01 mg L-1 on Day8 to 0.187 ± 0.01 mgL^{-1} on Day10. On Day14, water in the tank of PLs fed with CF (0.219 ± 0.01mgL^{-1}) showed the highest PO_4-P value compared to other treatment throughout the experiment, followed by CH75 (0.184 ± 0.00mgL^{-1}), CH25 (0.125 ± 0.01mgL^{-1}) and CH50 (0.204 ± 0.00mgL^{-1}) respectively (Figure 10c).





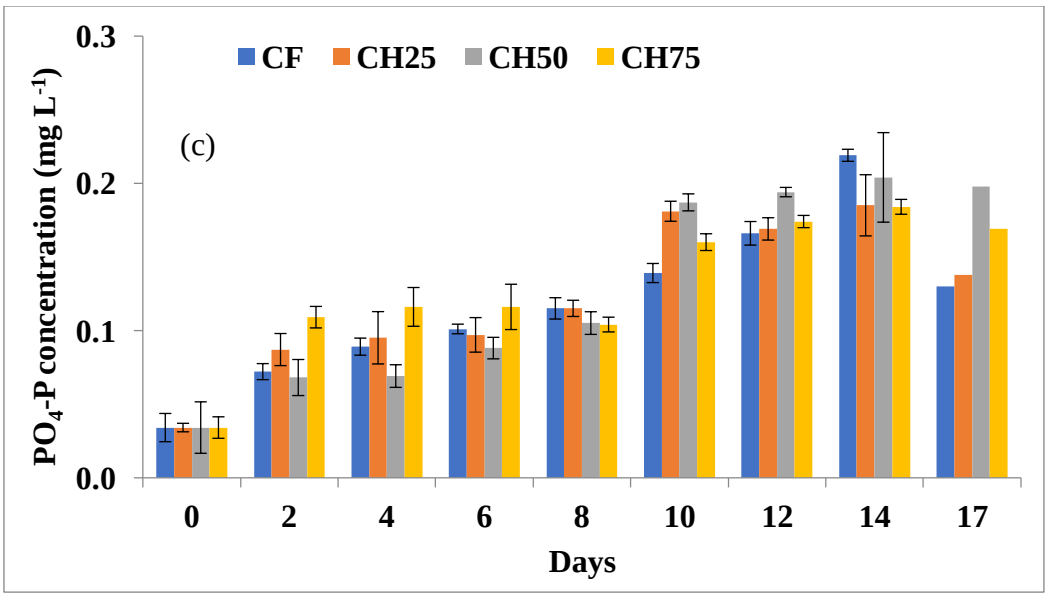


Figure 10: Total ammonia nitrogen (mgL^{-1}), NO_2-N concentration (mg L^{-1}) and PO_4-P concentration (mg L^{-1}) of all treatments during the entire culture period fed with *Chlorella vulgaris* as experimental diet.

4.5 Shrimp Survival and Specific Growth Rate

Figure 11(b) represented the survival rate of PLs fed with different types of formulated feed containing varying percentages of *T. chuii*. The highest survival of shrimp was achieved in tanks of PLs fed with T25 (64.44 ± 1.91%), CF (52.85 ± 2.37 %) and followed by T50 (53.22 ± 2.91%). Shrimp PLs cultured in tanks fed with 25% replacement of *T. chuii* (T25) showed significantly ($p<0.05$) higher survival compared

to CF. However, no significant difference ($p>0.05$) in SGR was observed between all treatments.

