Project ID - 333

Competitive Research Grant

Sub-Project Completion Report

on

Feasibility of Green Mussel *Perna viridis* Culture in the Coastal Areas of Bangladesh

Project Duration
May 2017 to September 2018

Department of Marine Bioresource Science
Faculty of Fisheries
Chittagong Veterinary and Animal Sciences University
Chittagong, Bangladesh

Submitted to

Project Implementation Unit-BARC, NATP-2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

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Acronyms

DO	-	Dissolve Oxygen
NO_2	-	Nitrite
NO_3	-	Nitrate
PO_4	-	Phosphate
NH_4	-	Ammonia
mL	-	Milliliter
M	-	Miter
ppt	-	Parts Per Thousand
mg/L	-	Milligram Per Liter
°C	-	Degree Celsius
µg/L	-	Microgram Per Liter
m/s	-	Minute Per Second
<	-	Less than
>	-	Greater than
e.g	-	Example
et al.	-	And his associates
etc.	-	Et cetera
%	-	Percentage
ppm	-	Parts Per Million
ft	-	Feet
Cm	-	Centimeter
NTU	-	Nephelometric Turbidity Unit
min-max	-	Minimum-Maximum
S	-	Station
EPA	-	Eicosapentaenoic Acid
DHA	-	Docosahexaenoic Acid
Sig.	-	Significance
NS	-	No Significant difference

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Executive Summary

Although there is a tremendous potential of Green mussel aquaculture in the coastal areas of Bangladesh, until now there are no research report about the biology, culture feasibility, reproduction and culture technology of this species. Therefore, the project aims to provide all the basic information needed to develop the culture technique of Green mussel (*P. viridis*) under the coastal areas of the country. In order to carry out the feasibility study, special emphasis was given to identify the potential sites, feeding behaviour, reproduction, growth and culture potential of *P. viridis* in the coastal areas. Initially, the project investigated the feasibility of Green mussel culture in the coastal land locked channels and estuary like Rezu Khal, Maheshkhali Channel and The Naf river estuary by applying site capability rating system which includes all the biophysical parameters and phytoplankton abundance. The results demonstrated that Maheshkhali channel was the best suitable habitat for Green mussel aquaculture followed by the The Naff River estuary. However, the Rezu khal was found not suitable for Green mussel aquaculture.

A thorough study on the reproductive biology of *P. viridis* and defining the accurate times of collecting spat is essential to develop the Green mussel culture technology in Bangladesh. The histological analysis of 242 Green mussels revealed five stages of gonadal development with peak spawning season from January to April. The feeding biology study demonstrated that *P. viridis* selectively ingest *Coscinodiscus* spp round the year. The results also demonstrated that feeding biology of Green mussel largely varied with the size, season, sex and gonad developmental stages.

Mussel spat settlement densities were studied by monthly setting on the ropes of on a bamboo made Mussel horizontal fixed frame in three locations of Maheskhali channel by hanging ropes and rope made nets as spat collectors. Larval occurrence was noticed from December and spat settlement began from the January. February and March were the pick months for spat settlement in the Maheshkhali Channel. At next stage, three pilot-scale culture sites were established in the Maheskhali Channel. Each culture site consists of 4 different culture systems such as hanging rope culture, hanging rope-net culture, cage culture and pole culture systems. We observed a large number of Green mussel spat settlement on the experimental substrates. Our results initially suggested that Green mussel can be cultured in hanging rope, rope net and cage culture. The monthly growth data showed satisfactory growth performances of the Green mussel. Growth performance data also showed that cage and rope culture could be the best options for Green mussel culture.

Nutritional profiling data indicated that Mussel meat is enriched with high protein and lipid. The lipid of Green mussel contains higher percentages of unsaturated and polyunsaturated fatty acids along with DHA and EPA, and the highest amount of these nutrients are found during spring and summer. The protein contents also vary with the season. The amino acid content in Mussel meat is very high during spring and summer and the highest percentage of glutamic acid is found in Mussels. Heavy metal analysis data showed that the heavy metal contents of Green mussel was found within the safe limit for human consumption.

In conclusion, it can be stated that there is huge potential for the culture of Green mussel in the coastal areas of Bangladesh.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the CRG sub-project: Feasibility of Green mussel *Perna viridis* Culture in the Coastal Areas of Bangladesh.

2. Implementing organization:
   Department of Marine Bioresource Science, Faculty of Fisheries, Chittagong Veterinary and Animal Sciences University, Khulshi-4225, Chittagong, Bangladesh.

3. Name and full address with phone, cell and E-mail of PI/Co-PI (s):

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4. Sub-project budget (Tk):
   4.1 Total: 50,00,000

5. Duration of the sub-project
   5.1 Start date (based on LoA signed): 07 May 2017
   5.2 End date: 30 September 2018

6. Justification of undertaking the sub-project:

Asian Green mussel (*Perna viridis*), an estuarine and intertidal bivalve species, is widely distributed in the tropical and sub-tropical areas of the Indo-Pacific region. It is a large and fast growing commercially important species, and has demonstrated prodigious culture feasibilities in many countries. The global production of marine Mussels (Mytilidae) has been increasing at an average rate of 5% per year since 1990, reaching about 1.1 million tonnes in 2015 of which 85% comes from the Asian countries (FAO, 2018). Since marine Mussels are a sustainable form of protein production, it is expected that the global production of Mussels, particularly in Asia, will continue to grow in order to fulfill part of the protein demand of the growing world population. In Bangladesh, the natural distribution of *P. viridis* is confined to the south-eastern coasts, particularly in Moheshkhali Channels and Naaf river estuaries (Sahabuddin *et al.,* 2004). The coastal water is one of the most productive zones in Bangladesh, and offers an ideal environment for natural growth and recruitment as well as farming of *P. viridis* (Sahabuddin *et al*., 2004). In support of blue economy, Department of Fisheries (DoF) is also now giving special focus on the expansion of coastal aquaculture of non-traditional species such as edible Oysters *(Crassostrea* sp. *Saccostrea* sp.), Pearl oyster, (*Anadra* sp.), Green mussel (*Perna viridis*), Clam (*Meretrix meretrix*, *Marcia opima*), Sea snails, Swimming crab, Squid, Cuttle fish, Sea weeds, Sea cucumber, Star fish, etc. However, very limited researches were accomplished on feasibility study, culture system, reproductive biology and nutritional profiles of these species in Bangladesh.

The production of aquaculture in our country can be increased to a certain extent by diversifying from only fish culture to shrimps, crabs, mollusk etc. However, there was little attempt made for mollusk like Green mussel culture in Bangladesh due to lack of proper knowledge on distribution and abundance of mollusks populations in our coast and ignorance of food value. To counter balance the stress exerted by exploitation of natural stock, technologies can be developed for culturing Green mussel in coastal areas which will ensure the conservation of shellfish biodiversity in nature as well as keeping harmony with the future fast-growing industry.

This potential Green mussel industry will offer employment opportunities for the unemployed youth and for the shrimp PL and Jatka collectors ensuring "Conservation with the community participation". It is not yet realized that there are about two million tribal people who regularly take mollusks meat in their daily diet. Since the distribution of mollusks is patchy and collection procedure is labor-intensive and supply is dependent on seasons. Therefore, culture of mollusks especially Mussels and oysters may be considered as an urgent option for coastal aquaculture. Side by side an export market in the neighboring Southeast countries needs to be explored so that excess product can be sold at a higher price. Upon proper processing, Green mussel flesh can be exported to foreign countries as a new item with shrimp and crabs. Prospective export markets also exist in Japan, Thailand, Malaysia, Cambodia and China where these species are widely eaten. In addition, the shells of the Green mussels can be used for making poultry and fish feed as well as lime making. Therefore, proposed project will provide future employment opportunities, alternative protein to 0.2million tribal people, earning foreign currencies and open a new arena in coastal aquaculture of Bangladesh.

Although there may be a tremendous potential of Green mussel aquaculture, until now there is no exclusive study on the culture potential for Green mussel aquaculture at coastal areas of Bangladesh. Therefore, the proposed project aims to provide all the basic farming information of *P. viridis* in the coastal areas through a series of research (site suitability, feeding and breeding biology, nutrient analysis, spat settlement) on different aspects. Initially, the project identifies the potential site for successful cultivation of *P. viridis* considering the availability of sustainable wild stocks and abundance of seed along with favorable environmental conditions. The proposed project also focused on the seasonal variation of spat settlement density on different substrates and measurement of the growth rate through a pilot-scale rope culture experiment.

7. Sub-project goal:

The ultimate goal of the proposed project is to conduct a feasibility study for gathering all basic information needed to develop a culture technique of Green mussel (*P. viridis*) in the coastal areas of Bangladesh. In order to conduct the feasibility study, special emphasis was given to identify the potential sites, to know the food & feeding behaviour, reproduction, growth etc. of *P. viridis* in coastal areas of Bangladesh.

8. Sub-project objective (s):

- To identify the potential farming sites (in terms of biophysical variables and food availability) of Green mussel

- To obtain near real-time data on some biological aspects (feeding and breeding biology, seasonal variation of spat settlement density) required to represent the feasibility of Green mussel culture

* To conduct pilot scale experiment on different culture systems of Green mussel at potentially suitable site particularly at the Maheshkhali channel

* To know the seasonal variation of nutritional profiles and heavy metal contents of Green mussels available in the coastal areas of Bangladesh.

9. Implementing location (s)

The proposed project has been carried out at different coastal regions of Cox's Bazar such as Rezu Khal, Maheshkhali Channel and The Naf River in Cox's Bazar. Five sub-stations have been selected from each of these locations for feasibility study. Other studies were carried out at Maheshkhali Channel. The details site position maps are shown below-

Fig. 1: Location of the sub-project in different region of Cox's Bazar, Bangladesh

10. Methodology:

10.1 Site capability rating system for fast and effective evaluation of potential farming sites in coastal areas of Bangladesh

10.1.1 Biophysical condition:

The monthly variation of temperature, salinity, alkalinity, pH, water current, water depth, dissolved oxygen, transparency, chlorophyll-*a*, dissolved nutrients such as nitrite, nitrate, phosphate, ammonium and ammonia were measured from all the stations at the study area following standard methods and using specialized devices.

10.1.2 Analysis of physico-chemical water quality parameters:

Water quality parameters like temperature (Celsius Thermometer), dissolved oxygen (Digital DO Meter), pH (Portable pH meter), salinity (Refractometer), transparency (Secchi Disc), water depth (Weight and Rope), water current speed (Digital Current meter) were monitored *in situ* during Day time on a monthly basis.

Three water samples were collected in sampling bottles from each station and were carried to laboratory as soon as possible for turbidity (Digital turbidity meter), alkalinity (Titrimetric method), chlorophyll-α and nutrient (NO_2, NO_3, PO_4, NH_3 and NH_4) analysis. After turbidity determination, the water samples were filtered through microfiber filter paper (Whitman GF/C), using a vacuum pressure air pump (Rocker filtration pump). The filtered water samples were used for alkalinity and nutrient analysis. The filter papers were preserved in acetone for chlorophyll determination.

Fig. 2: Water sample collection and parameter determination in coastal regions

10.1.3 Procedure of Turbidity measurement:

The device that is used for measuring turbidity is called Digital Turbidity Meter (Turb 430 IR). The zero adjustment of the turbidity meter was done before measurement using distilled water. The water samples were taken into the glass vials after vigorous shaking. After wiping properly, the vials were inserted into the turbidity meter. The reading appeared on the digital screen and was recorded afterwards.

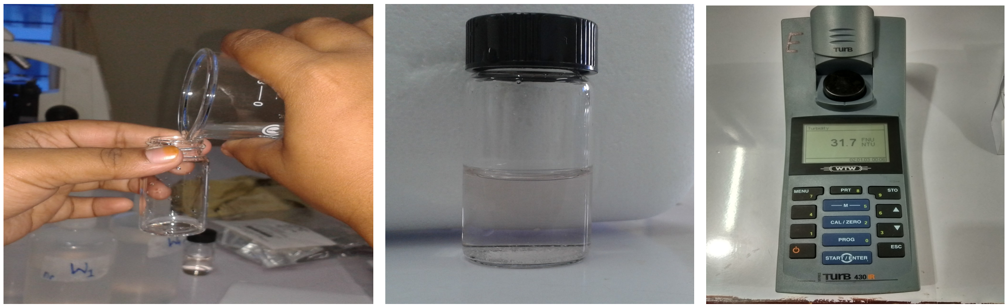


Fig. 3: Turbidity determination

10.1.4 Procedure of Alkalinity measurement:

100 mL of sample water was taken into a conical flask. Then 2-4 drops of Phenolphthalein indicator were added in the sample. As the color of the sample didn't change, it indicated that phenolphthalein alkalinity was absent. After that fresh 100 mL water sample was taken into another flask and 2-4 drops of Methyl Orange indicator were added in the sample. The color turned into yellow. Then the sample was titrated against standard H_2SO_4 (0.02N) until the yellow color turned into pink. The required amount of acid (H_2SO_4) was recorded and the result was calculated by the following formula:

$$\text{Alkalinity} = \frac{\text{Acid used (mL)} * 0.02\text{N}\left(\begin{matrix}\text{Normality}\\ \text{of Acid}\end{matrix}\right) * 50\left(\begin{matrix}\text{Gram Equivalent}\\ \text{Weight of } CaCO_3\end{matrix}\right) * 1000}{\text{Sample Volume (V)}}$$

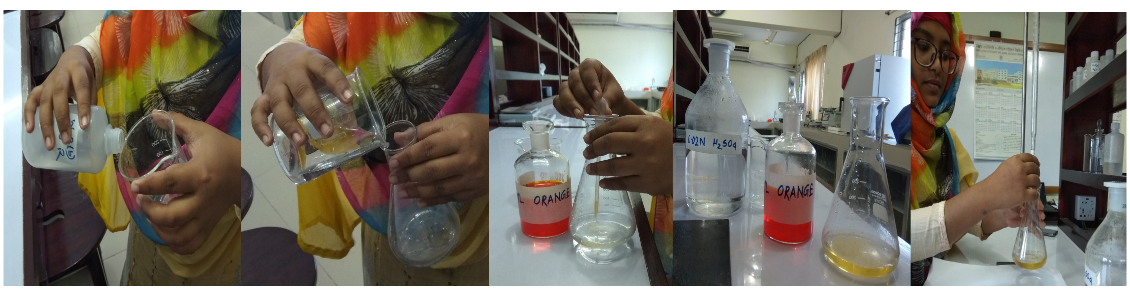


Fig. 4: Alkalinity determination

10.1.5 Procedure of Chlorophyll-a measurement:

500 mL of 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. The filtered papers were then mixed thoroughly with the acetone by using a glass/metal rod. Then centrifugation at 3500 RPM for 2.30 minutes was performed. The supernatant contents (extract) were taken into cuvettes and the absorbance of extracts was determined at 664, 647 and 630 nm comparing with blank acetone. The chlorophyll-α concentration was calculated by the following equation:

Chlorophyll-α= (11.85 A_{664}-1.54 A_{647}-0.08 A_{630}) *(V/S) *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)

Fig. 5: Chlorophyll - α test

10.1.6 Procedure of Nitrite measurement:

The program 305 was set before in the photometer and zero adjustment was done by using distilled water. VARIO Nitri 3 F10 Powder pack of chemical content was used to measure the nitrite in sample water. First 10 mL of sample water was taken in empty cell by using pipette. The contents of the powder pack were added and the cell was closed with screw cap. The cell was shaken and allowed to react for 15 minutes. The cell was inserted in the photometer and the reading was recorded afterwards.

