CHAPTER ONE

INTRODUCTION

1.1 Background

The identification and selection of species suitable for aquaculture are key objectives of aquatic resources management. Currently there is a need to promote aquaculture as a food source, a genetic reserve and an additional mechanism to diminish production of commercial species by extractive fisheries. Bangladesh is endowed with unique aquatic resources for aquaculture development. The fisheries of Bangladesh are very diverse and are comprised of inland open water capture fisheries, inland and coastal aquaculture and marine fisheries. The coastal water is one of the most productive zones in the world and rich in fish and shellfish. Most of our marine fishery resources are still unexploited, with a considerable size (301 species) of shellfish, which has economic importance as food (Ahmed, 1990). There are three species of mollusc that occur naturally in the coastal waters of Bangladesh, namely, Green mussel (Perna viridis), Oyster (Crassostrea sp.) and Clam (Meretrix meritrix) (Ahmed, 1990). Bangladeshi coastal tribal communities and small-scale fishermen living near the coast collect these bivalve and univalve for domestic consumption as well as for economic purpose. Around 850 ton/year mollusks are harvested by tribal communities and small-scale fishermen from Satkhira, Barishal and Chittagong-Cox's bazar regions every year (Shahabuddin, 2010)

Green mussel is known as the green-lipped mussel; an economically important bivalve species belongs to the family Mytilidae which is widely distributed in the higher latitude regions (Bayne, 1976; Hickman, 1992). It is an intertidal filter feeder and fast growing large warm water marine bivalve (Rajagopal et al., 2006). *P. viridis* mostly distributed in the tropical and sub-tropical areas of the Indo-Pacific region (Sivalingam, 1977; Siddall, 1980). Its distribution extends from Asian region including China, Singapore, Thailand, India and Philippines. A significant positive factor of green mussel farming development is the natural availability of seed. The green mussel is also a good candidate for cultivation because reproduction can be induced throughout the entire year (Sivalingam, 1977). In many Asian countries and Indo-Pacific region, different species of the green mussel have been cultivated successfully (Chatterji et al., 1984). Nowadays green mussels are considered as a delicious food item in Europe and North America (Boyel, 1981).

Like other Asian countries, Bangladesh can develop commercial shellfish farming in small scale to attain the viability and meet the local demand. In recent years, competition for fisheries resources has increased particularly in Cox's Bazar region due to resource conflict and increasing demand for food production and decrease in traditional finfish fisheries, development of shellfish farming practices can create a new horizon to support this situation. Green mussel provides the highest conversion of primary producers (phytoplankton) to human food as a low-priced source of animal protein, and culture of mussels in the water column can boost the seafood production to several folds (Pillai et al., 2000).

Bangladesh has vast coastal area which is one of the most productive zones in the world Varieties of marine habitats such as sandy, muddy and rocky grounds, mangrove areas and coral reefs are suitable place for bivalves, and thus are potentially viable for development of shellfish fishery. The high tide amplitude, sufficient tidal current, absence of pollutants and high phytoplankton abundance offer an ideal environment for the development of mollusc culture around coastal waters of Bangladesh (Ahmad, 1990). Recently, culture of shrimp, crab, mollusc etc. is increasing day by day in coastal area of Bangladesh to contribute in blue economy. However, there was little attempt made for mollusc like green mussel (*P. viridis*) culture in Bangladesh due to lack of proper knowledge on distribution and abundance of mollusc populations in our coast and ignorance of mollusc as food value etc.

The very high levels of production, obtained by raft culture in some countries, has aroused the expectation that mussel culture may be a reliable means of solving the animal protein needs of the population in the Third World (Pillay, 1993). Mussels are also used as bait, larval feed, fish meal and occasionally in cottage industries (Boyle, 1981). Besides the economic importance, they are being widely used in fundamental ecological, physiological and pollution research (Phillips, 1977). The export market in the neighbouring Southeast countries needs to explore so that excess product can be sold at a reasonable price. The shells of the green mussel can be used for making poultry, fish feed and lime production.

Information on growth and eco-physiological requirements in a given environment is very important for a successful mussel culture programme (Rivonker et al., 1993). The eco-physiological requirements, survival and growth of *P. viridis* in natural beds and

various culture regimes have been studied abroad (Rao et al., 1975; Qasim et al., 1977; Parulekar et al., 1982; Cheong and Chen, 1980; Chatterji et al., 1984; Chaitanawisuti and Menasveta, 1987; Joseph and Joseph, 1988; Rivonker et al., 1993;).

This study is done to analyse the growth performance of green mussel (*P. viridis*) cultured in raft and longline culture system in the Cox's Bazar coast of Bangladesh. The findings of the study are expected to contribute to the development for good planning and management practices of a sustainable green mussel aquaculture. Development of green mussel (*P. viridis*) culture system can be a good initiative to use the concept of blue economy and it will be an ideal species for mariculture, which is already established in many Asian countries.

1.2 Research objectives

The specific objectives of the study were as follows-

1. To compare the growth performance of green mussel (*P. viridis*) cultured in raft system and long-line system.

2. To investigate the suitability of environment factors for green mussel in cox's bazar coast of Bangladesh

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Importance of green mussel in Bangladesh

The coastal area of Bangladesh is suitable for mollusks habitats as it is enriched with sandy and rocky ground, mangrove and coral reefs (Shahabuddin et al., 2010). Mollusks species in the Bay of Bengal can be an important part of our economy. Among the shellfish species green mussel (*Perna viridis*) have potentiality to be exported and being an economically important species (Shahabuddin et al., 2010). Kamal and khan, 1998 said that cultivation and export of Caulerpa and mollusks like green mussel (*Perna viridis*), *Crassostrea* sp. and *Meretrix meretrix* could enrich the country's economy immensely. He also said, shellfish flesh can be exported to foreign countries along with shrimp and crabs and technologies can be developed for culturing shellfish in coastal area to ensure the conservation of shellfish biodiversity in nature as well as keeping harmony with the future fast-growing industry which will provide future employment opportunities, alternative protein to 0.2 million tribal people, earn foreign currencies and open a new arena in coastal aquaculture of Bangladesh.