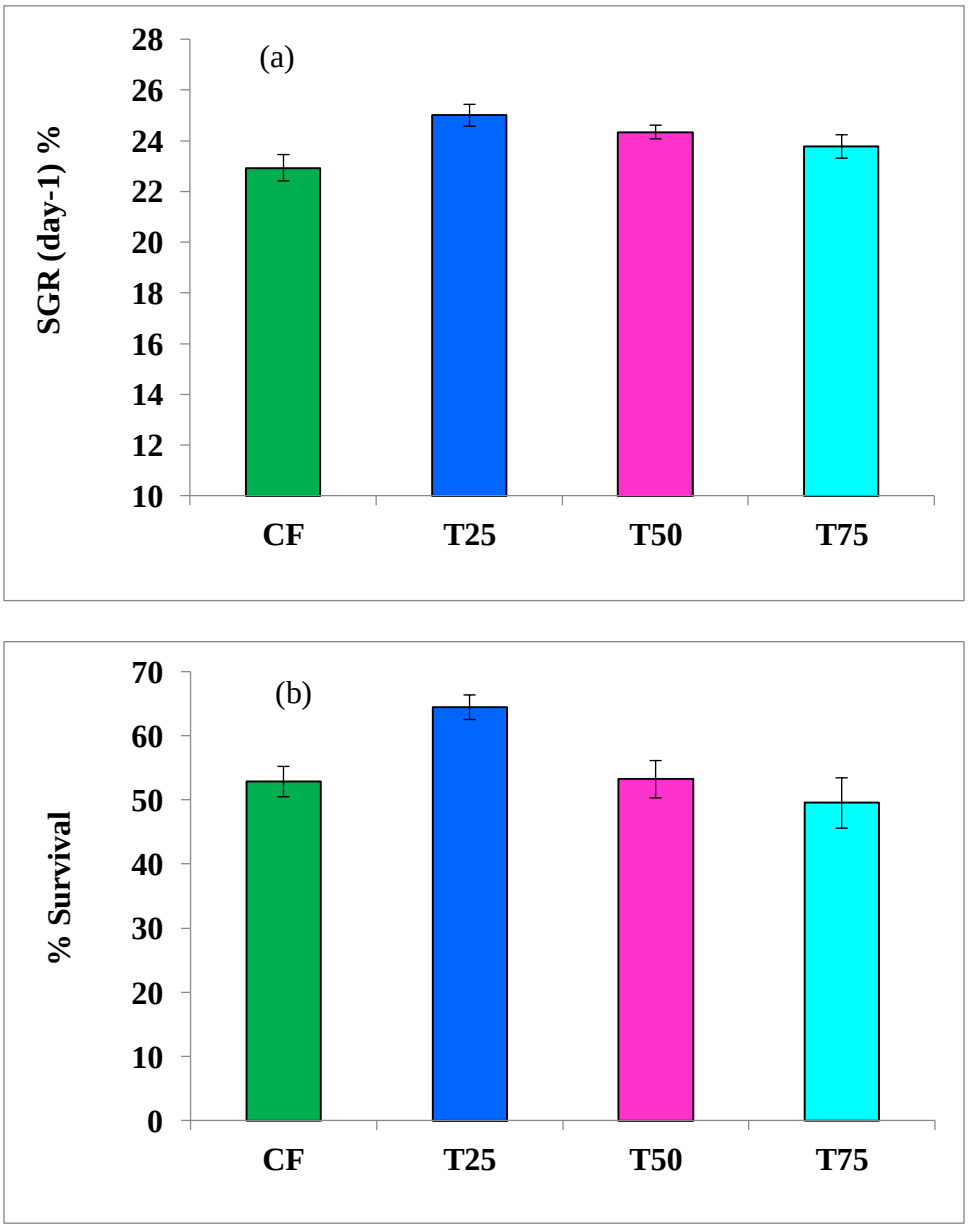


Figure 11: (a) SGR (Day^{-1}) % and (b) Survival rate of *P. monodon* in all treatments after fed with *T. chuii*.

Figure 12 shows the survival rate of PLs fed with four types formulated feed containing different percentages of *C. vulgaris*. The highest survival of shrimp was achieved in tanks of PLs fed with CH25 (62.38 ± 1.91%), CH50 (55.07 ± 2.37%) and followed by CF (52.85 ± 2.91%), respectively. Shrimp PLs cultured in tanks fed with inclusion of *C. Vulgaris* CH25 and CH50 showed significantly ($p<0.05$) higher

survival compared to CF. However, no significant difference in SGR was observed between all treatments.