Fig. 6: Nitrite test of sample

10.1.7 Procedure of Nitrate measurement:

The program 314 set before in the photometer and zero adjustment was done using distilled water. VARIO Nitrate Chromotropic Powder pack of chemical content was needed to measure the nitrite in sample water. First 1 mL of sample water was taken in empty cell using pipette. The contents of the powder pack were added and the cell was closed with screw cap. The cell was carefully shaken and allowed to react for 15 minutes. The cell was inserted in the photometer and the reading was recorded afterwards.

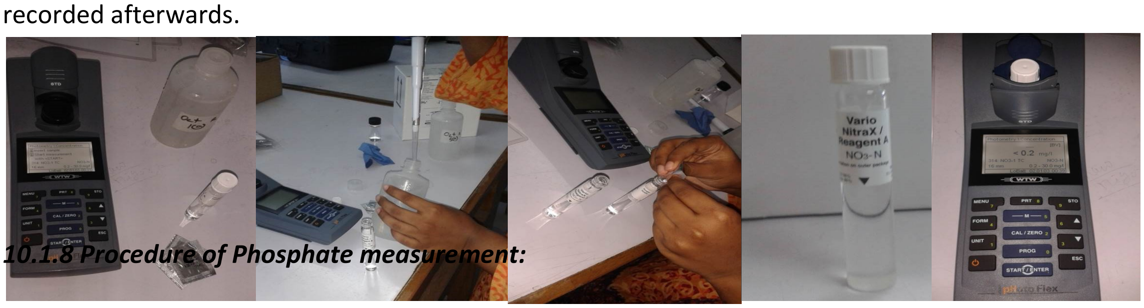


Fig. 7: Nitrate test of sample

10.1.8 Procedure of Phosphate measurement:

The program 306 set before in the photometer and zero adjustment was done by using distilled water. VARIO Phos3 F10 Powder pack of chemical content was needed to measure the nitrite in sample water. First 10 mL of sample water was taken in empty cell by using pipette. The contents of the powder pack were added and the cell was closed with screw cap. The cell was shaken and allowed to react for 15 minutes. The cell was inserted in the photometer and the reading was recorded afterwards.

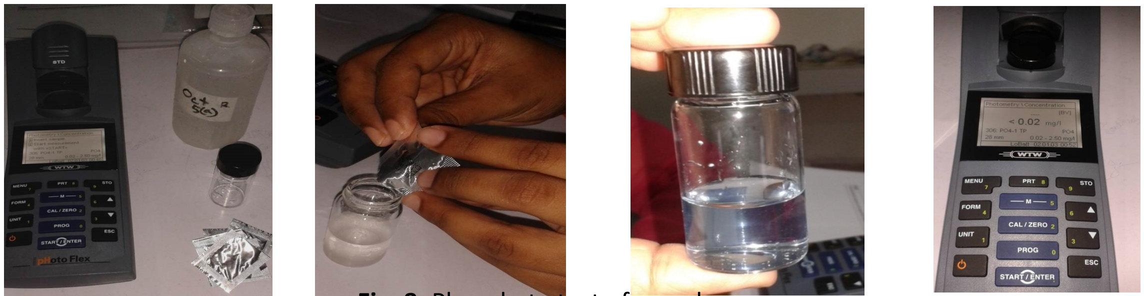


Fig. 8: Phosphate test of sample

10.1.9 Procedure of Ammonia measurement:

The program 341 set before in the photometer and zero adjustment was done by using distilled water. The pH value of the sample was checked. Desired value: approx. pH 7. VARIO AMMONIA Salicylate F5 Powder pack chemical content was needed to measure the nitrite in sample water. Firstly, 02 mL of sample water was taken in empty cell using a pipette. The contents of the powder pack were added and the cells were closed with screw cap. The cells were shaken and allowed to react for 15 minutes and inserted in the photometer to get reading.

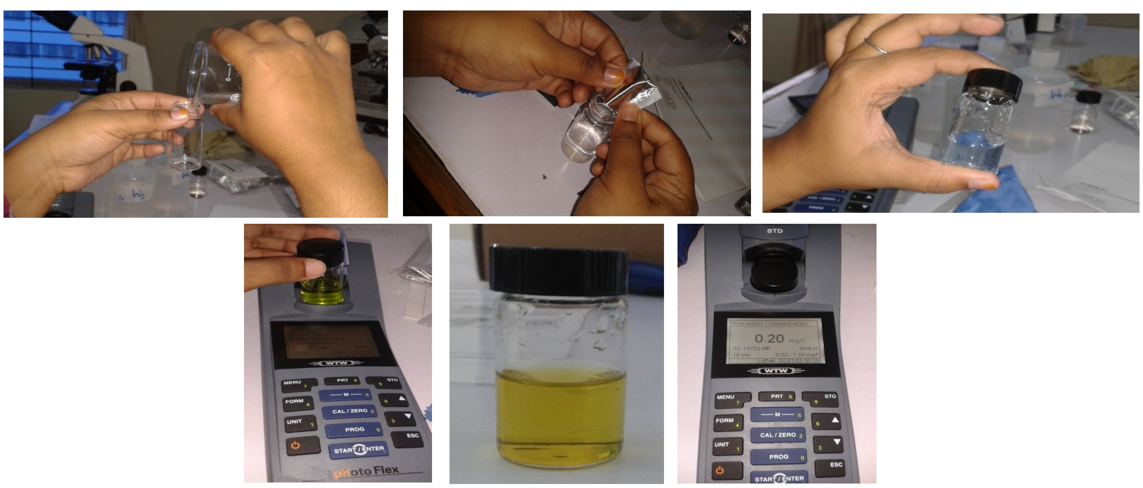


Fig. 9: Ammonia test of sample

10.1.10 Procedure of Ammonium measurement:

The program 71 set before in the photometer and zero adjustment was done by using distilled water. The pH value of the sample was checked. Desired value: approx. pH 7. NH_4^{-1} Solution, NH_4^{-2} Powder and NH_4^{-3} Solution was needed to measure the ammonium in sample water. First 10 mL of sample water was taken in empty cell by using pipette. Then 1.20 ml of NH_4^{-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 was added and 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 were added, the cells were closed with screw cap and mixed it well. The cells were shaken and allowed to react for 5 minutes. The cells were inserted in the photometer to get reading.

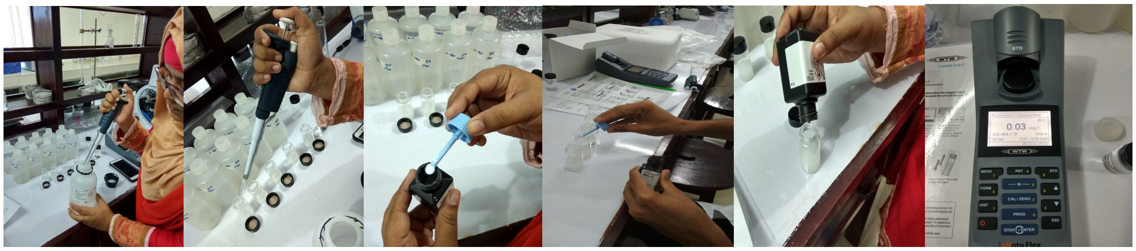


Fig. 10: Ammonium test of sample

10.1.11 Plankton composition

Plankton samples were collected monthly by pouring 50 liters of water from five different locations and passing them through 45 µm mesh plankton net. The concentrated samples were preserved in small plastic bottles with 5% buffered formalin. Qualitative and quantitative estimations of plankton were performed using a Sedgewick-Rafter Cell containing 1000 $1mm^3$ cells.

A 1 mL sample was taken in the S-R cell and left for 15 minutes undisturbed to allow plankton to settle. The plankton in 10 randomly selected cells were identified up to family level and counted under a binocular microscope with imaging facilities.

Plankton Abundance was calculated by using this formula:

N= (P*C*100)/L

Where,

- ✓ N= Number of Plankton cells or units per liter of original water (Counted by using Sedgewick-Rafter cell)
- ✓ P= The number of plankton counted in 10 fields
- ✓ C= The volume of final concentrate of the sample (mL)
- ✓ L= The volume (L) of the water sample

Fig. 11: Observing plankton under the digital microscope with imaging facility

10.1.12 Site suitability detection for P. viridis farming:

The site suitability selection for Green mussel farming is generally based on environmental conditions of the site, reflecting through the examination of a range of biophysical parameters. Assessing each and every parameter is quite not much feasible. Therefore, to assess potential sites a simple weighted system was developed by the scientists working on Green mussel culture, through which the proper site could be evaluated on the basis of minimum requirements of environmental parameters. These environmental indicators including water temperature, pH, dissolved oxygen, salinity, transparency, current speed and water depth were given a weighted value range from 0.00 to 0.90 based on the how it affects the growth or survival of the cultured species. The total weighted value of the parameters was 1.00 (Table 1). Then the environmental indicators of the potential site were rated based on its feasibility for Green mussel culture from 1 (unsuitable) to 10 (optimal) as exemplified in Table 1. In the interim, the maximum and minimum were graded based on the rating system as the parameters of a site are always declaimed as a range. The final rating score of a particular parameter was the average of its minimum and maximum. Finally, the rated value of each parameter of the studied site was multiplied with the weighted value for the parameter to get the parameter weighted value of the site. The total of the parameters weighted value was used to categorize the suitability of the site. If the parameters weighted value is high the site is considered suitable for Green mussel farming and *vice versa*. The rating of site suitability is shown in Table 2 (Tan and Ransangan, 2014).

Table.1. The weighted value and rating point for the range of environmental parameters for Mussel farming based on FIGIS (2005), Saxby (2002), Hickman (1992), Aypa (1990), Lovatelli (1990), Sivalingam (1977)

Rating Point	Salinity (ppt)	Dissolved Oxygen (mg/L)	pH value	Temperature (°C)	Chlorophyll-α (µg/L)	Water Current (m/s)	Water Depth (m)
10	27-32	>8	7.9-8.2	26-32	2.0-3.0	0.1-0.3	>8
09	25-33	6-7	7.8-8.3	25-33	1.8-3.5	0.15-0.35	8
08	24-34	5-6	7.7-8.4	24-34	1.6-4.0	0.2-0.4	7
07	23-35	4-5	7.6-8.5	23-35	1.4-4.5	0.25-0.45	6
06	18-36	3-4	7.5-8.6	22-36	1.2-5.0	0.3-0.5	5
05	15-40	-	7.4-8.7	21-37	1.0-5.5	0.35-0.6	4
04	12-45	-	7.3-8.8	20-38	0.8-6.0	0.4-0.7	3
03	10-50	3-2	7.0-8.9	19-39	0.6-6.5	0.6-0.9	-
02	5-55	2-1	6.9-9.0	18-40	0.4-7.0	0.9-15	-
01	0-65	-	6.8-9.1	17-41	<0.4 ->7.0	>1.5	1
Weighted Value	0.15	0.15	0.1	0.15	0.15	0.15	0.15

Table 2. Recommendation for site evaluation

Weighted Category	Site Evaluation	Recommendation
1.0-2.5	Not advisable	Not suitable for Green mussel farming; cannot support the culture
2.6-5.0	Poor	May support Green mussel but not recommended
5.1-7.5	Medium	Capable and moderately suitable for Green mussel farming
7.6-10.0	Good	Suitable for Green mussel farming and highly recommended

10.1.13 Data Analysis

The environmental parameter data were calculated for each station's once-a-month average value and results were displayed graphically by using Microsoft excel program 2013. All the parametric tests along with water quality and plankton composition variation were statistically analyzed by using the IBM-SPSS Windows Statistical Package (version 23); comparing the data by using descriptive one-way ANOVA, followed by Duncan's multiple comparison test for homogenous subsets with SPSS version 22.0 software and displayed as table and graphical form. Tests were judged to be significant at $p < 0.05$ level. Normality and homogeneity of variances of all the variables were tested prior to analysis using One-way ANOVA. All the data of variation was expressed into descriptive graphs along with the stations means and with the individual standard error showing the effectiveness of the statistical analysis.

10.2 Breeding biology of Green mussel

10.2.1: *Sample collection and transportation*:

Monthly samples were collected randomly once in every month from naturally bottom settled *P. viridis* in the month of October 2017 to September 2018 from the Maheshkhali Channel, Choufaldandi, Cox's bazar, Chittagong. 20 samples were collected in each month and the collected live samples were immediately kept in the ice box with ice ratio of 1:2 (Mussel: ice) and transported to the laboratory for further research.

10.2.2: *Morphometric data collection:*

Twenty Mussels were used to determine the individual total length, weight and sex. Mussels were initially cleaned from all the encrusting organisms and their byssus were removed. Biometric measurements such as length (maximum length along the anterior-posterior axis), height (maximum length along the dorsal-ventral axis), and width (maximum length through both valves) of each individual were measured using a Vernier caliper to the nearest 0.1 mm. The total weight of each individual was recorded after the inter-valval (or mantle) fluid was drained.
They were then dissected and the wet soft tissue weight and the shell weight were according to the nearest 0.001g. Dry tissue weight was measured after drying the sample tissue at 105°C for 12 hours in hot air oven and was cooled in desiccators. CI was calculated following Yep *et al*. (2003).

$$\mathbf{CI\ (g/cm^3)} = \frac{total\ soft\ tissue\ dry\ weight\ (g)}{shell\ volume\ (cm^3)} \times 1000$$

Fig. 12: Morphometric data collection

10.2.3: *Collection of gonads*:

For characterizing the gonad development stages of *P. viridis*, it is necessary to dissect the samples and collect the gonads from both sexes. After length, weight and width data collection, the mantle of Mussel was opened by knife to collect soft tissue and blotted. After removing the unwanted dirt, digestive parts and intestine, the gonad of the Mussel was collected carefully using forceps.