2.2 Biology of green mussel, *Perna viridis* (Linnaeus, 1758)

The green mussel or green lipped mussel, *Perna viridis* belongs to the family Mytilidae (NIMPIS, 2002) and also the solely family within the order Mytiloida. The Mytilidae consists of 32 genera. The green mussel belongs to the genus *Perna*. The genus *Perna* comprises of solely four species, *P. canaliculus, P. picta, P. perna* and *P. viridis*. The species *Perna viridis* is extensively apportioned in tropical and sub-tropical areas of the Indo-Pacific region (NIMPIS, 2002). Other members of the *Perna* are found in New Zealand (*Perna canaliculas*) and in coastal South America and Africa (*Perna perna*) (Sallih, 2005).

The green mussel (*P. viridis*) may be a giant mussel having average size of 80-100mm in length and it has been indicated sometimes to achieve a length of 150-165 mm (NIMPIS, 2002; FIGIS, 2005). It has two identical shell valves with a pear-shaped and smooth exterior surface characterized by concentric growth lines and slightly concave ventral margin. The shell surface of *P. viridis* is concocted by a swish and firm

periostracum, which is bright green in juvenile and brown with green margins in adult (Sallih, 2005).

Spawning happens with regards to environmental triggers like high food levels, temperature fluctuations and physical abundance. The stages following fertilization begin with the formation of free-swimming larvae or trochophore larvae after 7-8 hours, and growing to last larvae stage, veliger larval with the growth of ciliated velum after 16-19 hours and finish metamorphosis in 8-12 days (Tan, 1975). At metamorphosis, an eye spot and extended foot comes about, takes out the vellum and releases byssal threads as aids to selection of site for settlement. This phenomenon is usually known as mussel spat fall. Once the location is selected, the larvae which are about 2-5 weeks old and of 0.25-0.30 mm in size (Aypa, 1990) get connected by anchoring with byssus thread (Spencer, 2002). The young mussels usually adverted to as juvenile mussels then grow swiftly and achieve 3-4 mm shell length within 4-8 weeks (Aypa, 1990). The spawning season takes place doubly a year between early spring and late autumn (Rajagopal, 1998).

2.3 Culture and growth aspects of green mussels

Aquaculture provides an alternative means of increasing fish production which contribute to the protein food supply and contributing the socio-economic development of nation. Green mussels even have numerous characteristics that contribute to the potential of mussels in cultivation (Hickman, 1992). The high fecundity and a mobile free-living phase contribute to the widespread distribution of the relatively few mussel species, and at the same time has greatly influenced the technology and practice of mussel farming. The availability of natural seed has been an important positive factor in mussel farming development. It is a species which have rapid growth rate and reproduce throughout the year (Sivalingam, 1977). It additionally has ability to live in dense beds. *P. viridis* is commercially important because of its rapid growth rate and high population densities (Rajagopal et al., 1998). Its larvae and spat are settled through the year round. But the highest peak is found in October and the second highest in March (Hossain et al., 2004; Amin et al., 2005). The green mussel can form dense populations of 35,000 individuals per m² on different form of structures (NIMPIS, 2002). And this can help in the easy collection of seed for cultivation.

Bivalve growth depends on the environmental quality of the cultivation area. Due to the rapid growth rates, mussels are well suited for culture on a commercial scale in subtidal biotopes (Rivonker et al., 1993; Rajagopal et al., 2006). Mussel growth is a function of a number of environmental parameters, mainly food and temperature (Bautista, 1989). The environment influences the somatic and reproductive tissue growth of marine bivalves both directly and indirectly (Griffiths and Griffiths, 1987; Lodeiros and Himmelmam, 2000). The availability and quality of food can be considered an important factor since it affects physiological processes linked to growth (Bayne; Newel, 1983). Suspended food particles for bivalves vary in quality and quantity and, in general, are composed of seston which itself is a complex mixture of pelagic organisms and suspended detritus (Navarro and Thompson, 1995; Cranford and Hill, 1999; Hawkins et al., 2001). Additionally, the high selection capacity for particle quality might be independent of phytoplankton abundance and may have also a direct effect on growth. Selection processes of high-quality particles have already been reported in other bivalves, particularly when seston is abundant (Velasco and Navarro, 2002; Carmichael et al., 2004). In many tropical countries, P. viridis shows high performance and growth under culture conditions. It is the tropical mussel species with highest worldwide production, particularly in Asia and adjacent areas (Gallardo et al., 1992; Sreenivasan et al., 1989). In coastal areas of India, this mussel is considered to be suitable for culturing purposes on a commercial scale (Rivonker et al., 1993; Rajagopal et al., 1998), in which animals may grow up to 79.8 mm in 11 months (Sreenivasan et al., 1989). Commercial cultivation of the mussel P. viridis was started in late 1995 at Anthakaranazhi (Alleppey district) by local fishermen on long lines. This type of commercial activity along the southwest coast of India has expanded greatly since 1997 in different parts of the country (Appukuttan et al., 2001).

2.3.1 Site selection for green mussel culture

It is essential to select a proper location to culture green mussel. The site for green mussel cultivation should be well protected or sheltered coves and bays rather than open unprotected areas (Aypa, 1990). Sites are affected by strong wind and big waves must be avoided because this causes damage to stock and culture materials. The locations ought to be clear from serving as catchments basins for excessive flood waters. Flood water is instantly changing the temperature and salinity of the seawater, which is detrimental to the mussels. Water depth, water movement, turbidity, pH, dissolved

oxygen, and food availability are also the most important in the selection of a suitable culture site (Lovatelli, 1988).

2.3.2 Water depth

For green mussel culture, water depth should be below 1 m mean tide level at least. Water depth varies with culture methods. Bottom culture can be practiced in area where the mean tide level is less than 1.5 m (Lovatelli, 1988). For off bottom culture ways like raft and long line generally need a minimum water column height at the time of low water spring tide. The hanging ropes with mussel seeds of those culture types should be at least 1 m above the sea floor during extreme low water spring tides (Lovatelli, 1988) to stop ground predators, ocean floor high water turbidity and friction with the bottom. The favourable water depth for each seed collection and mussel cultivation is 2 m or more (Aypa, 1990).