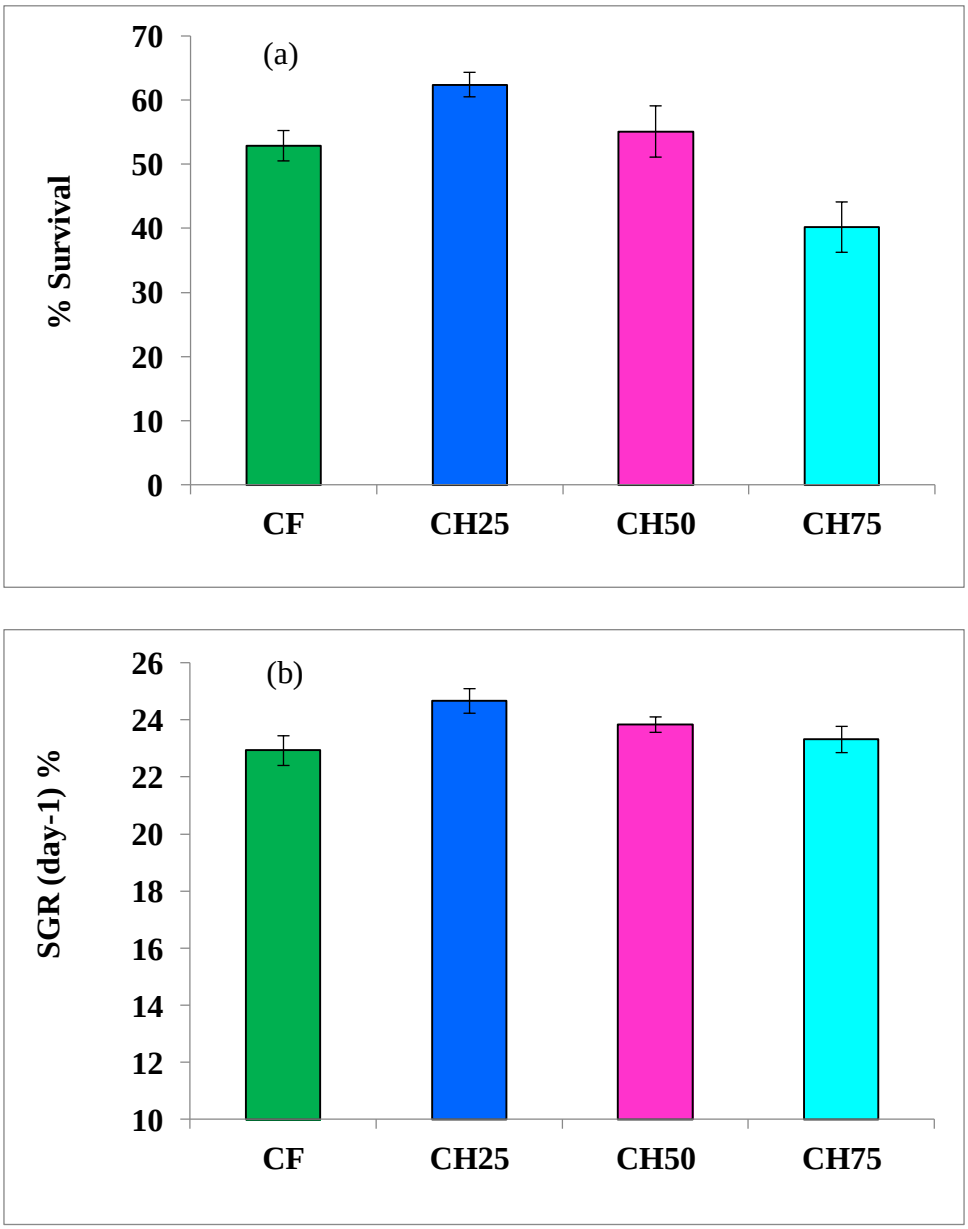


Figure 12: (a) Survival rate and (b) SGR (Day^{-1}) % of *P. monodon* in all treatments after fed with *Chlorella* sp.