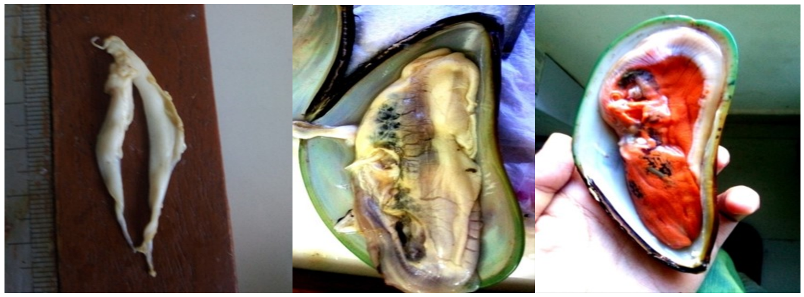


Fig. 13: Green mussel Gonad, Gravid Female Mussel and Matured Male Mussel

10.2.4: Gonadosomatic Index (GSI):

GSI is the ratio of samples gonad weight to body weight, which is particularly helpful to identify the spawning seasons. To determine the spawning period, gonadosomatic index (GSI) plays a major role as there is a cyclic change in gonad weight in relation to total body weight (Nieland and Wilson, 1993; Jons and Miranda, 1997; Smith 2008). The GSI was calculated by month-wise and sex-wise using the equation (Vladykov, 1956).

$$GSI=\frac{\text{Weight of gonad}}{\text{Total weight of mussel}}\times 100$$

10.2.5: Histological process

The histological processes involved several steps as described below. A small part of the gonad from the mesosoma and the mantle lobe from each dissected Mussel was sub-sampled by cutting a transverse section midway along the anteroposterior axis. The gonads were fixed in Bouiuns fixative for 24 hours and then post-fixed in 70% alcohol prior to dehydration after washing in 50% alcohol for 2-3 hours. All procedures were followed manually following the procedure described in the table 3.

Table 3: Protocol of histological procedure of Green mussel gonad

STEPS	**CHEMICALS**	**TIME**
A. Fixation	Bouins Fixative	24 hours
B. Dehydration	I. ethanol 50%	2-3 hours
	II. Ethanol 70%	2-3 hours
	III. Ethanol 80%	2-3 hours
	IV. Ethanol 90%	2-3 hours
	V. Ethanol 95%	2-3 hours
	VI. Ethanol 100%	2-3 hours
	VII. Ethanol 100%	2-3 hours
C. Clearing	1. Alcohol (50%) +Xylene (50%)	2hours or overnight
	2 Xylene	2 hours
	3 Xylene	2 hours or overnight
D. Infiltration	a) Parafin + xylene b) Paraffin c) Paraffin	2 hours or overnight 2 to 4 hours 2 to 4 hours

E. Embedding

The gonadal tissues were then embedded in paraffin blocks. After embedding the tissues, the paraffin blocks were trimmed to facilitate accurate sectioning.

F. Trimming:

Trimming is a process in which the undesirable wax layers of the embedded blocks are trimmed by knife to obtain suitable blocks. It helps for easy sectioning. After proper embedding, trimming was done to trim undesirable wax layers.

G. Sectioning

The blocks were then sectioned at 5 to 7 µm thickness using microtome. The sections were then mounted on slides and dried overnight in an incubator at around 40̊ C. Before staining, the sections were dewaxed through immersing them into different bathes of xylene, alcohol, and water.

H. Staining and mounting

Staining was performed using Hematoxylin (Harris Hematoxylin) and Eosin through standard methods (Bancroft and Stevens, 1996). Upon reaching the desired staining levels, the slides were mounted permanently with D. P. X. mounting media.

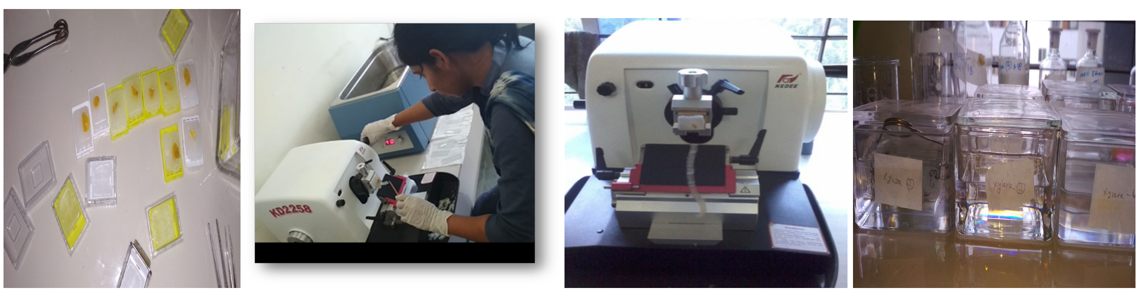


Fig. 14: Tissue Embedding, Tissue sectioning, Sectioned tissue in microtome and Staining

Table 4. The staining schedule

Sl. No.	Solutions	Time	Process
1	Xylene	10 minutes	Clearing
2	Xylene	10 minutes	
3	Xylene	10 minutes	
4	100% alcohol	5 minutes	Rehydration
5	100% alcohol	5 minutes	
6	90% alcohol	3 minutes	
7	80% alcohol	3 minutes	
8	70% alcohol	3 minutes	
9	50% ethyl alcohol	2 minutes	Staining
10	Distilled water	15 dips	
11	Haematoxylene	3 minutes	
12	Wash in tap water	15 minutes	
13	50% ethyl alcohol	10-15 dips	

Sl. No.	Solutions	Time	Process
14	95% ethyl alcohol	30 seconds	
15	Eosin Y	1 minute	
16	95% ethyl alcohol	2 minutes	Dehydration
17	100% ethyl alcohol	1 minute	
18	100% ethyl alcohol	3 minutes	
19	100% ethyl alcohol	1 minute	
20	Xylene	20 minutes	Clearing
21	Xylene	20 minutes	
22	Drying at room temperature	Overnight	Drying

I. Microscopic observation:

The mounted slides were observed under a microscope, which was connected to computer with Digital camera. By the help of this mechanism several photographs were taken at different magnifications.

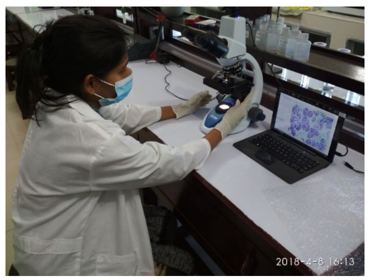


Fig. 15: Microscopic observation of gonad

10.2.6: Gonadal index and stages of development

The maturity stage of each sample was determined by screening the histological slides following Grizel (2003) and the gonad index (GI) was calculated following Buchanan (2001).

$$GI = \frac{\text{Number in each stage} \times \text{Numerical ranking of that stage}}{\text{Number of animals in the samples}}$$

10.3 Study on the feeding biology of Green mussel in coastal areas of Bangladesh

10.3.1: ***Sampling:***

Prior to experimentation, sampling was driven from October 2017 to September 2018 and was conducted from the Maheshkhali channel once in a month. The samples were always collected at the noon and every time it almost took 1-2 hours. The samples were taken randomly for different sizes and weight. Mainly the samples were gathered from the infra of Maheshkhali bridge. Each month 20 individuals of Green mussel were collected. The Green mussels were brought from the bottom of the collected area. The collectors were plunged to the water with oxygen tube and then collected the Mussels from the bottom for about 5-10 minutes. Immediately after collection, 20% buffered formalin was injected to each Green mussel and then these were kept to that solution. During that period, the Green mussels were brought back to the laboratory and acclimated for one week. Plankton, both phytoplankton and zooplankton, were collected simultaneously by plankton net (45µm) by towing the net at the vicinity of Mussel sample collection by pouring 50 liters of water. The water sample was preserved by 5% formaldehyde and also brought back to the laboratory for counting the plankton concentration (both phytoplankton and zooplankton).



Fig. 16: Green mussel sampling

10.3.2: ***Sample Preparation:***

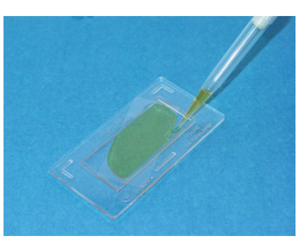
After that, the Green mussels were again kept in 70% alcohol for about a week (7 days). After 7 days, these were again kept in distilled water for another 4 days. Then each individual was weighed and measured. For observation of stomach content each individual was dissected and the black color stomach were appeared. After that, the stomach content was diluted to 50 ml water.



Fig. 17: Sample preparation

10.3.3: ***Analysis of stomach content:***

After the preparation of samples each individual of *P. viridis* were weighed and measured. Prior to dissection, Mussels were opened by cutting the adductor muscle using surgical scalpel. Diet content was removed by using a glass Pasteur pipette through a small slit beneath crystalline style. After that, the whole gut content was diluted to 50 ml water. The total volume of a food category taken by the Mussel population is usually given as a percentage of the total volume of all stomach contents (Hunt and Jones, 1972; Ikusemiju and Olaniyan, 1977; Pedley and Jones, 1978). Some authors have opted to use only stomachs of a particular fullness in volumetric determinations (Le Drew and Green, 1975; Sauvonsaari, 1971). Mean stomach volumes have been used to indicate seasonal changes in feeding activity (Voigtlander and Wissing, 1974). Then 1ml of diluted water was taken into a sedgewich rafter cell (Stirling, 1985; Tan and Ransangan, 2015) and 10 cells were counted. The diluted gut content was observed under microscope (Optica B-190TB digital LCD microscope) with 10x magnification.



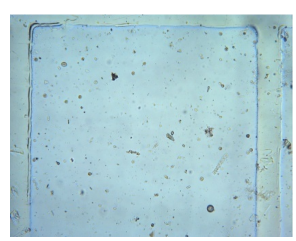


Fig. 18: Observation of stomach content

10.3.4 Analysis of water sample and plankton number of gut content

The plankton in 10 randomly selected cells were identified up to family level and counted under a binocular microscope with imaging facilities.
Plankton Abundance was calculated by using this formula:
N= (P*C*100)/L
Where,

- ✓ N= Number of plankton cells or units per liter of original water (Counted by using Sedgewick-Rafter cell)
- ✓ P= The number of plankton counted in 10 fields
- ✓ C= The volume of final concentrate of the sample (mL)
- ✓ L= The volume (L) of the water sample

10.3.5 Determination of Feeding Selectivity

The feeding selectivity of Mussel was measured by using Ivlev's selectivity indices (E') (Ivlev, 1961).

E' = (ag-aw) / (ag+aw),

Where,
E' = Ivlev’s selectivity indices,
ag= relative abundance of food item in the gut content and
aw= relative abundance of the same food item in the water column.
The value of Ivlev’s selectivity indices ranges from -1 to +1. A negative value indicates as a rejected food item, zero value indicates as randomized food item, and positive value is active selection on that food item on total count.

10.4 Determination of spat settlement densities on rope culture system

Mussel spat settlement densities were studied by monthly setting on the ropes of on a bamboo made Mussel horizontal fixed frame in three locations of Maheskhali channel. The hanging ropes and rope made nets were used as spat collectors. The spat collectors were hanged at three different areas at same depth. The average lengths of the ropes were around 120 centimeters. All the ropes were hanged vertically, in the same way the culture ropes were hanged at the horizontal fixed frame. After around one month of submersion, the ropes were retrieved and new ropes were diploid (Rajagopal *et al.*, 1998a; Alfaro and Jeffs, 2003). The ropes were properly labeled and brought back to the laboratory where they were kept in freezer until ready to be analyzed.

The removal of the spats was started by cutting the top 50 cm of each rope (Alfaro and Jeffs, 2002). The 50 cm was further divided into two portions, top 25 cm and bottom 25 cm. All Mussels were removed from the ropes by vigorous agitation in water, and the removal of remaining Mussels was achieved with forceps (Buchanan and Babcook, 1997). This spat-removing process is considerably easier when samples were previously frozen, compared to fresh samples (Alfaro and Jeffs, 2003). The Mussels were then sorted into 2 size classes (< 0.99 mm and > 1.0 mm) by sieving the Mussels through different-sized sieves (Figure 5.1) (Alfaro and Jeffs, 2002).

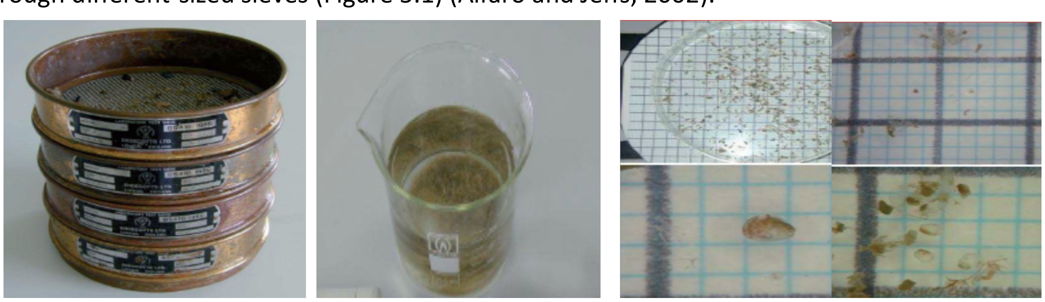


Fig. 19: Larvae of Green mussel collected from the Maheskhali channel of Cox’s Bazar

The first size class (<0.99 mm) were counted under Optika imaging microscope. Samples from this size class were first poured into a measuring cylinder and adjusted to 100 ml by adding water. Then it was transferred to a 250 ml glass beaker, where three replicates of sub-sample of 10 ml from a water suspension of Mussels were taken with an adjustable pipette. The 10 ml sub-sample was taken after it was thoroughly stirred to ensure the homogeneousness of the Mussel suspension. When the sample was dense, the water suspension volume was increased to 200 ml or more to avoid the clogging of the Mussels on the pipette tube. The 10 ml then was poured in petri dish with graded bottom to facilitate accurate counting and give a size indication of the spat. The other size classes (> 1.0 mm) were counted under laboratory magnifying glass on a white try. Analysis of variance tests were used to analyze the mean spat densities during the periods of study for all the four size categories.

10.5 Establishment of pilot scale culture system for investigating spat settlement and growth performances of Green mussel in coastal areas of Bangladesh

10.5.1 *Culture sites and systems*

Three (3) sub-stations were chosen to establish the culture system. The locations of the corresponding stations are-

a. South Khurushkul
b. North Khurushkul
c. Chaufaldandi

The locations of the sub-stations were chosen by hypothesizing the abundance of the Mussels in the bottom; as far known from the local collectors. These areas are perfect for culture system settlement as the bottom structure is quite muddy and the boring of the poles in the bottom is secured in here.

Total four (4) kinds of Green mussel culture systems had been established in these sub-stations.