2.3.3 Turbidity of water

Turbidity is essential for green mussel culture. It determines the presence of suspended, organic and inorganic matters in the culture area. High levels of the suspended materials have a bad effect on mussel culture due to failure of filtering activity. And also, these materials reduced penetration of sunlight in the water column, which will result in low primary productivity. As a result, the cultured species may face slow rate of growth because of the limited food availability. A practical method for determining the turbidity level is the use of the Secchi disc. Culture site having a disc reading of less than 25 cm must be considered not suitable for mussel culture (Lovatelli, 1988).

2.3.4 Salinity

Green mussel can tolerate a high range of salinity. The species has 50% survival salinity tolerance at 24 ppt and 80 ppt for a period of 2 weeks in a laboratory experiment (Sivalingam, 1977). Tropical green mussel occurs generally in estuarine or coastal water that is rich in plankton and has high salinity (27 ppt to 33 ppt). The green mussel shows a good growth performance in estuarine habitats with salinities ranging from 18 ppt to 33 ppt as reported in FIGIS, 2005 and this species shows a huge salinity and temperature tolerance in investigational testing. The salinity of 27 ppt to 35 ppt is ideal for mussel farming (Aypa, 1990). The green mussel can grow in salinity ranging from 5.2 ppt to 39.8 ppt (Rajagopal et al., 1998).

2.3.5 Temperature

The growth of green mussel culture is also affected by water temperature (Sivalingam ,1977) demonstrated that the green mussel has 50% survival temperature tolerance from 10°C to 35°C under experimental testing. It was reported that the optimal temperature for green mussel culture ranges from 26°C to 32°C (Hickman, 1989), 27°C to 30°C (Aypa, 1990), 25.3°C to 34.6°C (Rajagopal et al., 1998). It can tolerate a range of temperature 11°C to 32°C (FIGIS, 2005).

2.3.6 Food organisms

As Green mussel is filter feeders, primarily it feeds on a wide range of phytoplankton species, small zooplankton and other suspended fine organic particles. High primary productivity areas cause high productivity and biomass of mussels. The chlorophyll-a distribution varies from 0.7 mg/m3 to 17 mg/m3 in potential green mussel cultivation (Rajagopal et al., 1998).

2.3.7 Plankton composition

Phytoplankton species are the most favourite food item for green mussel. Coscinodiscus is the most favourite phytoplankton species of *P. viridis* (Tan and Ransangan, 2017). High amount of *Prorocentrum, Navicula, Rhizosolenia, Ditylum, Thalassionema* spp. may also be found in the *P. viridis* stomach (Tan and Ransangan, 2017). A little amount of *Proboscia, Protoperidinium, Pleurosigma, Entomoneis, Odentella, Nitzschia* also found in the *P. viridis* stomach (Tan and Ransangan, 2017). Interestingly, *Chaetoceros* spp. and *Bacteriastrum* spp. were selectively rejected by *P. viridis* in both high and low seston conditions (Tan and Ransangan, 2017). In the diet of *P. viridis* zooplankton has also significant. High numbers of copepod and bivalve larvae were found in the *P. viridis* stomach (Tan and Ransangan, 2017).

2.4 Culture methods

Mussel culture, as practiced in many countries, is carried out by using a variety of culture methods based on the prevailing hydrographical, social and economic conditions. In the wild, mussels are mostly found in the littoral zone, attached in clusters on various substrates. Being a filter-feeder on phytoplankton and detritus, it is considered the most efficient converter of nutrients and organic matter, produced by

marine organisms in the aquatic environment, into palatable and nutritious animal protein. It's very short food chain (one link only), sturdy nature, fast growth rate and rare occurrence of catastrophic mass mortalities caused by parasitic micro-organisms, makes it possible to produce large quantities at a very reasonable price (Korringa, 1976). Likewise, its ability to attach to substrates with the byssus, makes it an ideal aquaculture species using different culture systems. According to Bardach et al. (1972), mussel culture is the most productive form of saltwater aquaculture and its proliferation is virtually a certainty. Raft culture system and long-line culture system are both off-bottom culture system which are used for mussel culture in the intertidal and deepwater zones (Aypa, 1980).

2.4.1 Raft culture

Mussel raft culture has been practiced in Spain for a long time. Mussel seeds that settle freely on rocks or on rope collectors are suspended from a raft. When the weight of the bivalves on a given rope exceeds a certain limit, the rope is taken out and again distributed over a greater length until marketable size. It is a continuous thinning of the mussel stock to provide ample space to grow (Aypa, 1980). Marketable shellfish are detached from the rope, purified in basins before marketing. The raft may be an old wooden boat with a system of outrigger built around it. Other kinds of rafts could be a catamaran-type boat carrying some 1000 rope hangings, or just an ordinary plain wooden raft with floats and anchors. Floats can be made of plastic, wood, oil drums, etc. The raft is transferred from one place to another using a motor boat. Production of mussels from this type of culture is high. From a catamaran-type raft with 1,000 rope, 6–9 m in length, about 4,666–5,333 MT of marketable mussel can be produced (Korringa, 1976). Advantages of this type of culture are: high durability, reduce predation, utilization of planktonic food at all levels of water and minimum siltation (Aypa, 1980).

2.4.2 Long-line culture

Long-line culture is an alternative to raft culture in areas less protected from wave action. A long-line supported by a series of small floats joined by a cable or chain and anchored at the bottom on each end is employed. Collected mussel spats on ropes or strings are suspended on the line. The structure is fairly flexible (Aypa, 1980).