CHAPTER-5

DISCUSSION

CHAPTER-5

DISCUSSION

Microalgae play a crucial nutritional role to marine species both in aquaculture and natural condition. Microalgae are utilized as live feed during all the developmental stages of bivalve molluscs (e.g., oysters, scallops, clams and mussels), from larval/early juvenile stages of molluscs, crustaceans and some fish species, and for zooplankton used in hatchery and commercial aquaculture. Microalgae act as the primary producer for the entire aquatic food chain which makes it a very important nutrient source in aquaculture, especially during the culture of shrimp, fish and molluscs (Muller-Feuga, 2000). Researchers suggested different cost-effective protein sources for the preparation of feed to achieve optimum growth of fish (Tacon & Metian, 2008). Microalgae species are used in this study due to their importance as feed for both larval and growth stages of bivalves, shrimp and certain fish species in aquaculture (Guedes and Malcata, 2012). The present study has shown that between the two species studied, *T. chuii* and *C.vulgaris* showed better growth performance, nutritional profile and survival rate compared to controlled feed. In this study, PL of *Peneaus monodon* was fed with different formulated feed with a replacement of 25%, 50% and 75% of fish meal with two indigenous marine microalgae species, *Tetraselmis chuii* and *Chlorella vulgaris* as sources of protein.

5.1 Proximate Composition of Microalgae and Formulated Feed

During this study, all microalgae biomass was harvested at the stationary. *T. chuii* was harvested on Day 7. On the other hand, *C. vulgaris* was harvested during the stationary phase on Day 9. Six different formulated feeds for the PLs with the inclusion of *T. chuii* and *Chlorella* sp. at 25% (T25), 50% (T50), 75% (T75) and 25% (CH25), 50% (CH50), 75% (CH75) respectively were prepared as treatment feed. Custom made feed containing all the rest ingredients except algae was used as a control. *T. chuii* and *C. vulgaris* were selected in this study as the microalgae, for the PLs have been shown to contain a sufficient level of protein, lipid and carbohydrate for growth and survival of commercial species. According to Loya-Javellana (1989), *Tetraselmis* is considered a good food source for several species of penaeid shrimp. In a diet, protein is considered as a vital component. In this study, higher protein (57%), lipid (19%) found in *Tetraselmis* sp. which similar to the result of Miller and Brown

(1992). Unicellular algae, *Chlorella* sp. used in commercial hatcheries as rotifer feed and help to maintain good water quality (Juario and Storch, 1984). Increased feed costs can result from higher reliance on artificial feeding if shrimps are cultivated intensively. In the present study, the protein content of custom-made feed was found to be higher than the treatment feeds with *T. chuii* and *Chlorella* sp. inclusion. The protein content of the treatment feed is, however, still within the range recommended for shrimp growth and survival. According to Biedenbach et al. (1989), higher dietary protein is required for postlarvaes during the nursery phase than the later stages.

The total lipid content of the formulated feed composed with *T. chuii* (10.73% - 10.90%) and *Chlorella* sp. (11.87% - 12.56%) inclusions were also within the recommended for shrimp. Higher lipid levels have been identified in post larvae-fed *Chlorella* diets. Similar results were also reported when various species of microalgae were included in *P. monodon* larvae diets (D'Souza et al., 2000). A study showed 40 to 50 percent protein with 20 percent carbohydrate and (5 – 10)% lipid gave *Penaeus monodon* juvenile the best growth and survival (Bautista, 1986). According to Sick and Andrews (1973), an improvement in the growth of *Penaeus duorarum* fed with a diet contained 10% lipid.

In addition, the total content of the carbohydrate in feed formulated with inclusion of *T. chuii* and *Chlorella* sp. was also shown to be within the range of recommendations of Bautista (1986). The results of this study were consistent with the findings of Piña et al. (2006), which showed that *Tetraselmis* sp. was richer in carbohydrates (24.8%). Carbohydrate was found to be 23 percent in the case of *Chlorella* sp. The addition of *T. chuii* and *Chlorella* sp. into PL feed could therefore provide a sufficient amount of protein and lipid as well as the required carbohydrate as well as an antioxidant source for PLs.

5.2 Proximate Composition of Shrimp Postlarvae

T. chuii and *Chlorella* sp. were selected in the study as the microalgae, for the shrimp postlarvae has been shown to contain a sufficient level of protein, lipid and carbohydrate for growth and survival of aquaculture organisms. From the result it was found that, PL of *Penaeus monodon* which was fed with *Chlorella vulgaris* in 25% replacement of fish meal had greater protein content than all other treatments. Also, highest protein (34%) and lipid (18%) level was found in larvae fed with *Tetraselmis*