These are

i. Bamboo pole culture
ii. Rope culture
iii. Cage culture
iv. Net-rope culture

Fig.20: Maheshkhali Channel

Fig.21: Discussion with local communities

Fig.22: Culture area demarcation

Fig.23: Preparation of cage made of ropes and bamboo

Fig.24: Cutting of ropes for rope culture

Fig.25: Attachment of rope in bamboo

Fig.26: Preparation for culture system establishment

Fig. 27: Established culture system of Green mussel in Maheskhali channel

Fig 28: Four different culture techniques of Green mussel

10.5.2 *Sampling techniques for spat settlement and growth measurement*

Three culture stations with four types of substrate (rope, cage, cage made of net and pole) in each station have already been established in three different locations (e.g. South Khuruskul, North Khuruskul and Caufoldandi) in Moheskhali channel. After the installment of the substrates we had to wait for two months till March from the end of January for debris settlement in the substrate. Debris settlement is essential for the spat settlement. The observation of the distribution of Green mussel spat and juvenile was started at the end of March.

- An engine boat along with two divers were hired by the research team for sampling at different locations.
- After reaching the culture site the divers dived into the channel and cut the rope from the anchor tied to the substrate under the water
- After cutting the rope diver brought each substrate onto the deck of boat
- Then we have counted the number of spat attached with substrate
- We have collected 15 samples from each substrate and preserved them into zipper bag and brought to the laboratory for growth observation

The above collection protocol had been followed in low tide because it is easy to bring the substrate from the bottom during low tide.

Figure 1: Going to culture station

Figure 2: Diver ready for diving

Figure 3: Diver pulled out substrate from the bottom

Figure 4: Counting the spat number

Figure 5: collected sample kept in zip bag

Figure 6: Growth study

Fig. 29: Observation of Green mussel spat settlement and growth

10.6. Seasonal variation in nutrients composition of Green mussel

10.6.1: ***Sample collection:***

Green mussels (*Perna viridis*) samples were collected in each month from Maheshkhali channel, Chawfaldandi, Cox's Bazar by diving into the water of the channel by local people. The collected monthly samples were used to analyse seasonal variation of nutrient composition in terms of proximate composition, amino acid profile and fatty acid profiles.

10.6.2: Sample Preparation:

Once Green mussel is collected from Maheshkhali channel, it is brought to the lab. In the lab shell length, shell weight and muscle weight were measured. After these initial task, muscle of Mussel was blended and dried for analysing moisture content in wet basis. After drying sample were used for further analysis such as protein, lipid, and ash on dry weight basis. Some samples were sent to Fish Inspection and Quality Control (FIQC) and Bangladesh Council of Scientific and Industrial Research (BCSIR) for analysing essential fatty acid profile and essential amino acid profile respectively.

Fig. 30: Sample preparation

10.6.3 Proximate composition analysis:

a. Moisture content determination:

Moisture content influences the taste, texture, weight, appearance, and shelf life of foodstuffs. Even a slight deviation from a defined standard can adversely impact the physical properties of a food material. The rate of microbial growth increases with total water content, possibly resulting in spoiled batches that need to be disposed off. However, water is also an inexpensive ingredient adding to the weight of the final product. Hence, obtaining an optimal analytical value for moisture is of great economic importance to a food manufacturer.

Materials:

- Weighing machine
- Foil paper
- Marker
- Blender
- Spatula

Methodology:

- First, fresh sample was blended and then weighed.
- Then, blended sample was taken into foil paper and weighed.
- Weighed sample was kept in hot air oven for overnight in 105°C.
- After drying in oven, sample was weighed again.

Calculation:

Moisture content determination: $\frac{Sample\ weight\ before\ drying - Sample\ weight\ after\ drying}{Sample\ weight\ before\ drying} \times 100$

b. Protein analysis:

Proteins are polymers of amino acids. Twenty different types of amino acids occur naturally in proteins. Proteins differ from each other according to the type, number and sequence of amino acids that make up the polypeptide backbone. Proteins are important constituents of foods for a number of different reasons. They are a major source of *energy*, as well as containing essential amino-acids, such as lysine, tryptophan, methionine, leucine, isoleucine and valine, which are essential to human health, but which the body cannot synthesize.

Protein was analyzed by the Kjeldahl method, where sample was digested with a strong acid so that it releases nitrogen which can be determined by a suitable titration technique. The amount of protein present is then calculated from the nitrogen concentration of the food. This method consists of three steps, digestion, distillation, and titration.

Materials:

- Weighing machine
- Spatula
- Pipette
- Pipette filler
- Wash bottle
- Beaker
- Burette etc.

Chemicals:

- Sulphuric acid (H_2SO_4)
- Digestion mixture (Potassium sulphate: Copper sulphate = 9:1)
- Sodium hydroxide (NaOH)
- 0.2N Hydrochloric acid (HCL)
- Distil water
- Mixed indicator (Methyl red and Bomocrecol green in Boric acid)

Methodology:

Digestion:

- 0.3g sample of dried Green mussel muscle was measured by weighing machine and then it was taken to digestion tube.
- Then about 4g of digestion mixture is added into the tube with the sample.
- After that, 5ml of sulphuric acid was added in the tube and set into the digestion unit for 1.5h in 550^{o}C.

Distillation:

- Digested sample were left solo for cooling.
- Each tube was then used for distillation where before installing the tube into the distillation unit 25ml of distilled water was added.
- 25ml of sodium hydroxide was needed for each sample during distillation which was supplied by a pipe into the unit from beaker.
- A beaker was set with 10ml of mix indicator in the unit which turn greenish colour after completion of distillation.
- Distillation was done for 3-5 minutes for each sample.

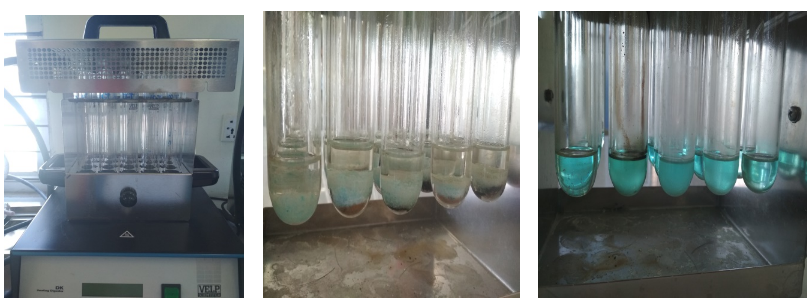


Fig. 31: Digestion and distillation

Titration:

- After distillation, samples were set in the titration unit and titrated against 0.2N HCL.
- Titration was done until colour changes into slight pinkish from greenish colour of sample

Calculation:

Crude protein content = $\frac{Normality\ of\ HCL \times Molecular\ weight\ of\ Nitrogen \times Acid\ used\ in\ titration}{Sample\ weight} \times 100 \times 6.25(conversion\ factor)$

c. Lipid analysis:

Lipids are usually defined as those components that are soluble in organic solvents (such as ether, hexane or chloroform), but are insoluble in water. This group of substances includes triacylglycercols, diacylglycercols, monoacylglycercols, free fatty acids, phospholipids, sterols, caretonoids and vitamins A and D. The lipid fraction of a fatty food therefore contains a complex mixture of different types of molecule. Even so, triacylglycercols are the major component of most foods, typically making up more than 95 to 99% of the total lipids present.

Materials:

- Measuring cylinder
- Weighing machine
- Thimble paper
- Spatula

Chemicals: Di-ethyl ether

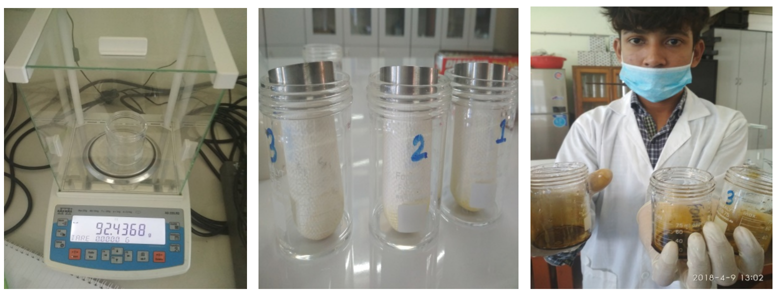


Fig. 32: Lipid extraction

Methodology:

- First about 2g of dried sample was measured and taken into thimble paper.
- Then beaker was weighed and filled with about 70ml of di ethyl ether.
- Lipid extraction consists of three steps- decocting, rinsing, and evaporation.

Decocting:

- Thimble paper was hanged by magnet in the machine with the sample.
- It is then lifted down by a screw so that it remains hanged in the beaker into the ether solvent

Rinsing:

- The next step was rinsing where extra lipid of the sample was extracted.
- Thimble paper was lifted up from the beaker so that solvent can rinse away the extra lipid from the thimble into the beaker.
- This step was also run in $100^{o}C$ for 20mins.

Evaporation:

- It took 10-30mins until all the solvent is evaporated.
- After evaporation beaker contains the extracted lipid in it.
- It was then left a while into desiccator for cooling and then weight was measured.

Calculation:

Crude Lipid content (%) = $\frac{Weight\ of\ the\ beaker\ after\ solvent\ extraction - Weight\ of\ empty\ beaker}{Sample\ weight} \times 100$

d. Ash content determination:

Ash is the inorganic residue remaining after the water and organic matter had been removed by heating in the presence of oxidizing agents, which provided a measure of the total amount of minerals within a food. The three main types of analytical procedure were used to determine the ash content of foods based on this principle: *dry* ashing, *wet* ashing and *low temperature plasma dry* ashing.

Materials:

- Crucible
- Weighing machine

Methodology:

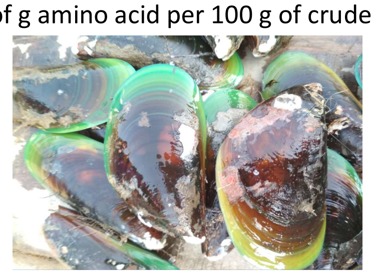
- First, dry sample was weighed through weighing machine.
- 2-4g sample was taken and three replications are taken.
- Then those samples were installed in muffle furnace.
- Samples were burned for five hours in 550^{o}C.
- Then, sample were taken out from muffle furnace and kept in desiccator.
- Finally, weight was measured by weighing machine.

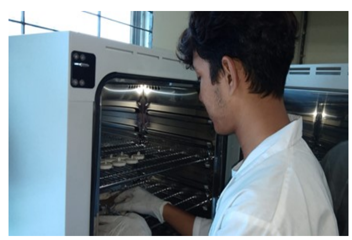
Calculation:

$$\text{Ash content} = \frac{\textit{Sample weight after burning}}{\textit{Sample weight before burning}} \times 100$$

10.6.4 Amino acid profile

Total amino acid composition was determined following the method of Ishida *et al.* (1981) using a Shimadzu chromatograph LC-10AT vp high performance liquid chromatography (HPLC) equipped with an ion exchange column, quaternary pump, a 20 ll injection valve and a fluorescence detector at BCSIR laboratory. Mobile phase A contained sodium citrate and ethanol (pH 3.5) and B had sodium citrate and NaOH (pH 9.8). The flow rate was kept constant at 0.4 ml/min, and the column temperature was set at 60° C. The fluorescence excitation and emission wavelengths were 340 and 450 nm, respectively. Samples were hydrolysed in 6 N HCl in evacuated sealed tubes at 110 C for 24 h. After derivatisation by O-phthalaldehyde, amino acids were identified and quantified by comparison of their retention times with those of standards (Sigma). The results were expressed in terms of g amino acid per 100 g of crude protein.





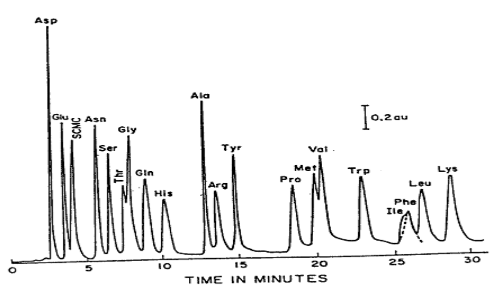


Figure 33: Sample preparation and HPLC ana

10.6.5 Fatty acids analysis

Green mussels (*Perna viridis*) samples were collected in each month from Maheshkhali channel, Chawfaldandi, Cox's Bazar by diving into the water of the channel by local people. After these initial task, muscle of Mussel is blended and dried for analysing moisture content in wet basis The dried samples of Green mussel from different seasons were analyzed for fatty acid (FA) composition. Quadruplicate samples (200-300 mg) were taken and the one-step method of FA analysis was carried out by combining the extraction and esterification processes using a single tube following the method described by Abdulkadir and Tsuchiya (2008). The fatty acid methyl esters (FAMEs) were separated and quantified by gas chromatography equipped with mass spectrometer (GCMS-QP2010 Ultra). Qualitatively (as a percentage), composition in terms of individual FAs was calculated by comparing the peak area of each FA with the total peak area of all FAs in the sample.

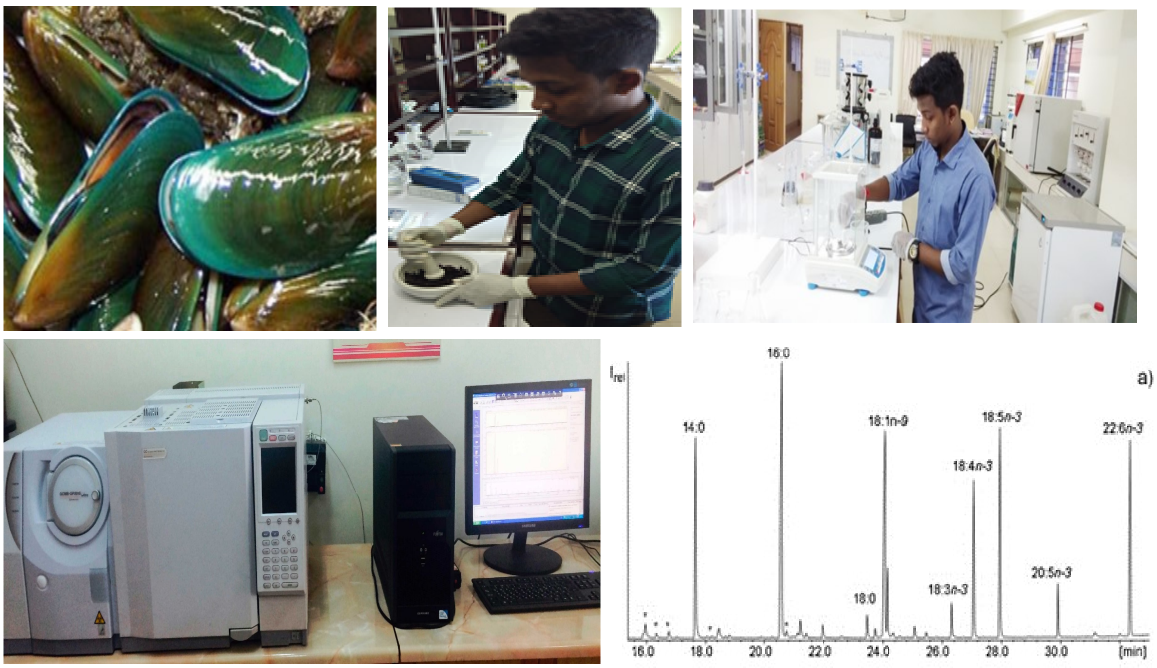


Figure 34: Sample preparation and GC-MS analysis for amino acid profiling of Green mussel

10.7. Data Analysis:

The environmental parameter data were calculated for each station's once-a-month average value and results were displayed graphically by using Microsoft excel program 2013. All the parametric tests were statistically analyzed by using the IBM-SPSS Windows Statistical Package (version 23); comparing the data by using descriptive one-way ANOVA, followed by Duncan's multiple comparison test for homogenous subsets with SPSS version 22.0 software and displayed as table and graphical form. Tests were judged to be significant at $p < 0.05$ level. Normality and homogeneity of variances of all the variables were tested prior to analysis using One-way ANOVA. All the data of variation was expressed into descriptive graphs along with the stations means and with the individual standard error showing the effectiveness of the statistical analysis.