Study on abundance, distribution, population dynamics and gametogenic cycle of *Perna viridis* has already been done by many authors (Kamal and Khan., 1998; Shahabuddin et al., 2010; Amin et al., 2005; Noor et al., 2020) but comparative growth study of green mussel (*P. viridis*) among different culture systems in Bangladesh has not yet been done. Study on comparative growth study is essential to develop culture and management strategies which will be beneficial in mussel culture.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This research work was conducted at Nuniar Chora, Cox's Bazar region located in the south-east direction of Bangladesh. The geographical location of the two culture stations is raft culture (21°47'31"N and 91°96'17"E) and Long-line Culture (21°47'45"N and 91°96'39"E (Fig. 1 and Fig. 2)



Fig. 1: Green mussel culture site (open view)



Fig. 2: Raft and longline culture stations of green mussel (close view)

3.2 Sampling frequencies and studied parameter

3.2.1 Sample collection

Water sample was collected from the 2 selected stations where the raft and long-lines were established. Water sampling was done twice a month basis for a period of 13 months from November 2019 to November 2020. Surface water samples collected during high tide condition for measuring water temperature, salinity, pH, water transparency, dissolve oxygen, alkalinity, turbidity, chlorophyll-a, dissolve nutrients such as nitrite, nitrate, phosphate and ammonia. Water from bottom, middle and surface was collected to measure total suspended solids (TSS) and total dissolved solids (TDS). After naturally spat settlement, ten ropes were randomly selected from each culture system from which *P. viridis* were sampled from February 2020 to November 2020 for observing and analysing growth performance.

3.2.2 Biophysical condition determination

The variation of temperature, salinity, pH, water current, total suspended solids, water depth, alkalinity, turbidity, dissolve oxygen, chlorophyll-a, dissolve nutrients such as nitrite, nitrate, phosphate, ammonia, ammonium were measured at the study area following standard methods (APHA, 2005).

3.2.2.1 Analysis of water quality parameters

Water quality parameters like temperature (Celsius Thermometer), dissolve oxygen (Digital DO Meter), pH (Portable pH meter), salinity (Refractometer), transparency (Secchi Disc) and depth (Weight and Rope) were monitored in-situ during morning at 10 am on monthly basis. Three replication of water samples were collected from each Station using water sampler and were taken to laboratory as soon as possible for the turbidity (Digital turbidity meter), alkalinity (Titrimetric method), Chlorophyll-a and nutrient (NO₂-N, NO₃-N, PO₄-P, NH₃, NH₄⁺) analysis in laboratory. After turbidity determination, water samples were filtered through microfiber filter paper (Whitman GF/C) using a vacuum pressure air pump (Rocker filtration pump). The filtered water was used for alkalinity and nutrient analysis. The filter paper was taken for chlorophyll-a determination, which is described in later part of this chapter.

3.2.2.1.1 Total suspended solids

Filtration apparatus was placed with weighed filter paper in filter flask. Water sample was mixed well and poured into a graduated cylinder to the selected volume. Suction was applied to filter flask and filter paper was seated with a small amount of distilled water. Selected volume was poured into filtration apparatus. Sample was drawn through filter paper into filter flask. Graduated cylinder was rinsed into filtration apparatus with three successive 10 mL portions of distilled water, allowing complete drainage between each rinsing. Suction was continued for three minutes after filtration of final rinse was completed. Filter paper was dried in an oven at 103-105°C for at least 1 hour. Filter paper was cooled in desiccator to room temperature. When cooled, the filter paper was weighed. TSS was calculated by using this formula:

Total Suspended Solids, $mg/L = (A-B) \times 1,000/C - Where: A is the combined weight of filter paper and residue in mg, B is the weight of filter paper in mg and C is the volume of sample filtered in ml.$

3.2.2.1.2 Alkalinity

Alkalinity was measured by titrimetric method. Phenolphthalein was used as indicator and the sample was titrated against 0.02N sulphuric acid. Alkalinity was calculated by using this formula: Alkalinity (mg/L) = (Volume of H_2SO_4 x Normality x 50 x 1000)/Volume of sample taken.

3.2.2.1.3 Nitrite

The program 305 was set before in the photometer (pHoto Flex; WTW, Germany) along with zero adjustment using distilled water. VARIO Nitri 3 F10 Powder pack of chemical content was needed to measure the nitrite concentration in sample. At first 10 ml of sample water Page 13 was taken in empty cell using pipette. The contents of the powder pack were added and the cell closed with screw cap. Then the cell was shaken and allowed to react for 15 minutes. Then the cell was inserted in the photometer and the photometric reading was recorded afterwards.

3.2.2.1.4 Nitrate

The program 314 was set in the photometer (pHoto Flex; WTW, Germany) and zero adjustment was done by using distilled water. VARIO Nitrate Chromotropic Powder

pack of chemical content needed to measure the nitrate. At first 10 ml of sample water was taken in vacant cell using pipette. The contents of the powder pack were added and the cell closed with screw cap. Then the cell was shaken and allowed to react for 5 minutes. Then the cell was inserted in the photometer and the photometric reading was recorded afterwards.

3.2.2.1.5 Phosphate

The program 306 was set in the photometer (pHoto Flex; WTW, Germany) with zero adjustment using distilled water. VARIO Phos3 F10 Powder pack of chemical content required to measure the phosphate in sample water. 10 ml of sample water was taken in empty cell using pipette. The contents of the powder pack were added and the cell closed with screw cap. Then the cell was shaken and allowed to react for 2 minutes. Then the cell was inserted in the photometer and the photometric reading was recorded afterwards.

3.2.2.1.6 Ammonia

For the determination of ammonia, the program 324 was set in the photometer (pHoto Flex; WTW, Germany) and zero adjustment was done using distilled water. The pH value of the sample was also checked whereas, the desired value; approx. pH 7. VARIO AMMONIA Salicylate F10 powder pack and VARIO AMMONIA Cyanurate F10 powder pack needed to measure the ammonia in water sample. At first, 10 ml of sample water was taken in empty cell using pipette. The contents of VARIO AMMONIA Salicylate F10 powder packs were added and the cell was closed with screw cap. Then, the cell was shaken and allowed to react for 3 minutes. After that the contents of VARIO AMMONIA Cyanurate F10 powder pack also added and the cell closed with screw cap. Then the cell was shaken and allowed to react for another 15 minutes. Then, the cell was inserted in the photometer and the photometric reading recorded afterwards.

3.2.2.1.7 Ammonium

The program 71 was set in the photometer (pHoto Flex; WTW, Germany) and zero adjustment was done by using distilled water. The pH value of the sample was checked. Desired value; approx. pH 7. NH₄ -1 Solution, NH₄ -2 powder and NH₄ -3 Solution was needed to measure the ammonium in sample water. At first 10 ml of sample water was taken in empty cell by using pipette. Then 1.20 ml of NH₄ -1 Solution was added into

the cell by using pipette and mixed it with the sample. Then, 2 level blue micro spoons of NH_4 -2 Powder were added. Then, the cell was closed with screw cap. Then, the cell was shaken and allowed to react for 5 minutes. After that, 8 drops of NH_4 -3 Solution was added and the cell was closed with screw cap and mixed it. Then, the cell was shaken and allowed to react for 5 minutes. Then, the cell was inserted in the photometer and the photometric reading was recorded afterwards.