sp. 25% replacement of fish meal that is strongly correlate with the result of Jamali et al. (2015) where diet supplemented with *Tetraselmis* sp. resulted in highest protein content in *Litopenaeus vannamei*. In case of *Chlorella vulgaris*, PL also showed higher protein content where fish meal was replaced at 50% by *Chlorella* sp. than the control that is almost similar with the result of Maliwat et al. (2017) who showed that *Chlorella* sp. are able to influence high growth and protein content in giant freshwater prawn *Macrobrachium rosenbergii*. In the present study, a slight reduction in protein levels was observed in *Penaeus monodon* postlarvae as *Chlorella* inclusion was increased. Similarly, slightly lower protein levels were reported in white-leg shrimp (*Litopenaeus vannamei* Boone) as higher inclusions of the marine algae *Tetraselmis* were incorporated into their diet (Kiron et al., 2012). Lowered protein content was also reported in juvenile *P. monodon* when *Chlorella* and other species of microalgae were incorporated into prawn diets (Sivakumar et al., 2011). Also, in the study, postlarvae-fed *Chlorella* diets were observed to contain higher lipid. Similar findings were also reported when different species of microalgae were incorporated in diets of *P. monodon* larvae (D'Souza and Loneragan, 1999).

5.3 Water Quality of the Culture Tanks

Microalgae have a high capacity for extracting inorganic nutrients from waste water, according to Samorì et al. (2013). The principal method for extracting algal nutrients from wastewater is their assimilation into cell biomass (Bich et al., 1999). The key benefits of using microalgae as a fertilizer are that the assimilated nitrogen and phosphorus can be recycled as an algal biomass. Ammonia is the main nitrogen waste product excreted by aquatic organisms from microbial activities and released by nitrogen-containing organic matter from the decomposition, or converted into nitrite, and nitrate in the water column. Ammonia is the most common waste products in water. Aerobic chemoautotrophic bacteria oxidize nitrate and nitrate in waters by nitrification processes. Nitrite, an aquatic animal toxic material, usually exits in waters at low levels, as it is normally converted into nitrate in the aerobic environment, the least toxic nitrogen compound for aquatic animals. When the ammonia oxidation rate exceeds the rate of nitrite oxidation and the denitrification phase of heterotrophic bacteria under anaerobic conditions, nitrite accumulates in water (Boyd and Tucker, 1998). Increased levels of TAN and NO_2-N with culture time are significant factors that affect the health, survival, and growth of shrimp post

larvae in a shrimp hatchery, according to Chin and Chen (1987). On the 17^{th} and 14^{th} days of the experiment, total ammonia nitrogen, nitrite, soluble reactive phosphorus was found to be the maximum in the control treatment. In water samples from the tank of PLs supplemented with *T. chuii* and *Chlorella* sp., significantly lower TAN, NO_2-N and PO_4-P were found compared to control treatment. This outcome was in accordance with Guedes and Malcata (2012), who presented that microalgae can stabilize a culture's water quality and boost it. This is possibly due to the "green water" found in PL tanks fed with the inclusion of microalgae that may be triggered during culture by the leftover feed. According to Chuntapa et al. (2003), it has been shown that the application of phytoplankton (green-water technique) to large varieties of aquaculture species produces better growth and survival, compared to clear water culture. Besides the maintenance of lower TAN, NO_2-N and PO_4-P throughout the culture, the culture medium of PLs fed with T25, T50 and T75 also showed a decrease in the TAN, NO_2-N and PO_4-P towards the end of culture. This result was in accordance with Chen et al. (2012) who found that compared to the other three species studied, *T. chuii* exhibited the highest TAN uptake that led to lower nutrients towards the end of culture. Minimal water exchange can be considered on PLs fed with *T chuii* inclusion, according to Thompson et al. (2002) and this is advantageous due to the expense and labor normally needed to maintain a good water quality for regular water exchange in aquaculture. In the case of *Chlorella* treated tanks PLs fed with CH25, CH50 and CH75, NO_2-N and PO_4-P increased gradually in lower amounts from 4^{th} days of the experiment but the TAN didn't increase. This findings was in accordance with Mujtaba et al. (2015) who reported that The highest TAN uptake was shown by *Chlorella vulgaris*, which contributed to lower nutrients at the end of culture relative to NO_2-N and PO_4-P.