11 Results and discussion:

11.1 Site capability rating system for fast and effective evaluation of potential farming sites in coastal areas of Bangladesh

11.1.1 Site suitability of Rezu Khal, Cox's Bazar

11.1.1.1 Water quality parameters

The temperature readings during the year round vary from 23-34ºC. No major fluctuation in temperature was observed throughout the study time. No significant difference ($p > 0.05$) was observed in temperature among different Stations. Temperature in June and July months was significantly higher ($p < 0.05$) than that in other months. The value of transparency fluctuated from 25.4-116 cm. The transparency of Station 5 was significantly higher ($p < 0.05$) than that in other Stations. Transparency in November and January months was significantly higher ($p < 0.05$) than that in other months. The value of turbidity fluctuated from 19.6-25NTU. No major fluctuation in turbidity was observed throughout the study time. The value of pH fluctuated from 7.3-8.3. No major fluctuation in pH was observed throughout the study time. But pH of Station 4 was significantly higher ($p < 0.05$) and Station 1 was significantly lower ($p < 0.05$) than that in other Stations. The dissolve oxygen readings during the year round vary from 6-8.1mg/l. No major fluctuation in DO was observed throughout the study time. But DO of Station 4 was significantly higher ($p < 0.05$) than that in other Stations and Station 1 was significantly lower ($p < 0.05$) than that in other Stations. The value of salinity highly fluctuated from 2-39.5ppt. But this fluctuation was observed in the monthly variation. In September (2017) salinity decreased significantly ($p < 0.05$). The depth readings during the year round vary from 1.9-4.6m. The depth of Station 5 was high because it was located at the downstream of the Rezu Khal. It was significantly higher ($p < 0.05$) than that in other Stations. Station 4 and 3 was significantly higher ($p < 0.05$) than Station 1 and 2. The value of alkalinity fluctuated from 22-313 ppm. Wide range of fluctuation was observed throughout the study time. But No significant difference ($p > 0.05$) was observed in alkalinity among the different Stations.

The value of nitrate fluctuated from 0.02-0.2 ppm. No major fluctuation was observed throughout the study time. The value of nitrite fluctuated from 0.002-0.2 ppm. No major fluctuation was observed throughout the study time. Also, no significant difference ($p > 0.05$) was observed in nitrite among the different Stations. During the study period, phosphate concentrations fluctuated widely from 0.02-3ppm. Highly significant difference ($p < 0.05$) was also observed in phosphate. The phosphate of Station 1 was significantly higher ($p < 0.05$) than that in other Stations. Highly significant difference ($p < 0.05$) of phosphate was also observed in the monthly variation. However, phosphate in April month was significantly higher ($p < 0.05$) than that in other months. The value of ammonia fluctuated from .045-0.5ppm. The ammonia of Station 1 and 2 was significantly higher ($p < 0.05$) than that in Station 3, 4 and 5. The Stations 3, 4 and 5 were significantly lower ($p < 0.05$) than that Station 1. The value of ammonium fluctuated from 0.04-2.8ppm. No significant difference ($p > 0.05$) was observed in ammonium among the different Stations. But highly significant difference ($p < 0.05$) of ammonium was observed in the monthly variation. The value of chlorophyll a fluctuated from 0.8-17.1. Wide range of fluctuation was observed throughout the study time. Highly significant difference ($p < 0.05$) was also observed in chlorophyll a. Chlorophyll a of Station 1 was significantly higher ($p < 0.05$) than that in other Stations. Station 5 had no significant difference from other Station. The Stations 2, 3 and 4 were significantly lower ($p < 0.05$) than that Station 1. Highly significant difference ($p < 0.05$) of chlorophyll a was also observed in the monthly variation.

Table 5. Water quality parameters (min-max) of five stations in Rezu Khal recorded from April 2017 to March 2018 (Station wise variation)

Parameters	Station 1	Station 2	Station 3	Station 4	Station 5	Sig.
Depth (m)	2.6±2.25^{c} (1.8-3.9)	2.62±2.29^{c} (1.9-4.2)	3.15±2.7^{b} (2.1-4.5)	3.2±2.9^{b} (2.1-4.8)	3.65±2.5^{a} (1.9-4.6)	***
Temperature (˚C)	29.06±2.8^{a} (24-33)	29.32±3.1^{a} (23-33)	29.31±3.03^{a} (23-33)	29.71±2.92^{a} (24-34)	29.42±3.0^{a} (23-33.5)	NS
Transparency (cm)	50.8±15.9^{c} (25.4-68.7)	55.5±17^{bc} (30.5-52)	58.3±20.6^{abc} (65.3-34.5)	61.9±18.9^{ab} (37-92)	67.34±28.4^{a} (32-116)	**
Turbidity (NTU)	21.9±24.7^{a} (4.16-90.3)	21.7±24.4^{a} (3.9-88.7)	19.6±20.6^{a} (2.6-74.7)	25±29.2^{a} (5.75-92.3)	19.6±23.1^{a} (4.8-75.5)	NS
pH	7.5±.28^{b} (7.3-8.25)	7.6±.31^{ab} (7.3-8.34)	7.7±.29^{ab} (7.3-8.3)	7.7±.34^{a} (7.3-8.37)	7.7±.31^{ab} (7.3-8.3)	NS
DO(mg/l)	6.8±.49^{b} (5.8-7.72)	6.86±.37^{ab} (6.3-7.7)	6.9±.41^{ab} 6.2-7.8)	7.1±.45^{a} 6.33-8.1)	6.9±.34^{ab} (6.3-7.7)	NS
Salinity(ppt)	23.8±12.3^{a} (2-35.5)	24.8±12.21^{a} (3-37.5)	26.8±9.72^{a} (6.9-38)	27.4±10.04^{a} (5.9-38)	28.6±9.24^{a} (9-39.5)	NS
Alkalinity (mg/l)	82.5±62.3^{a} (37.5-281)	86.8±63.2^{a} (41-285)	86.7±52.6^{a} (25.5-247)	92.7±72.6^{a} (21.7-321)	99.8±68.5^{a} (41-315)	NS
Nitrite(ppm)	.093±.092^{a} (.002-.2)	.093±.092^{a} (.002-.2)	.093±.092^{a} (.002-.2)	.093±.092^{a} (.002-.2)	.093±.092^{a} (.002-.2)	NS
Nitrate(ppm)	.142±.083^{a} (.02-.2)	.142±.083^{a} (.02-.2)	.142±.083^{a} (.02-.2)	.142±.083^{a} (.02-.2)	.142±.083^{a} (.02-.2)	NS
Phosphate (ppm)	.4±.81^{a} (.02-3)	.17±.22^{b} (.02-.8)	.18±.23^{b} (.02-.67)	.22±.38^{ab} (.02-1.23)	.13±.15^{b} (.01-.52)	*
Ammonia (ppm)	.24±.173^{a} (.07-.75)	.23±.173^{a} (.04-.61)	.17±.059^{b} (.1-.27)	.16±.055^{b} (.09-.27)	.14±.071^{b} (.04-.28)	***
Ammonium (ppm)	.75±1.10^{a} (.068-2.85)	.67±.96^{a} (.04-2.75)	.62±.86^{a} (.03-2.5)	.63±.94^{a} (.02-2.55)	.62±.90^{a} (.015-2.45)	NS
Chlorophyll a	5.41±8.33^{a} (2.04-32.1)	4.8±3.16^{b} (1.02-14.57)	3.96±3.25^{b} (.82-14.57)	4.61±4.09^{b} (1.1-17.1)	5.26±4.13^{ab} (.84-15.8)	*

Here, "* " indicates the level of significance,

* → <0.05; ** → <0.01; *** → <0.001; NS → Not Significant

Table 6. Water quality parameters (min-max) in Rezu Khal recorded from April 2017 to March 2018 (Month wise variation)

Parameter	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Sig.
Depth (m)	8.32±1.5	11.1±2.32	9.12±2.83	10.6±2.81	11±1.96	14.2±1.01	13.1±.92	11.4±1.5	7.5±1.4	7.47±1.4	7.2±.96	8.4±1.6	***
Temperature	30±.35	31.8±.8	33.2±.41	33.2±.4	28.2±.4	30±0.0	31.7±.7	29±.92	26.2±.4	23.5±.5	25.8±.5	29.7±.5	***
Transparency	58.6±4.5	38.9±6.71	41±1.88	46.4±1.45	36.4±3.64	35.4±8.65	74.8±8.88	89.2±15.5	52.9±6.4	89.5±15.8	77.7±12.5	64.1±4.36	***
Turbidity	6.9±1.39	8.3±.54	55.7±7.96	29.3±16.8	84.2±7.85	20.3±10.5	4.4±1.2	6.9±1.4	16±5.5	7.6±2.5	9.2±3.69	10.1±2.76	***
pH	7.3±0.0	7.7±0.7	7.76±.07	7.6±0.6	7.3±0.0	7.5±0.5	7.4±0.6	7.6±1.9	7.88±.3	8.3±0.4	7.9±1.8	8±.04	***
DO(mg/l)	6.9±.52	6.4±.2	7.4±.2	6.8±.3	7±.19	7.1±.1	6.3±.1	6.8±.1	7±.19	7±.16	6.9±.1	7.3±.5	***
Salinity (ppt)	30.5±1.1	31.7±1.5	25.4±1.6	31.8±.79	6.2±1.77	13.7±9.5	21±2.07	10.9±2.25	34.6±.5	35.8±.45	37.4±1.4	36.2±.88	***
Alkalinity	90.2±1.8	72.6±6.6	86±7.5	50.8±4.6	34.4±9.1	79.4±16.2	68.6±1.1	66.4±15.1	78.8±7.1	97.2±15.7	288.8 ±27.5	263.9±8.4	***
Nitrite(ppm)	.002±0.	.02±0.	.02±0.	.02±0.	.002±0	.2±0.0	.2±0.0	.2±0.0	.2±0.0	.044±0	.2±0.0	.005±.	***
Nitrate(ppm	.02±0.0	.02±0.	.02±0.	.2±0.0	.2±0.0	.2±0.0	.2±0.0	.2±0.0	.2±0.0	.044±0	.2±0.0	.2±0.0	***
Phosphate	1.13±0	.06±.2	.097±.	.5±.16	.03±.0	.03±.0	.02±.0	.02±.0	.35±.0	.1±.02	.14±.0	.21±.1	***
Ammonia	.14±.02	.3±.26	.21±.1	.2±.16	.27±.0	.16±.0	.12±.0	.14±.0	.25±.0	.24±.0	.11±.0	.12±.1	***
Ammonium	.06±.03	2.1±.1	.04±.0	.04±.0	.27±.0	.14±.0	.1±.06	.1±.05	.2±.01	.21±.0	2.2±.1	2.6±.9	***
Chlorophyll	3.7±1.9	5.75±1	7.25±	1.8±1.	2.2±1.	3.3±.7	3.31±1	13.1±4	3.11±.	5.5±1.	3±.24	4.14±.	***

Here, "* " indicates the level of significance,

* → <0.05; ** → <0.01; *** → <0.001; NS → Not Significant

11.1.1.2 Qualitative Abundance of Plankton

In Rezu Khal both phytoplankton and zooplankton were observed. A total of 59 phytoplankton genera, representatives of five classes were identified. In Rezu Khal 16 genera of zooplankton were identified.

The contribution of phytoplankton was 87.3% of the total count of plankton. The observed five class of phytoplankton was Bacillariophyceae, Chlorophyceae, Cyanophyceae, Dinophyceae and Pyrrophyceae. The phytoplankton community in Rezu Khal was dominated by the class Bacillariophyceae (83% of the total count) consisting of 39 genera. *Skeletonema* and *Coscinodiscus* were the most dominant genus of Bacillariophyceae. The contribution of *Coscinodiscus* was 7.8%, *Skeletonema*8.2% and *Thalassiosira* 5.3% of the total count. No significant difference ($p > 0.05$) was observed in Bacillariophyceae among the different Stations. But highly significant difference ($p < 0.05$) was observed in Bacillariophyceae in the monthly variation. However, Bacillariophyceae in January month was significantly higher ($p < 0.05$) than that in other months.

The contribution of class Chlorophyceae was 1.2% of the total count consisting of 3 genera (*Spirogyra, Ulothrix* and *Oscillatoria*). No significant difference ($p > 0.05$) was observed in Chlorophyceae among the different Stations. But highly significant difference ($p > 0.05$) was observed in Chlorophyceae in the monthly variation. However, Chlorophyceae in the month of May was significantly higher ($p < 0.05$) than that in other months. The contribution of class Cyanophyceae was 1.63% of the total count consisting of 4 genera (*Cyanobacreria, Anabaena, Lossiusirap*and *Microcystis*). No significant difference ($p > 0.05$) was observed in Cyanophyceae throughout the Station. But highly significant difference ($p < 0.05$) was observed in Cyanophyceae in the monthly variation. However, Cyanophyceae in August month was significantly higher ($p < 0.05$) than that in other months. December to March no plankton were found. The contribution of class Dinophyceae was 2.4% of the total count consisting of 8 genera. No significant difference ($p > 0.05$) was observed in Dinophyceae among the different Stations. But highly significant difference ($p > 0.05$) was observed in Dinophyceae in the monthly variation. However, Dinophyceae in the month of July was significantly higher ($p < 0.05$) than that in other months.

The contribution of class Pyrrophyceae was 11.35% of the total count consisting of 5 genera (*Bacteriastrum, Ceratium, Prorocentrum, Protoperidinium* and *Pyrocystis*). No significant difference ($p > 0.05$) was observed in Pyrrophyceae among the different Stations. But highly significant difference ($p < 0.05$) was observed in Pyrrophyceae in the monthly variation. However, Pyrrophyceae in September month was significantly higher ($p < 0.05$) than that in other months.The contribution of zooplankton was 12.52% of the total count of plankton. The zooplankton community in Rezu Khal was dominated by the species Copepod (26.4% of the total count of zooplankton) fish larvae (24.6% of the total count of zooplankton). It was the main contributors to the bulk of the biomass. The contributions of the copepod at different Stations ranged from 25.6% to 26.9% during the investigation period.