3.2.2.1.8 Chlorophyll-a measurement

500 ml water samples were filtered through membrane filter (0.45µm) with the help of a vacuum pump. The filtered membranes were taken into 10 ml of 90% acetone and kept overnight in refrigerator. The filtered papers were mixed thoroughly with acetone using a glass rod. Then centrifugation at 3500 RPM for 2.30 minutes was performed. The supernatant contents (extract) were taken into corvettes and the absorbance of extract was determined at 664, 647 and 630 nm comparing with blank acetone. The chlorophyll-a concentration was calculated by following equation: Chlorophyll-a = $(11.85 A_{664} - 1.54 A_{647} - 0.08 A_{630}) \times (V/S) \times 1000$ Where, A_{664} = absorbance at 664 nm, A_{647} = absorbance at 647 nm, A_{630} = absorbance at 630 nm, V = volume of acetone used (ml) S = volume of sampled filter (ml)

3.2.3 Determination of length and weight of P. viridis

Sampled 180 green mussels were used to determine the individual live weight and shell length from February 2020 to November 2020. Mussels were initially cleaned from all the encrusting organisms and their byssus were removed. Shell length was measured individually using a Vernier callipers to the Vernier constant of 0.01. The live weight of each individual was recorded keeping the shell intact with electronic weight meter (AS 220.R2, Radwag, Poland).

3.3 Data Analysis

The water quality data were investigated for each station monthly and results were demonstrated by using Microsoft Excel 2016. All experimental results of water quality parameters, shell length and body weight of green mussel were analysed by using paired sample t-test with SPSS version 25.

CHAPTER FOUR

RESULTS

4.1 Water quality parameters

The water quality parameters from these selected two culture stations were recorded over 13 months period. These physico-chemical parameters include temperature, salinity, pH, dissolve oxygen, turbidity, alkalinity, water depth, current speed, total suspended solids and transparency. The range of nutrient substance such as nitrate, nitrite, phosphate, ammonia, ammonium and chlorophyll-a were also recorded. The range of water quality parameters and nutrient substance from the two culture sites in Cox's Bazar coast, near Nunia Chora is summarized in Table 1.

4.1.1 Temperature

The temperature varied from approximately 25-31° C (Fig. 3) in both the culture stations. Highly significant difference (p < 0.05) was observed in the monthly variation. However, temperature in April, 2020 was significantly higher (p < 0.05) than other months of the study period.



Fig. 3: Temperature variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.2 Transparency

The value of transparency fluctuated approximately from 29-51 cm (Fig. 4) in both culture stations. Highly significant difference (p < 0.05) of transparency was observed in the monthly variation. However, transparency in October, 2020 was significantly higher (p < 0.05) and transparency of July, 2020 was significantly lower than that other months.



Fig. 4: Transparency variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.3 Turbidity

The range of turbidity varied from approximately 18-129 NTU (Fig. 5) in both culture stations. Highly significant difference (p<0.05) was observed in turbidity in the monthly variation. However, turbidity in August, 2020 was significantly higher (p<0.05) than that in other months.



Fig. 5: Turbidity variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.4 pH

The level of pH fluctuated from 7.4-8.4 (Fig. 6) in both culture sites. Highly significantly difference (p< 0.05) was observed in pH in the monthly variation. However, pH in February, 2020 was significantly higher (p< 0.05) than that in other months.



Fig. 6: pH variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.5 Dissolve oxygen (DO)

The dissolve oxygen reading during the sampling time varied approximately from 7-10 mg/l (Fig. 7). Highly significant difference (p< 0.05) in the monthly variation was observed in DO. However, DO in September, 2020 was significantly higher (p< 0.05) than that in other months.



Fig. 7: Dissolved oxygen variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.6 Salinity

The concentration of salinity varied from approximately 26-33 ppt (Fig. 8). Significant difference (p < 0.05) in salinity was observed in monthly variation. Salinity decreased significantly (p < 0.05) in July, 2020 and increased significantly (p < 0.05) in November, 2019 and November, 2020.



Fig. 8: Salinity variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.7 Depth

The depth reading varied from approximately 2-4 m during the study time during high tide (Fig. 9) in both culture sites. Highly significant difference (p< 0.05) in the monthly variation was observed in depth. However, depth in July, 2020 was significantly higher (p< 0.05) than that in other months.



Fig. 9: Depth variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.8 Alkalinity

The range of alkalinity fluctuated approximately from 140-315 ppm (Fig. 10) in both stations. Significant difference (p< 0.05) in alkalinity was observed in monthly variation. Alkalinity decreased significantly (p< 0.05) in June, 2020; August, 2020 and January, 2020. But alkalinity increased significantly (p< 0.05) in May, 2020.



Fig. 10: Alkalinity variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.9 Nitrate

The value of nitrate fluctuated from 0.22-0.75 ppm (Fig. 11) in both stations. Highly significant difference (p< 0.05) in the monthly variation was observed in nitrate. However, nitrate in November, 2020 was significantly higher (p< 0.05) than that in other months.



Fig. 11: Nitrate variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.10 Nitrite

The value of nitrite fluctuated from 0.02-0.18 ppm (Fig. 12) in both culture stations. Highly significant difference (p < 0.05) in the monthly variation was observed in nitrite. However, nitrite in June, 2020 was significantly higher (p < 0.05) than that in other months.



Fig. 12: Nitrite variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.11 Phosphate

During the study time, the concentration of phosphate fluctuated approximately from 0.35-1.08 ppm (Fig. 13) in both stations. Highly significant difference (p< 0.05) in the monthly variation was observed in phosphate. However, phosphate was significantly higher (p< 0.05) in April, 2020 and August, 2020 than other months.