5.4 Specific Growth and Survival Rate of *Penaeus monodon* Postlarvae

In the feeding experiments with the *Penaeus monodon* postlarvae, after 17 days of the culture period, 25% inclusion of *T. chuii* into the diet showed a significantly higher survival (64.4 percent) compared to PLs fed with custom made feed (control). In the case of the inclusion of *Chlorella* sp. in the diet, 25 percent substitution also demonstrated significantly higher survival. This finding is almost similar Tejera et al. (2007) who showed no significant difference in growth and survival after 4 months of feeding experiment from multiple sources of astaxanthin. *Penaeus monodon*

(Fabricus), a juvenile shrimp species, was found to show increased growth when fed with *Chlorella* as live feed Kong et al. (2011). As compared to shrimp fed commercial shrimp meal, higher growth rates were also seen in Indian white shrimp (*Fenneropenaeus indicus*) fed *Chlorella* as live feed which also similar to the result of Pakravan et al. (2018), fish meal substitution by Chlorella vulgaris meal in the diet of Pacific white shrimp improved growth performance. In addition, in brine shrimp Artemia fed *Chlorella*-containing diets, the same growth increase was evident in (Kayim, Ates and Elekon 2010). Growth promoters, such as adequate quantities of macronutrients and naturally occurring bioactive ingredients found in *C. vulgaris*, can be linked to comparable growth success in postlarvae fed *Chlorella* diets and fishmeal-based diets Yamaguchi (1996). Up to now, no research has reported any negative effects on growth efficiency of introducing *Chlorella* at high levels in other crustaceans' diets. In the present analysis, the highest specific growth rate was shown by the PLs supplied with 25 percent fish meal replacement by *T. chuii*. The highest specific growth rate in the case of *Chlorella* sp. was found in 25% replacement of fish meal by *Chlorella*, which is almost identical to the outcome of Kong et al. (2002); Maliwat et al. (2017) who showed that when *P. monodon* was fed with different levels of *Chlorella* sp., their growth output improved with the increase in algae percentage in fish diets compared to control diets. The reliable growth success in all treatments demonstrated that the post larvae of shrimp can readily accept and use *Chlorella*.

CHAPTER-6

CONCLUSIONS

CHAPTER-6

CONCLUSIONS

Microalgae contain adequate number of various types of nutrient which improve the growth performance, survival rate and give the ability to survive against bacterial infestation. The results of this study showed that dietary replacement of fish meal with microalgae had beneficial effects on the survival and growth efficiency of *P. monodon* PL at 25% *Tetraselmis* sp. and *Chlorella* sp. levels. In addition, supplementation of *Tetraselmis* sp. and *Chlorella* sp. also provides the PLs with sufficient nutrients and increases the quality of water throughout the culture. The findings of this study make use of the species of microalgae as a substitute for fish meal that can be used and has a potential role to play in increasing growth and survival. This knowledge would be useful for feed producers to realize that the aforementioned microalgae can be used as a cost-effective feed for 25% replacement of fishmeal aquaculture species. This result showed that the marine microalgae *Tetraselmis chuii* and *Chlorella* sp. protein from plant sources would minimize reliance on fish meal protein.

CHAPTER-7

RECOMMENDATIONS AND FUTURE PERSPECTIVES

CHAPTER-7

RECOMMENDATIONS AND FUTURE PERSPECTIVES

The aim of this study was to observe the effects of inclusion of selected dried microalgae on growth rate, survival, and nutritional profile in *Penaeus monodon*. This study will be helpful for the feed preparation for aquatic organisms where microalgae can be used as an alternative feedstuff for fish meal which will compensate the cost of feed. Although the experiment was followed to explore the objective of the research, there are some limitations of the study which can be minimized by following the recommendations:

- ✓ Algal culture should be done by collecting clean seawater which will reduce the cost of filtration as filter paper and vacuum pump are costly and time consuming.
- ✓ The algae should be extracted in stationary phase of algae so that nutritional value of algae can remain the same.
- ✓ There is need for a better approach to mass production of the microalgae species.
- ✓ It should be possible to extract algal biomass in less time by using large centrifugal machine or develop a method to extract algal biomass in large amount with minimum time.
- ✓ It should be carefully drying algae in hot air oven will prevent algae from burning.
- ✓ There should be a separate lab for algae cultivation so that there is no possibility to cross contamination.
- ✓ The rate of growth can be seen in the future by feeding algae in different stages of shrimp.
- ✓ Future studies should also be done on the effect of the microalgae combination of *Tetraselmis chuii* and *Chlorella* sp. in different percentages.
- ✓ There is an opportunity to establish the effects of shrimp growth fed with other algae by following this method.