No significant difference ($p > 0.05$) was observed in zooplankton among the different Stations. But highly significant difference ($p < 0.05$) was observed in zooplankton in the monthly variation. However, zooplankton in November month was significantly higher ($p < 0.05$) than that in other months.

Table 7. Phytoplankton composition in Rezu Khal, recorded from April 2017 to March 2018

Phytoplankton	Mean count (×10^3cells/L)	%	S1	S1 (%)	S2	S2 (%)	S3	S3 (%)	S4	S4 (%)	S5	S5 (%)
Actinastrum	0.8222	3.18	833.33	0.51	444.44	1.77	805.56	3.17	1305.56	4.89	1700	6.75
Attheya	0.050	0.19	138.89	3.30	0.0	0.00	55.56	0.22	0.0	0.00	55.56	0.22
Asterionella	0.8778	3.39	888.89	2.57	805.56	3.20	944.44	3.72	611.11	2.29	1138.8	4.53
Asterionellopsis	0.6444	2.49	694.44	0.62	638.89	2.54	777.78	3.06	611.11	2.29	500	1.99
Biddulphia	0.3889	1.50	166.67	0.72	638.89	2.54	277.78	1.09	444.44	1.66	416.67	1.66
Bacillaria	0.0389	0.15	194.44	2.06	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00
Chaetoceros	0.627	2.42	555.56	0.51	694.44	2.76	361.11	1.42	472.22	1.77	1055.6	4.19
Cocconeis	0.2167	0.84	138.89	0.00	305.56	1.21	83.3	0.33	277.78	1.04	277.78	1.10
Corethron	0.0222	0.09	0.0	7.72	27.78	0.11	83.33	0.33	0.0	0.00	0.0	0.00
Coscinodiscus	2.02	7.80	2083.33	0.82	2388.9	9.49	2000	7.87	1638.89	6.14	1972.2	7.84
Cyclotella	0.25	0.97	222.2	0.31	250	0.99	166.6	0.66	222.2	0.83	388.89	1.55
Cylindrotheca	0.094	0.36	83.33	0.72	55.56	0.22	27.78	0.11	277.78	1.04	27.78	0.11
Diatoma	0.072	0.28	194.4	0.21	167	0.66	0.0	0.00	0.0	0.00	0.0	0.00
Detonula	0.011	0.04	56	0.41	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00
Diploneis	.1389	0.54	111.11	1.75	166.67	0.66	277.78	1.09	138.89	0.52	0.0	0.00
Ditylum	0.517	2.00	472.22	6.18	416.67	1.66	388.89	1.53	611.11	2.29	694.44	2.76
Fragilaria	1.217	4.70	1666.67	0.31	722.22	2.87	1333.3	5.25	1416.67	5.31	944.44	3.75
Golenkinia	0.033	0.13	83.33	0.00	27.8	0.11	0.0	0.00	56	0.21	0.0	0.00
Guinordia	0.02778	0.11	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.00	133.89	0.53
Leptocylindricus	0.072	0.28	0.0	7.11	139	0.55	139	0.55	83.3	0.31	0.0	0.00
Licomphora	1.644	6.35	1916.67	1.03	1611.1	6.40	1527.8	6.01	1583.33	5.93	1583.3	6.29
Melosira	0.3111	1.20	277.78	5.87	333.33	1.32	194.44	0.77	472.22	1.77	277.78	1.10
Navicula	1.422	5.49	1583.33	4.22	1305.56	5.19	1361.1	5.36	1555.56	5.83	1305.56	5.19
Nitzschia	1.056	4.08	1138.89	0.00	1027.8	4.08	1055.6	4.15	1111.1	4.16	944.44	3.75
Odontella	0.0556	0.21	0.0	0.00	0.0	0.00	0.0	0.00	83.3	0.31	194	0.77
Paralia	0.0111	0.04	0.0	0.00	0.0	0.00	56	0.22	0.0	0.00	0.0	0.00
Plagioselmis	0.0444	0.17	0.0	4.74	0.0	0.00	83.33	0.33	55.56	0.21	83.33	0.33
Pleurosigma	1.022	3.95	1277.78	2.47	1027.8	4.08	1000	3.93	1055.5	3.95	750	2.98
Polykrokos	0.4222	1.63	666.67	2.27	333.33	1.32	305.56	1.20	416.67	1.56	388.89	1.55
Pseudo-nitzschia	0.461	1.78	611.11	4.94	388.89	1.55	250	0.98	333.33	1.25	722.22	2.87

Table 7 Cont'd...

Phytoplankton	Mean count (×10^3 cells/L)	%	S1	S1 (%)	S2	S2 (%)	S3	S3 (%)	S4	S4 (%)	S5	S5 (%)
Rhizosolenia	1.45	5.60	1333.33	6.80	1583.3	6.29	1361.1	5.36	1694.4	6.35	1277.8	5.08
Skeletonema	2.1222	8.20	1833.33	8.72	2055.6	8.17	2194.4	8.63	2472.2	9.26	2055.6	8.17
Surirella	0.1389	0.54	194.44	0.10	194.44	0.77	55.56	0.22	166.67	0.62	83.33	0.33
Tetradron	0.0611	0.24	27.78	0.10	0.0	0.00	166.67	0.66	111.11	0.42	0.0	0.00
Triceratium	0.333	1.29	27.8	5.05	0.0	0.00	167	0.66	111	0.42	0.0	0.00
Thalassiosira	1.361	5.26	1361.11	0.72	1500	5.96	1527.8	6.01	1111.1	4.16	1305.6	5.19
Thalassionema	0.338	1.31	194.44	2.99	305.56	1.21	361.11	1.42	305.56	1.14	527.78	2.10
Thalassiothrix	0.772	2.98	805.56	3.19	555.56	2.21	972.22	3.83	833.33	3.12	694.44	2.76
Tropidoneis	0.7111	2.75	861.11	3.51	750	2.98	805.56	3.17	583.33	2.19	555.56	2.21
Total Bacillariophyceae	21.5111	83.11	22555.56	83.63	20889	83.00	20916	82.29	22167	83.04	21028	83.55
Spirogyra	0.133	0.51	138.89	1.03	55.56	0.22	111.11	0.44	194.44	0.73	166.67	0.66
Ulothrix	0.1667	0.64	277.78	0.00	111.11	0.44	111.11	0.44	111.11	0.42	222.22	0.88
Oscillatoria	0.033	0.13	0.0	1.24	0.0	0.00	56	0.22	83	0.31	27	0.11
Total Chlorophyceae	0.3167	1.22	333.3	0.00	167	0.66	277	1.09	389	1.46	417	1.66
Cyanobacreria	0.00556	0.02	0.0	0.41	0.0	0.00	27.78	0.11	0.0	0.00	0.0	0.00
Anabaena	0.2889	1.12	111.11	0.00	333.33	1.32	361.11	1.42	250	0.94	388.89	1.55
Lossiusirap	0.0111	0.04	0.0	0.93	0.0	0.00	0.0	0.00	0.0	0.00	56	0.22
Microcystis	0.1222	0.47	250	1.17	83.33	0.33	0.0	0.00	138.89	0.52	138.89	0.55
Total Cyanophyceae	0.4222	1.63	316.11	0.31	388.89	1.55	388.89	1.53	388.89	1.46	583.33	2.32
Amphidinium	0.0667	0.26	83.33	0.00	55.56	0.22	27.78	0.11	27.78	0.10	138.89	0.55
Boreadinium	0.0222	0.09	0.0	0.00	0.0	0.00	55.56	0.22	55.56	0.21	0.0	0.00
Cerataulina	0.022	0.08	0.0	0.00	0.0	0.00	0.0	0.00	83	0.31	27	0.11
Ceratium	0.0333	0.13	0.0	1.65	167	0.66	0.0	0.00	0.0	0.00	0.0	0.00
Dinophysis	0.3944	1.52	444.44	0.41	361.11	1.43	416.67	1.64	361.11	1.35	388.89	1.55
Entomoneis	0.033	0.13	111.11	0.62	55.56	0.22	0.0	0.00	0.0	0.00	0.0	0.00
Gymnodinium	0.0889	0.34	166.67	0.00	27.78	0.11	0.0	0.00	166.67	0.62	83.89	0.33
Oxyphysis	0.022	0.1	0.0	2.99	0.0	0.00	83	0.33	28	0.10	0.0	0.00
Total Dinophyceae	0.6833	2.64	805.56	0.00	666.67	2.65	583.33	2.30	722.22	2.71	683.33	2.72
Bacteriastrum	0.0278	0.11	0.0	0.21	0.0	0.00	83	0.33	0.0	0.00	56	0.22
Ceratium	0.0222	0.09	56	0.10	28	0.11	28	0.11	0.0	0.00	0.0	0.00

Table 7 Cont'd...

Phytoplankton	**Mean count (×10^3 cells/L)**	**%**	**S1**	**S1 (%)**	**S2**	**S2 (%)**	**S3**	**S3 (%)**	**S4**	**S4 (%)**	**S5**	**S5 (%)**
Prorocentrum	0.1111	0.43	27.78	9.47	277.78	1.10	222.22	0.87	27.78	0.10	0.0	0.00
Protoperidinium	2.5055	9.68	2555.56	1.24	2583.3	10.26	2472.2	9.73	2611.1	9.78	2305.6	9.16
Pyrocystis	0.3167	1.22	333.33	10.92	222.22	0.88	444.44	1.75	305.56	1.14	277.78	1.10
Total Pyrrophyceae	2.9388	11.35	2944.44	0.51	3083.3	12.25	3250	12.79	2888.9	10.82	2527.8	10.04
Total Phytoplankton	25.883		26972.22		25166.7		25416.7		26694.44		25166.67	

Table 8. Zooplankton composition in Rezu Khal, recorded from April 2017 to March 2018

Zooplankton	**Mean count (×10^3 cells /L)**	**%**	**S1**	**S1 (%)**	**S2**	**S2 (%)**	**S3**	**S3 (%)**	**S4**	**S4 (%)**	**S5**	**S5 (%)**
Acetes	.01111	0.3	0.00	0	0.00	0	55.56	1.61	0.0	0	0.0	0
Amphipoda	0.11556	3.11	222.2	5.68	222.22	5.93	138.89	4.001	138.89	3.51	55.5	1.61
Balanus	0.00556	0.15	0.0	0	27.78	0.75	0.0	0	0.0	0	0.0	0
Copepoda	0.97778	26.4	1055.5	26.9	972.22	25.9	888.8	25.61	1055.56	26.6	916.67	26.6
Crab zoea	0.200	5.39	55.56	1.42	277.78	7.41	111.11	3.2	222.22	5.6	333.33	9.68
Daphnia	0.050	1.35	166.67	4.26	0.0	0	27.78	0.801	55.56	1.41	0.0	0
Fish egg	0.42222	11.4	555.56	14.2	500	13.3	500	14.41	361.11	9.11	194.44	5.65
fish larvae	0.9111	24.6	805.56	20.57	694.44	18.52	777.78	22.41	1250	31.5	1027.78	29.9
Isopoda	0.15556	4.2	222.22	5.68	194.44	5.19	222.22	6.41	55.56	1.41	83.33	2.42
Lucifer	0.03333	0.9	0.0	0	27.78	0.74	55.56	1.6	55.56	1.41	27.78	0.81
Moina	0.24444	6.59	333.33	8.52	277.78	7.41	166.67	4.81	277.78	6.99	166.67	4.85
Mollusc larvae	0.10556	2.85	55.56	1.42	138.89	3.71	166.67	4.81	55.56	1.41	111.11	3.23
Mysids	0.06667	1.8	27.78	0.71	27.78	0.7408	83.33	2.4	0.0	0	194.	5.65

											44	
Rotifer	0.07778	2.1	27.78	0.71	166.67	4.45	111.11	3.2	27.78	0.71	55.56	1.61
Shrimp larvae	0.21667	5.84	194.44	4.97	194.44	5.19	83.33	2.4	361.11	9.11	250	7.3
Tintinnopsis	0.07222	1.95	166.67	4.26	27.78	0.7408	82.33	2.37	55.56	1.41	27.78	0.81
Total zooplankton	3.71111		3916.67		3750		3472.22		3972.22		3444.44	

Plankton Composition

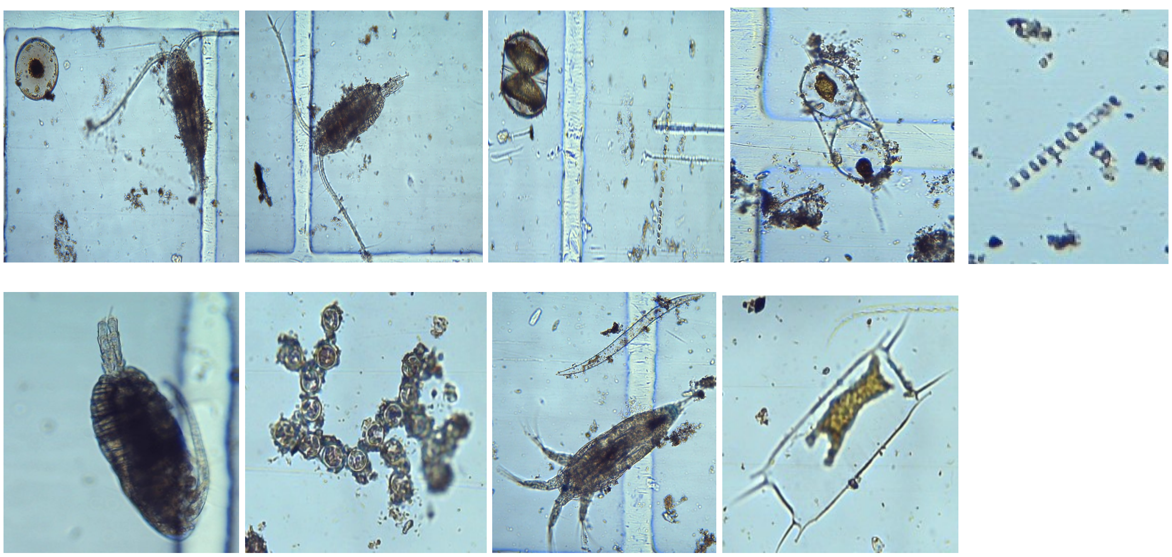


Fig. 35: Some planktons observed under microscope

Table 9. Site suitability rating system for Rezu Khal, Cox's Bazar

Parameter	Station 1		Station 2		Station 3		Station 4		Station 5	
	Rating	**Score**	**Rating**	**Score**	**Rating**	**Score**	**Rating**	**Score**	**Rating**	**Score**
Salinity	2	0.3	3	0.45	4	0.6	5	0.75	5	0.75
DO	9	1.35	9	1.35	9	1.35	9	1.35	9	1.35
pH	4	0.4	4	0.4	4	0.4	4	0.4	4	0.4
Temperature	9	1.35	8	1.2	9	1.35	9	1.35	9	1.35
Chlorophyll-a	10	1.5	10	1.5	10	1.5	10	1.5	10	1.5
Water depth	3	0.45	3	0.45	4	0.6	4	0.6	4	0.6
Weighted Category	6.2		6.2		6.67		6.83		6.83	
	Medium		Medium		Medium		Medium		Medium	

Conclusion

Based on our result, it is concluded that Rezu Khal is not that much good site for Green mussel culture but it is capable and moderately suitable for Green mussel farming. The result of the site evaluation was summarized in table 9. Environmental variables in all Station were categorized as medium, in term of suitability for Green mussel farming. But the Stations 4 and 5 might be considered as cultural site for Green mussel culture. In the environmental variables table (3 and 4), it was clearly shown that the water depth and salinity fluctuation was the major issue that made Rezu Khal unfavorable for Green mussel culture.