Fig. 13: Phosphate variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.12 Ammonia

The value of ammonia varied approximately from 0.01-0.44 ppm (Fig. 14) in both stations. Significant difference (p< 0.05) in ammonia was observed in monthly variation. Ammonia decreased significantly (p< 0.05) in November, 2019 and October, 2020. But ammonia increased significantly (p< 0.05) in June, 2020 and July, 2020.



Fig. 14: Ammonia variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.13 Ammonium

The value of ammonia varied from 0.02.-0.31 ppm (Fig. 15) in both culture sites. Highly significant difference (p< 0.05) in the monthly variation was observed in ammonium. However, ammonium was significantly higher (p< 0.05) in May, 2020 and June, 2020 than other months.



Fig. 15: Ammonium variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.14 Chlorophyll-a

The value of chlorophyll-a fluctuated approximately from 2.55-5.38 (Fig. 16) in both sites. Highly significant difference (p < 0.05) in the monthly variation was observed in chlorophyll-a. However, chlorophyll-a was significantly higher (p < 0.05) in September, 2020 than other months.



Fig. 16: Chlorophyll-a variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.15 Total suspended solids

The value of Total suspended solids fluctuated from 17-45 ppm (Fig. 17) in both stations. Highly significant difference (p < 0.05) in the monthly variation was observed in total suspended solids. However, total suspended solids were significantly higher (p < 0.05) in November, 2019; February, 2020 and October, 2020 than other months.



Fig. 17: Total suspended solids variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

4.1.16 Current Speed

The value of current speed fluctuated from 0.1-0.45 m/s (Fig. 18) in both sites. Significant difference (p< 0.05) in current speed was observed in monthly variation. Current speed increased significantly (p< 0.05) in May, 2020 and July, 2020.



Fig. 18: Current speed variations in raft and longline system in Nuniar Chora during November 2019 to November 2020

Table 1: Water quality parameters (mean \pm SD; min-max value is presented within the parenthesis) of two stations in Nuniar chora recorded from November, 2019 to November, 2020

Parameter	Station-1 (Raft Culture)	Station-2 (Longline Culture)
Dissolved Oxygen (ppm)	8.83±0.67	8.75±0.71
	(7.62-10.12)	(7.45-10.11)
pН	7.81±0.26	7.81±0.26
	(7.40-8.40)	(7.40-8.40)
Water Temperature (°C)	28.58±1.83	28.58±1.83
	(25.30-31.00)	(25.30-31.00)
Total Suspended Solids	30.19±6.90	29.96±5.89
(ppm)	(17.00-45.00)	(18.00-41.00)
Alkalinity (ppm)	208.73±53.27	210.38±52.54
	(142.00-315.00)	(150.00-315.00)
Transparency (ppm)	39.75±5.41	39.67±5.30
	(29.58-51.53)	(29.57-51.12)
Turbidity (NTU)	41.00±26.28	41.07±26.21
	(18.95-129.27)	(18.77-129.13)
Nitrite (ppm)	0.05±0.04	0.05±0.04
	(0.02-0.18)	(0.02-0.18)
Nitrate (ppm)	0.49±0.12	0.49±0.12
	(0.22-0.75)	(0.22-0.75)
NH ₃ (ppm)	0.18±0.11	0.18 ± 0.10
	(0.01-0.44)	(0.01-0.41)
NH ₄ (ppm)	0.15±0.09	0.16±0.09
	(0.02-0.31)	(0.02-0.31)
PO ₄ (ppm)	0.77±0.18	0.77±0.18
	(0.35-1.07)	(0.38-1.08)
Chlorophyll-a	3.67±0.60	3.62±0.56
	(2.64-5.38)	(2.55-5.15)
Salinity (ppt)	30.94±1.86	30.94±1.87
	(26.85-33.51)	(26.85-33.55)
Depth (m)	3.00±0.37	3.06±0.34
	(2.50-3.80)	(2.60-3.80)
Current Speed (m/s)	0.23±0.09	0.24±0.08
	(0.10-0.45)	(0.13-0.41)

4.2 Growth performance of P. viridis

To analyse the growth of green mussel, shell length and live weight of cultivated *P*. *viridis* from raft and longline were recorded over 10 months period. The range of f shell length and live weight of cultivated *P*. *viridis* from raft and longline in Cox's Bazar coast, near Nunia Chora is summarized in Table 2 and Table 3, respectively.

4.2.1 Shell length of *P. viridis*

No significance difference (p>0.05) was observed in shell length of *Perna viridis* among the two culture stations (Fig. 19). The growth increment (shell length) of green mussel was higher in August, 2020 than other months (Table 4).



Fig. 19: Average shell length comparison of P. Viridis cultured in raft and longlines

Table 2: Shell length of *P. viridis* (mean \pm SD; min-max value is presented within the parenthesis) of two stations in Nuniar chora recorded from February, 2020 to November, 2020

Month	Shell length (cm) in Raft	Shell length (cm) in				
	Culture	Longline Culture				
February	0.62±0.19	0.49±0.14				
	(0.20-0.90)	(0.30-0.70)				
March	0.87±0.07	0.83±0.07				
	(0.04-0.09)	(0.70-0.90)				
April	1.14±0.14	1.13±0.09				
	(0.90-1.40)	(1.00-1.30)				
May	1.47±0.15	1.44±0.1				
	(1.20-1.70)	(1.20-1.60)				
June	2.55±0.3	2.49±0.30				
	(2.10-3.20)	(2.10-3.10)				
July	4.41±0.61	4.37±0.57				
	(3.30-5.20)	(3.50-5.20)				
August	6.11±0.43	6.09±0.36				
	(5.40-6.80)	(5.50-6.70)				
September	8.00±0.37	7.97±0.38				
	(7.50-8.60)	(7.30-8.70)				
October	9.40±0.39	9.30±0.40				
	(8.80-10.10)	(8.70-10.00)				
November	11.17±0.53	10.92±0.34				
	(10.30-12.20)	(10.30-11.30)				

4.2.2 Live weight of *P. viridis*

No significance difference (p>0.05) was observed in live weight of *Perna viridis* among the two culture stations (Fig. 20). The growth increment (live weight) of green mussel was higher in August, 2020 than other months (Table 5).