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APPENDICES

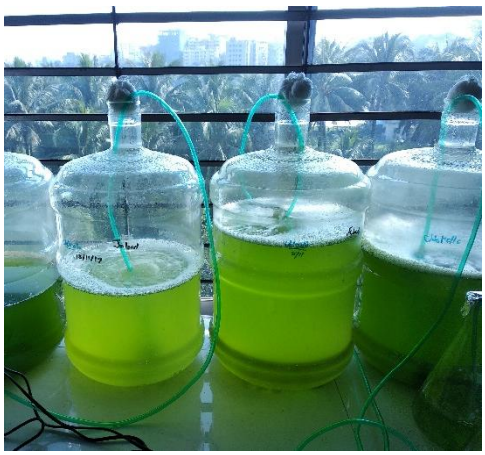
APPENDICES

Appendix 1: Culture of *Tetraselmis chuii*

Appendix 2: Mass culture of *Tetraselmis chuii*

Appendix A.3: Culture of *Chlorella vulgaris*

Appendix 4: Mass culture of *Chlorella vulgaris*



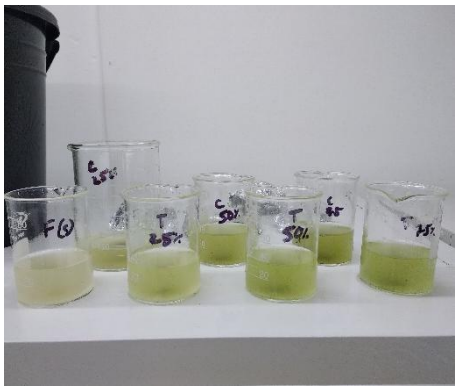
Appendix 5: Centrifuging to collect biomass of microalgae

Appendix 6: Dry biomass of microalgae

Appendix 7: Formulated feed (CF, T25, T50, T75, CH25, CH50 and CH75)

Appendix 8: Experimental setup for feeding experiment

Appendix 9: Feeding with formulated feed (CF, T25, T50, T75, CH25, CH50 and CH75)



Appendix 10: PLs counted at the end of experiment

Appendix 11: One-way Analysis of Variance examining the water quality of treatment tanks of *Penaeus monodon* after the microalgae used as diet

ANOVA

TAN

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.289	3	.430	27.495	.000
Within Groups	.500	32	.016		
Total	1.789	35			

ANOVA

NO_2O

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.825	3	.275	17.489	.000
Within Groups	.503	32	.016		
Total	1.329	35			

Appendix 12: One-way Analysis of Variance examining the protein, lipid and carbohydrate content of treatment tanks of *Penaeus monodon* after the microalgae used as diet

ANOVA

PL Protein

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	232.571	6	38.762	22.578	.000
Within Groups	24.035	14	1.717		
Total	256.606	20			

Protein

	VAR0000 1	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	3.00	3	29.0000			
	6.00	3	29.0000			
	1.00	3	30.0000	30.0000		
	7.00	3		32.6667	32.6667	
	2.00	3			34.0000	
	4.00	3			34.0000	
	5.00	3				39.0000
	Sig.		.960	.233	.864	1.000
Duncan[a]	3.00	3	29.0000			
	6.00	3	29.0000			
	1.00	3	30.0000			
	7.00	3		32.6667		
	2.00	3		34.0000		
	4.00	3		34.0000		
	5.00	3			39.0000	
	Sig.		.390	.256	1.000	
Scheffe[a]	3.00	3	29.0000			
	6.00	3	29.0000			
	1.00	3	30.0000	30.0000		
	7.00	3	32.6667	32.6667		
	2.00	3		34.0000		
	4.00	3		34.0000		
	5.00	3			39.0000	
	Sig.		.141	.090	1.000	

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA

Pl Lipid

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	256.286	6	42.714	87.583	.001
Within Groups	6.828	14	.488		
Total	263.114	20			