11.1.2 Site suitability of Maheskhali Channel, Cox's Bazar

The temperature data ranged during the year varied from 20.7-28.2ºC. During all over the study time there was no major fluctuation observed. The temperature variation had no significant difference ($p > 0.05$) comparing all the research stations in the study area. But monthly variation of temperature was significant ($p < 0.001$) in Maheshkhali Channel. Nevertheless, temperature in January was significantly lower than that of other months during the study. The value of transparency fluctuated from 21-79 cm. No significant difference ($p > 0.05$) was observed in turbidity among the stations overall. But significantly higher ($p < 0.05$) transparency of Station 5 was observed rather than that in other Stations. However, transparency in November and March was significantly higher ($p < 0.05$) than transparency in other months in the mean value range of all the stations.

The value of turbidity oscillated from 7.1-372 NTU. Turbidity in January was significantly higher from October and November and the turbidity got higher in the following months with lowering fluctuation on February-April. The array of pH readings ranged from 6.81-8.3. No major fluctuation in pH was observed throughout the study time. But pH of Station 1 was significantly lower ($p < 0.001$) and Station 5 was significantly higher. pH in November was relatively higher but the readings throughout the year was more or less close to the mean value of all the stations.

The dissolve oxygen (DO) readings during the year round vary from 5.5-7.2 mg/l. No significant difference ($p > 0.05$) was observed among the stations overall. During January the station mean DO was lower than that of the other months in the station mean value. The value of salinity moderately fluctuated from 19.5-36.5 ppt. In October-November, 2017 and September, 2018 mean salinity of the stations decreased significantly ($p < 0.05$). The depth of water in Maheshkhali Channel was measured during the time of high tide. The year-round depth readings vary from 5.09-9.85 m. The depth of Station 5 was high and it was located at the downstream, at the mouth of the Maheshkhali Channel Estuary. Mean water depth of the stations in March was significantly higher than that of other months. The value of alkalinity fluctuated from 65.8-280.4 ppm. Wide range of fluctuation was observed throughout the study time. During February, March and April the alkalinity was higher than the other months of the year. The current speed readings during the year of study vary from 0.17-0.72 m/s. The current velocity of Station 5 which was located at the downstream of the Maheshkhali Channel; at the mouth of the estuary was consecutively the highest one among all the stations. Current speed at Station 1 was significantly higher ($p < 0.05$) than Station 2 and 4. Station 3 had the lowest current speed of all. The value of nitrate fluctuated from 0.1-0.2 ppm throughout the year. No major fluctuation was observed throughout the study time.

Likewise, no significant difference ($p > 0.05$) was observed in nitrate among the different Stations. No significant difference ($p > 0.05$) of nitrate was observed in the monthly variation too. The value of nitrite fluctuated from 0.001-0.002 ppm. No major fluctuation was observed throughout the study time. However, nitrite in April, August and March months was significantly lower than the other months. Phosphate content oscillated extensively from 0.13-2.75 ppm. The phosphate reading of Station 1 and 5 was significantly higher ($p < 0.05$) than that in other Stations. The Stations 2, 3 and 4 were significantly lower ($p < 0.05$) than that of Station 1 and 5. However, phosphate in January, March and May was significantly higher ($p < 0.05$) than that in other months. The value of ammonia fluctuated from 0.11-0.36 ppm. The ammonia of Station 1 and 2 was significantly higher ($p < 0.05$) than that in Station 3, 4 and 5. Drastic fall of the ammonia reading was observed during November and February and significant rise was observed during October and January at the station mean values of ammonia. The value of ammonium fluctuated from 0.01-0.22 ppm. The ammonium values of the stations fluctuated highly from month to month. The value of chlorophyll a fluctuated from 2.02-28.2 µg/L. Wide range of fluctuation was observed throughout the study time. Chlorophyll a of Station 1 was significantly higher ($p < 0.05$) than that in other Stations. Significant difference ($p < 0.001$) of chlorophyll a was also observed in the monthly variation.

Table: 10. Water quality parameters (min-max) of five stations in Maheshkhali Channel (Station wise variation)

Parameters	Station 1	Station 2	Station 3	Station 4	Station 5	Sig.
Depth (m)	6.42±.81 (5.09-7.69)	6.83±.65 (5.64-7.99)	7.12±.76 (5.98-8.3)	7.65±.72 (6.68-8.94)	8.4±.01 (6.56-9.85)	NS
Temperature (˚C)	24.78±1.15 (22.4-26.4)	24.98±1.5 (21.7-27.2)	24.69±1.6 (21.4-27.1)	25.16±1.84 (21.6-27.2)	25.08±2.16 (20.7-28.2)	NS
Transparency (cm)	59.89±7.28 (51-71.14)	62.10±8.51 (50-74)	61.88±6.93 (52-73)	62.25±9.41 (49-76)	63.38±10.46 (48-79)	NS
Turbidity (NTU)	76.18±51.5 (11.1-166)	83.73±55.86 (7.1-183)	90.17±69.09 (8.1-188)	96.51±95.25 (6.8-250.2)	109.66±99.7 (14.4-275.2)	NS
Current Speed (m/s)	.42±.03 (.36-.47)	.4±.10 (.23-.53)	.36±.09 (.17-.46)	.4±.05 (.33-.5)	.46±.07 (.33-.56)	NS
pH	7.19±.28 (6.85-7.77)	7.35±.38 (6.86-8.2)	7.35±.38 (6.85-8.08)	7.55±.42 (6.78-8.12)	7.54±.38 (7.0-8.16)	*
DO(mg/l)	6.60±.38 (5.7-7)	6.23±.38 (5.7-6.9)	6.45±.41 (5.8-7.1)	6.42±.48 (5.7-7.2)	6.515±.52 (5.2-7.1)	NS
Salinity(ppt)	31.75±5.53 (19.5-36.5)	31.25±4.91 (21.5-35.5)	30.88±5.91 (20.5-36.5)	31.13±5.69 (19.5-36.5)	31.25±5.24 (21.5-36.5)	NS
Alkalinity (mg/l)	166.75±72.89 (67.3-280.2)	159.03±75.34 (65.8-280.4)	167.75±81.7 (71.8-270.2)	167.30±87.08 (67.8-280.2)	173.63±77.63 (91.8-278.2)	NS
Nitrite(ppm)	.002±.001 (.001-.002)	.002±.001 (.001-.002)	.002±.001 (.001-.002)	.002±.001 (.001-.002)	.002±.001 (.001-.002)	NS
Nitrate(ppm)	.15±.04 (.1-.2)	.15±.04 (.1-.2)	.15±.04 (.1-.2)	.15±.04 (.1-.2)	.15±.04 (.1-.2)	NS
Ammonia (ppm)	.24±.07 (.11-.34)	.26±.07 (.13-.36)	.24±.06 (.12-.31)	.23±.06 (.13-.33)	.24±.06 (.13-.32)	NS
Ammonium (ppm)	.14±.15 (.04-.8)	.08±.06 (.01-.22)	.08±.04 (.01-.14)	.07±.02 (.03-.10)	.07±.04 (.01-.16)	*
Chlorophyll a	7.95±7.95 (2.02-28.23)	7.87±7.13 (3.6-26.1)	5.8±2.44 (2.54-10.35)	6.22±2.48 (2.98-10.14)	6.67±2.17 (3.80-10.32)	NS

Here, "* " indicates the level of significance,

* → <0.05; ** → <0.01; *** → <0.001; NS → Not Significant

Table 11. Water quality parameters (mean±SD) in Maheshkhali Channel (Month wise variation)

Parameter	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Sig.
Depth (m)	12	12	77	23	.88	93	7	75	58	31	09	8	***
Temperatu	.5	.56	.54	.62	.79	.72	.06	.51	.23	.35	.52	31	***
Transparen	.9	.5	.7	.6	.2	3	70.6±3	.1	42.8±2	9	.7	24±1.7	***
Turbidity	10.9±3 .3	11.8±5 .01	10.6±3 .5	181.4± 76.6	120.9± 4.76	41.6±2 9.23	86.4±1 7.08	206.4± 37.27	275.8± 27	268.1± 4.76	331.8± 24.28	366.4± 5.48	***
pH	.4	.19	.33	.1	.08	.13	.28	.12	.16	.04	.03	05	***
Current speed	0.37±0 .03	0.3±0. 1	0.3±0. 06	0.48±0 .05	0.43±0 .04	0.44±0 .05	0.44±0 .04	0.45±0 .03	0.46±0 .02	0.54±0 .04	0.58±0 .08	0.63±0. 07	***
DO (mg/l)	.1	17	.37	.34	.44	.24	.4	.2	.17	.2	.07	17	***
Salinity	.4	.27	1	.88	.14	.6	34±1.5	8	.7	.14	.14	14	***
Alkalinity	101.2± 9.7	81.6±1 6.5	98.8±8 .1	101.2± 21.3	241.2± 40.7	266.1± 15.5	262.2± 11	182.8± 12	171.6± 13	172.4± 18	180.6± 17	188.8± 4.3	***
Nitrite(ppm)	0.002± 0.0005	0.000 5	0.000 5	0.000 5	0.000 5	0.000 5	0.000 5	0.000 5	0.000 5	0.000 5	0.000 5	0.001± 0.0005	NS
Nitrate(pp	.04	04	.04	04	.04	.04	.04	04	.04	.04	.04	4	NS
Phosphate	.59	16	.13	19	.19	.89	.45	1	.07	.18	.08	07	***
Ammonia	0.27±0 .04	0.13±0 .01	0.2±0. 02	0.27±0 .03	0.19±0 .05	0.28±0 .04	0.28±0 .05	0.3±0. 03	0.28±0 .04	0.27±0 .03	0.31±0 .03	0.26±0. 04	***
Ammonium	0.09±0 .1	0.03±0 .01	0.09±0 .02	0.12±0 .02	0.05±0 .03	0.12±0 .19	0.1±0. 06	0.06±0 .02	0.04±0 .03	0.04±0 .02	0.05±0 .03	0.07±0. 02	***
Chlorophyll	.6	.7	.84	.6	.21	.64	.3	.24	.29	.38	.81	36	***

Here, "* " indicates the level of significance,

* ⟶ <0.05; ** ⟶ <0.01; *** ⟶ <0.001; NS ⟶ Not Significant

In Maheshkhali Channel both phytoplankton and zooplankton were detected. A total of 70 plankton genera, representatives of five classes were identified based on the given references. Besides 57 phytoplankton genera, 13 genera of zooplankton were identified among all the stations from month to month. From the total amount of plankton, the content of phytoplankton was 84.74% in all the stations. Of all the planktons Bacillariophyceae, Chlorophyceae, Cyanophyceae, Dinophyceae and Pyrrophyceae were the detected five classes of phytoplankton. The Phytoplankton count was higher in the Station 1 than that in others. Besides, during June and July the total phytoplankton count was higher than that in the other months throughout twelve months of study. The class Bacillariophyceae dominated the phytoplankton community in Maheshkhali Channel, (67.3% of the total count) consisting of 38 genera. The most dominant genus of Bacillariophyceae were *Skeletonema* and *Coscinodiscus*. The contribution of *Skeletonema* was 13.2%, *Coscinodiscus* 9.7%, *Ditylum* 4.6%, *Navicula* 4.5% and *Rhizosolenia* 3.9% of the total count. Nevertheless, during June and July month number of Bacillariophyceae was significantly higher ($p < 0.05$) than that in other months.

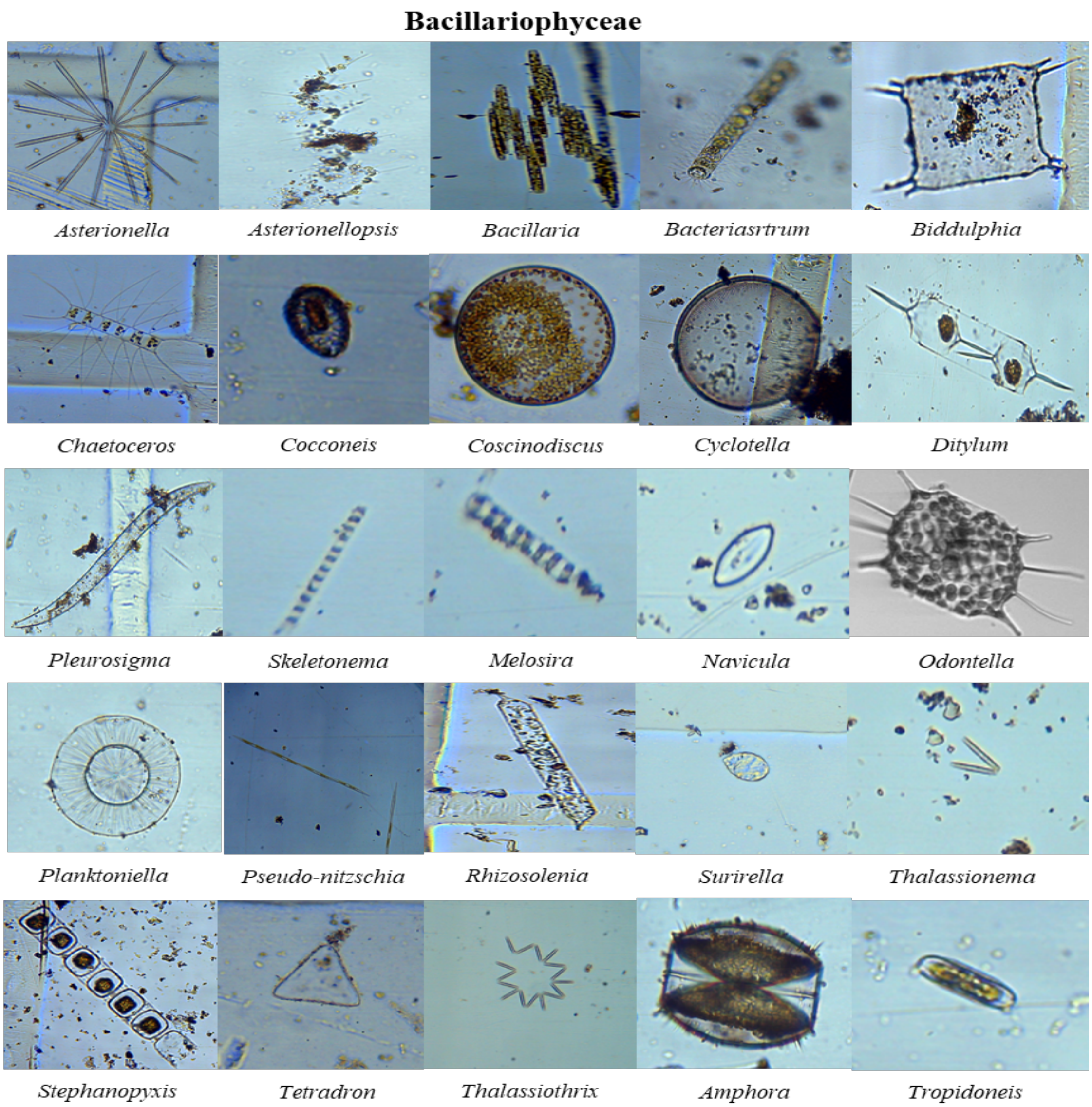


Fig. 36: Microscopic images of some phytoplankton under Bacillariophyceae Class; found in Maheshkhali Channel

The contribution of class Chlorophyceae was 4.78% of the total count consisting of 5 genera (*Chlorella*, *Dunaliella*, *Oscillatoria, Spirogyra,* and *Ulothrix*). No significant difference ($p > 0.05$) was observed in Chlorophyceae content among the different Stations. But in the monthly variation highly significant difference ($p < 0.05$) was observed. Yet, Chlorophyceae count in the month of July and September was significantly higher ($p < 0.05$) than that in other months.