Fig. 20: Average live weight comparison of P. Viridis cultured in raft and longlines

Table 3: Live weight of *P. viridis* (mean \pm SD; min-max value is presented within the parenthesis) of two stations in Nuniar chora recorded from February, 2020 to November, 2020

Month	Live weight (g) in Raft	Live weight (g) in
	Culture	Longline Culture
February	0.07±0.02	0.06±0.02
	(0.04-0.09)	(0.04-0.09)
March	0.11±0.02	0.10±0.02
	(0.08-1.00)	(0.08-0.14)
April	0.16±0.02	0.15±0.02
	(0.10-0.18)	(0.09-0.17)
May	0.21±0.04	0.20±0.04
	(0.18-0.29)	(0.17-0.30)
June	0.62±0.18	0.57±0.20
	(0.26-0.93)	(0.25-0.93)

July	3.01±0.75	2.96±0.75	
	(1.89-3.89)	(1.99-4.14)	
August	19.74±3.63	19.54±2.62	
	(15.07-25.01)	(15.47-23.01)	
September	29.56±5.06	29.07±5.38	
	(22.56-39.76)	(21.01-39.89)	
October	41.57±1.39	40.63±1.88	
	(39.86-44.34)	(35.86-42.43)	
November	50.19±2.51	47.99±2.03	
	(44.85-54.56)	(44.65-50.09)	

Table 4: Growth increment of *P. viridis* (mean \pm SD) in raft and longline

	Live weight	Live weight (g)	Shell length	Shell length (cm)
Month	(g) in Raft	in Longline	(cm) in Raft	in Longline
	Culture	Culture	Culture	Culture
March	0.04±0.01	0.04±0.01	0.25±0.16	0.34±0.19
April	0.05±0.01	0.05±0.01	0.27±0.02	0.3±0.02
May	0.05±0.02	0.05±0.02	0.33±0.05	0.31±0.05
June	0.41±0.04	0.37±0.04	1.08±1.84	1.05±1.82
July	2.4±0.75	2.39±0.73	1.86±0.83	1.88±0.87
August	16.73±3.83	16.58±3.86	1.93±0.89	1.91±0.83
September	9.82±2.65	9.53±2.67	1.89±1.09	1.88±1.08
October	12.01±3.35	11.56±3.32	1.4±0.75	1.33±0.74
November	8.62±1.84	7.36±1.82	1.77±0.91	1.62±0.87

Parameter	Observed Range	Suitable range (FIGIS, 2005)	Comments	
Dissolved Oxygen (ppm)	7.62-10.12	>6	Suitable	
рН	7.40-8.40	7.2-8.5	Suitable	
Water temperature (°C)	25.30-31.00	27-32	Suitable	
Total suspended solids (ppm)	17.00-45.00	15-50	Suitable	
Alkalinity (ppm)	142.00-315.00	150-350	Suitable	
Transparency (ppm)	29.58-51.53	30-60	Suitable	
Turbidity (NTU)	18.95-129.27	10-150	Suitable	
Nitrite (ppm)	0.02-0.18	0.01-0.1	Suitable	
NH ₃ (ppm)	0.01-0.44	0.05-0.5	Suitable	
NH ₄ (ppm)	0.02-0.31	0.05-0.5	Suitable	
PO ₄ (ppm)	0.35-1.07	0.5-1	Suitable	
Chlorophyll-a	2.64-5.38	2-3	Suitable	
Salinity (ppt)	26.85-33.51	25-35	Suitable	
Depth (m)	2.50-3.80	>2	Suitable	
Current speed (m/s)	0.10-0.45	0.1-0.3	Suitable	
Nitrate (ppm)	0.22-0.75	0.1-1	Suitable	

Table 5: Observed range and suitable range of water quality parameters for green mussel culture

CHAPTER FIVE

DISCUSSION

The purpose of this study was to investigate the relation of growth performance with the eco-physiological factors of green mussel (*P. viridis*) cultured in raft culture system and long-line culture system. To analyse seasonal variation of water quality parameters as well as the shell length and total growth of green mussel of the two culture systems. Furthermore, the gathered information will provide the best information to compare the growth performance of green mussel (*P. viridis*) cultured in raft culture system and long-line culture system.

5.1 Environmental variables

Ecological waters factors are important in supporting the growth of green mussel. Environmental factor is an important parameter because it affects the needs and feed intake of green mussel, and therefore contributes to the growth of mussels (Pattikawa and Ferdinandus, 2009). In this study, temperature, dissolved oxygen, salinity and pH in both culture stations were recorded within tolerance range of P. viridis culture and growth. (Sallih, 2005; Tan and Ransangan, 2015). There was no significant variation of water quality parameters among the raft culture station and long-line culture station. However, both stations experienced significant temporal variation in all water quality parameters except phosphate nutrient. It supports the study conducted by Pattikawa & Ferdinandus, 2009. Both stations experienced relatively higher temporal salinity and turbidity fluctuation in July 2020 and August 2020 during high rainfall and hill run off. The high turbidity might not prevent the predator (Aypa, 1990). P. viridis is a marine water mussel species that requires high salinity of 27-35 ppt for optimal growth (Aypa, 1990; Rajagopal et al., 2006; Tan and Ransangan, 2014). Low salinity caused by fresh water dilution during heavy rainfall might negatively affects the growth and survival of the bivalve (Saxby, 2002). The concentration of salinity varied from approximately 26-33 ppt in both stations. The favourable water depth for both seed collection and mussel cultivation is 2 m or more (Aypa, 1990). The depth reading varied from approximately 2-4 m during the study time during high tide in both culture sites. Depth increased in the month of June and July, 2020. From this consultation the water depth in the two culture sites can be considered as suitable for green mussel culture and growth but not perfect. The level of transparency is important in mussel culture, because it can determine the presence of suspended, organic and inorganic matters and also level of primary production in culture area (Lovatelli, 1988). Culture site having a disc reading of less than 25 cm should be considered unsuitable for mussel culture (Lovatelli, 1988). The value of transparency measured approximately from 29-51 cm from both culture sites during this study. Because of high runoff, low transparency was observed highest during the month of July. Organic effluents from land are known to be the main factor reducing the pH value in marine environments (Sany et al., 2004). Several previous studies mention the optimal temperature to support the growth of green mussel is 26 – 32 °C (Hickman, 1992). Based on the results of research in the tropics area, green mussel will die within 30 minutes at a temperature of 43°C (Nair et al., 2003). The temperature varied from approximately 25-31 °C in both the culture stations which is favorable for mussel growth (Saxby, 2002). The pH value fluctuated from 7.4-8.4 in both stations during study time. The level of DO in the study might not be the direct factor that induced culture potentiality and growth in P. viridis but it could be the consequences from the energy demanding selective feeding activities in P. viridis which requires high level of oxygen (Bayne, 1998). The level of DO varied from 7-10 mg/l which is very good for green mussel culture according to the range of environmental parameters for mussel farming (FIGIS, 2005). The value of chlorophylla fluctuated approximately from 2.55-5.38 in both sites which is very good and suitable for green mussel culture according to the range of water quality parameters for mussel farming (FIGIS, 2005). The value of current speed fluctuated from 0.1-0.45 in both sites which is also very good for green mussel culture according to the range of environmental parameters for mussel farming (FIGIS, 2005). The water quality parameters of the culture sites during culture period are considered in good condition and support the growth of green mussels.