Lipid

	VAR0000		Subset for alpha = 0.05				
	1	N	1	2	3	4	5
Tukey HSD[a]	1.00	3	8.0000				
	6.00	3		10.0000			
	7.00	3			12.0000		
	3.00	3			13.0000	13.0000	
	5.00	3				14.0000	
	2.00	3					18.0000
	4.00	3					18.0000
	Sig.		1.000	1.000	.595	.595	1.000
Duncan[a]	1.00	3	8.0000				
	6.00	3		10.0000			
	7.00	3			12.0000		
	3.00	3			13.0000	13.0000	
	5.00	3				14.0000	
	2.00	3					18.0000
	4.00	3					18.0000
	Sig.		1.000	1.000	.101	.101	1.000
Scheffe[a]	1.00	3	8.0000				
	6.00	3	10.0000	10.0000			
	7.00	3		12.0000	12.0000		
	3.00	3			13.0000		
	5.00	3			14.0000		
	2.00	3				18.0000	
	4.00	3				18.0000	
	Sig.		.126	.126	.126	1.000	

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA

Pl Carbohydrate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	68.571	6	11.429	14.681	.00
Within Groups	10.899	14	.778		
Total	79.470	20			

Carbohydrate

	VAR0000 1	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	1.00	3	7.0000			
	4.00	3		10.0000		
	3.00	3		11.0000	11.0000	
	7.00	3		11.0000	11.0000	
	5.00	3		12.0000	12.0000	
	6.00	3		12.0000	12.0000	
	2.00	3			13.0000	
	Sig.		1.000	.149	.149	
Duncan[a]	1.00	3	7.0000			
	4.00	3		10.0000		
	3.00	3		11.0000	11.0000	
	7.00	3		11.0000	11.0000	
	5.00	3			12.0000	12.0000
	6.00	3			12.0000	12.0000
	2.00	3				13.0000
	Sig.		1.000	.208	.221	.208
Scheffe[a]	1.00	3	7.0000			
	4.00	3		10.0000		
	3.00	3		11.0000	11.0000	
	7.00	3		11.0000	11.0000	
	5.00	3		12.0000	12.0000	
	6.00	3		12.0000	12.0000	
	2.00	3			13.0000	
	Sig.		1.000	.326	.326	

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix 10: One-way Analysis of Variance examining the SGR and Survival rate of treatment tanks of *Penaeus monodon* after the microalgae used as diet

ANOVA

SGR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.928	6	1.488	1.639	.009
Within Groups	12.707	14	.908		
Total	21.636	20			

ANOVA

Survival

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1188.617	6	198.103	16.567	.000
Within Groups	167.412	14	11.958		
Total	1356.029	20			

Survival

	VAR0000 1	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	7.00	3	40.1600			
	4.00	3	49.5200	49.5200		
	3.00	3		52.2200		
	1.00	3		52.8500	52.8500	
	6.00	3		55.0700	55.0700	55.0700
	5.00	3			62.3800	62.3800
	2.00	3				64.4400
	Sig.		.060	.474	.054	.059
Duncan[a]	7.00	3	40.1600			
	4.00	3		49.5200		
	3.00	3		52.2200		
	1.00	3		52.8500		
	6.00	3		55.0700		
	5.00	3			62.3800	

	2.00	3			64.4400	
	Sig.		1.000	.090	.478	
Scheffe[a]	7.00	3	40.1600			
	4.00	3	49.5200	49.5200		
	3.00	3		52.2200	52.2200	
	1.00	3		52.8500	52.8500	52.8500
	6.00	3		55.0700	55.0700	55.0700
	5.00	3			62.3800	62.3800
	2.00	3				64.4400
	Sig.		.164	.694	.111	.052

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

BRIEF BIOGRAPHY

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Mohammad Jabedul Islam completed B.Sc. in Fisheries (Hon's) from the Faculty of Fisheries of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh with CGPA 3.80 out of 4.00. He has strong passionate in research of fisheries and his research interests are on microalgae identification, isolation and mass culture, used feed for aquaculture and the extraction of pigments, carotenoids and lipid from the microalgae. Now, he is a candidate for the degree of MS in Aquaculture under the Department of Aquaculture, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University (CVASU).