5.2 Comparative Growth Performance in Raft and Longline Culture

The research shows that the highest shell length of green mussel collected from the longline method was 11.30 cm and raft method was 12.20 cm (table 2). It also shows that the highest live weight of green mussel collected from the longline method was 50.09 g and raft method was 54.56 g (table 3). The study also shows that the minimum *P. viridis* spat length recorded in longline and raft method were 0.3 cm and 0.2 cm, respectively. The minimum *P. viridis* live weight recorded in both longline and raft method was 0.04 g. The difference in size of green mussel spat can be caused by the

abundance of plankton in waters as a source of food and water environmental factors (Alfaro and Jeffs, 2002; Alfaro, 2005).

No significance difference (p>0.05) was observed in shell length of *Perna viridis* among the two culture stations. In case of live weight, no significance difference (p>0.05) was observed of *Perna viridis* among the two culture stations.

The growth increment (live weight) of green mussel was higher in August, 2020 than other months. Also, the growth increment (shell length) of green mussel was higher in August, 2020 than other months. The green mussel growth is influenced by environmental factors such as temperature, food availability and waters current (NIMPIS, 2002). All or a combination of several environmental factors can affect the growth, reproduction, and distribution of aquatic organisms (Sahin et al., 2006).

CHAPTER SIX

CONCLUSIONS

It is a critical matter and has always been a priority to farmers and investors to identify the most suitable culture method for successful and sustainable aquaculture activity. Green mussel (*P. viridis*) is one of the highest demandable species that is used as the main food to the tribal people of Bangladesh as well as neighbouring countries like Japan, Thailand, Malaysia, Cambodia and China. Fish processing industries, exporter and other associated stakeholders can export flesh of green mussel to those neighbouring countries. Developing P. viridis culture techniques can be a great source of livelihood for many people. Also, it can be a mean for developing the socioeconomic conditions. But it is critical for farmers and investors to accept new and nonconventional species for commercial aquaculture farming. This basic comparative growth performance study related to ecological parameters of green mussel cultivated in longlines and rafts will be the baseline information for aquaculture practitioners who will take steps for mussel culture establishment in the future. In this study, it has been shown that the water quality parameters such as temperature, pH, DO etc. in both culture stations were within the tolerance and suitable range of *P. viridis* culture. In all stations, the chlorophyll-a consideration was higher than the minimum recommendation. All the water quality parameters of the two culture sites were suitable for green mussel culture and had influence on the growth of green mussel. This study also prevails that there is no significant variation in growth performance of P. viridis cultivated on longline and raft. The growth increment of P. viridis is higher in August than the other months in both raft and longline culture methods. From an economic point of view, longline is more viable but raft method has more durability in harsh environments. Both longline and raft method of off-bottom green mussel farming can be successfully adopted by the coastal people of Cox's Bazar, Bangladesh by setting up small units near the coast adjacent to their homesteads; and more over the proximity of major mussel markets and high degree of mussel consumption in the area would synergize this endeavour. Furthermore, the technology of green mussel farming is simple, economically viable and eco-friendly, making the technique sustainable and easily adoptable.

CHAPTER SEVEN

RECOMMENDATION AND FUTURE PERSPECTIVES

Study on different aspects like site selection, growth performance, feeding behaviour, reproductive system, ecological impacts etc. are the primary need not only in green mussel culture of Cox's Bazar coast of Bangladesh but also in other coastal mussel habitats. Culture of mollusks especially mussels and oysters are considered as an urgent rising option of coastal aquaculture. Side by side an export market in the neighbouring Southeast countries needs to be explored so that the cultured excess product can be sold. A large quantity of mussel and oysters' meat is destroyed during the collection, which can be prevented through improving methods of exploitation, transportation and storage. Identification of a distinct local community in the southern region who consume mollusk to develop local market is very important. Proper processing technology can also ensure the quality and increase demand of mussel in local restaurants. This study provides the baseline information regarding the development of this culture. Culture development is necessary to reduce stress on natural stock of green mussel and will ensure the conservation of shellfish biodiversity as well as keeping harmony with the future fast-growing industry. Culture development will also provide economic support to the small-scale fishermen by creating employment opportunity.

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APPENDIX A: Paired sample t-test of shell length of *P. viridis* cultured in raft and longlines

Paired Samples Test									
Paired Differences			t	df	Sig. (2-				
		Mean	Std.	Std.	95% Confidence				tailed)
			Deviation	Error	Interval of the				
				Mean	Difference				
					Lower	Upper			
Pair 1	Raft-	0.07	0.20	0.02	0.03	0.11	3.54	99	0.14
	Long								
	line								

APPENDIX B: Paired sample t-test of live weight of *P. viridis* cultured in raft and longlines

Paired Samples Test									
Paired Differences				t	df	Sig. (2-			
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				(ancu)
					Lower	Upper			
Pair 1	Raft- Long line	0.39	1.34	0.13	0.12	0.66	2.94	99	0.19

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