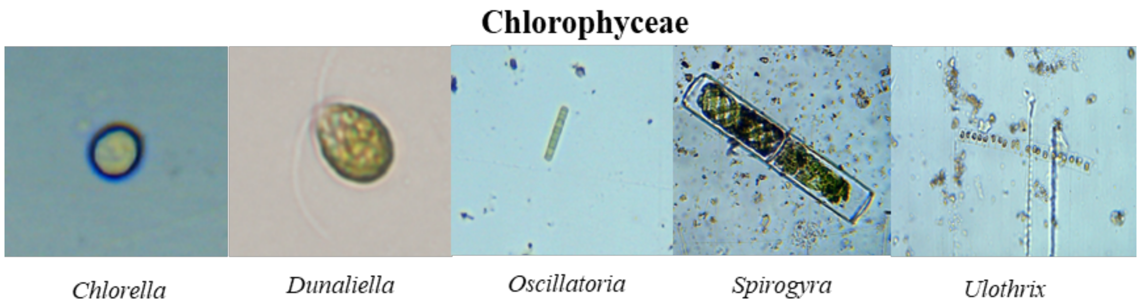


Fig. 37: Microscopic images of some phytoplanktons under Chlorophyceae Class; found in Maheshkhali Channel

Class Cyanophyceae contributed around 4.14% of the total count consisting of 5 genera (*Anabaena, Anabaenopsis, Aphanizomenon, Aphanocapsa* and *Nostoc*). Throughout the Stations no significant difference ($p > 0.05$) was observed in Cyanophyceae count. But in the monthly variation highly significant difference ($p <0.05$) was observed. Howsoever, during August month Cyanophyceae count was significantly higher ($p < 0.05$) than that in other months. During some months in some stations the Cyanophyceae count was close to zero in the collected samples.

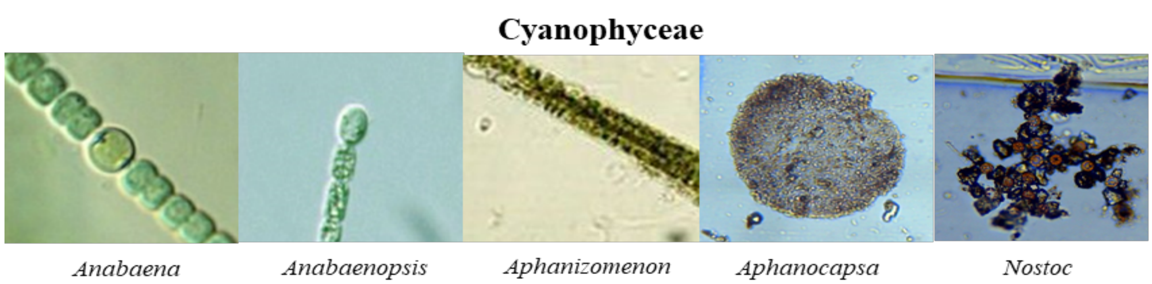


Fig. 38: Microscopic images of some phytoplanktons under Cyanophyceae Class; found in Maheshkhali Channel

Class Dinophyceae contribution in the total plankton count was 0.69% consisting of 5 genera (*Amphidinium, Boreadinium, Dinophysis, Entomoneis* and *Gymnodinium*). Among the different Stations no significant difference ($p > 0.05$) was evident in Dinophyceae. But in the monthly variation highly significant difference ($p < 0.05$) was observed. However, Dinophyceae count in the month of July and September was significantly higher ($p < 0.05$) than that in other months. From November to May no Dinophyceae were found.

Fig. 39: Microscopic images of some phytoplanktons under Dinophyceae Class; found in Maheshkhali Channel

The contribution of class Pyrrophyceae was 8.33% of the total count consisting of 4 genera (*Ceratium, Prorocentrum, Protoperidinium* and *Pyrocystis*). Among the different Stations no significant difference ($p > 0.05$) was observed in Pyrrophyceae count. But in the monthly variation highly significant difference ($p < 0.05$) was observed. However, Pyrrophyceae in September month was significantly higher ($p < 0.05$) than that in other months.

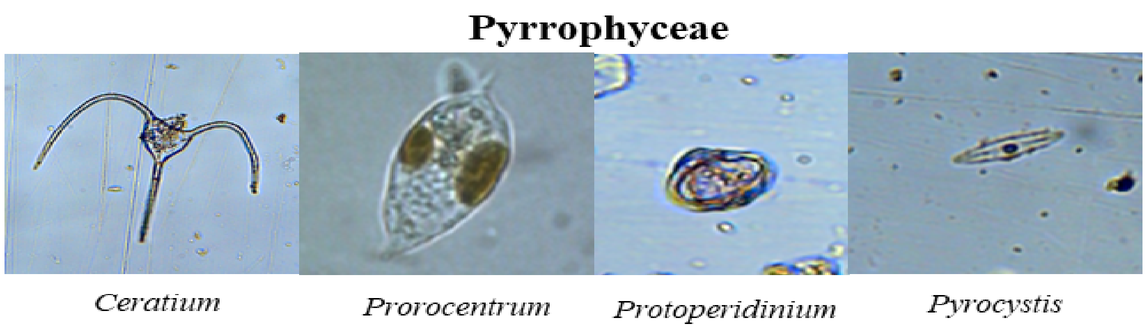


Fig. 40: Microscopic images of some phytoplanktons under Pyrrophyceae Class; found in Maheshkhali Channel

The contribution of zooplankton was 12.9% of the total count of plankton. The main contributors to the majority of the biomass were Copepods, Fish eggs and Rotifers. The zooplankton community in Maheshkhali Channel was dominated by Copepod (4.37% of Total Phytoplankton and 33.9% of the Total Zooplankton) and Fish egg (4.29% of Total Phytoplankton and 33.3% of the Total Zooplankton). The contributions of the copepod at different Stations ranged from 28.6% to 33.9% during the investigation period. No significant difference ($p > 0.05$) was observed in zooplankton among the different Stations. But highly significant difference ($p< 0.05$) was observed in zooplankton in the monthly variation. However, zooplankton in July month was significantly higher ($p < 0.05$) than that in other months.

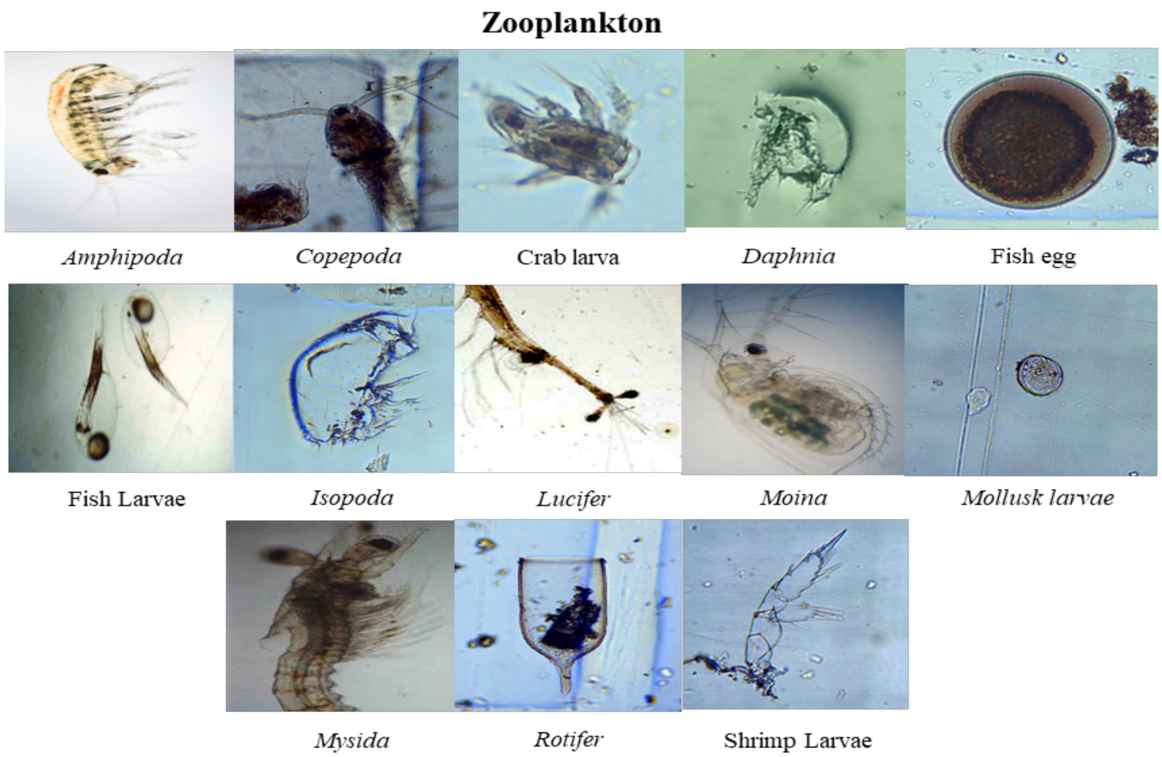


Fig. 41: Microscopic images of some zooplanktons; found in Maheshkhali Channel

Table 12. Phytoplankton composition in Maheshkhali Channel, Cox's Bazar

Phytoplankton	Mean count (×10^3 cells/L)	%	S1	S1 (%)	S2	S2 (%)	S3	S3 (%)	S4	S4 (%)	S5	S5 (%)
Amphora	0.03	0.1	0.03	0.1	0	0	0	0	0.08	0.3	0.06	0.19
Asterionella	0.42	1.5	0.36	1.23	0.44	1.53	0.33	1.18	0.53	1.8	0.44	1.52
Asterionellopsis	0.05	0.2	0.08	0.28	0.03	0.09	0.06	0.19	0.08	0.3	0	0
Biddulphia	0.19	0.7	0.19	0.66	0.17	0.58	0.33	1.17	0.11	0.4	0.14	0.48
Bacillaria	0.05	0.2	0.14	0.47	0	0	0.06	0.19	0.06	0.2	0	0
Bacteriastrum	0.13	0.5	0.22	0.75	0.06	0.19	0.17	0.58	0.06	0.2	0.17	0.57
Chaetoceros	0.42	1.5	0.39	1.32	0.36	1.24	0.5	1.76	0.39	1.3	0.47	1.62
Cocconeis	0.17	0.6	0.11	0.38	0.14	0.48	0.11	0.39	0.28	1	0.22	0.77
Corethron	0.02	0.1	0	0	0.06	0.19	0.03	0.09	0	0	0	0
Coscinodiscus	2.8	9.7	2.7	9.27	2.67	9.18	2.86	10.1	2.80	9.7	3	10.3
Cyclotella	0.06	0.2	0.06	0.19	0.03	0.09	0.08	0.29	0.11	0.4	0.03	0.09
Cylindrotheca	0.07	0.2	0.03	0.09	0.06	0.19	0.14	0.49	0.03	0.1	0.08	0.29
Diatoma	0.35	1.2	0.39	1.32	0.36	1.24	0.22	0.78	0.36	1.3	0.42	1.43

Table 12 Cont'd..

Phytoplankton	Mean count (×10³ cells/L)	%	S1	S1 (%)	S2	S2 (%)	S3	S3 (%)	S4	S4 (%)	S5	S5 (%)
Detonula	0.09	0.3	0.06	0.19	0.14	0.48	0.08	0.29	0.08	0.3	0.08	0.29
Diploneis	0.02	0.1	0	0	0.06	0.19	0.03	0.09	0	0	0.03	0.1
Ditylum	1.34	4.6	1.31	4.44	1.58	5.45	1.19	4.21	1.28	4.4	1.36	4.67
Fragilaria	0.07	0.3	0.06	0.19	0.11	0.39	0.03	0.1	0.11	0.4	0.06	0.19
Leptocylindricus	0.04	0.1	0	0	0.08	0.29	0.03	0.1	0.03	0.1	0.06	0.19
Licomphora	0.47	1.6	0.58	1.98	0.36	1.24	0.58	2.06	0.47	1.6	0.36	1.24
Melosira	0.22	0.8	0.28	0.95	0.19	0.67	0.17	0.59	0.25	0.9	0.22	0.76
Navicula	1.3	4.5	1.58	5.39	1.22	4.21	1.31	4.6	1.25	4.3	1.14	3.90
Nitzschia	0.11	0.4	0.14	0.47	0.06	0.19	0.08	0.29	0.06	0.2	0.22	0.76
Odontella	0.44	1.5	0.42	1.42	0.56	1.91	0.47	1.67	0.36	1.3	0.39	1.33
Planktoniella	0.13	0.5	0.19	0.66	0.14	0.48	0.11	0.39	0.17	0.6	0.06	0.19
Pleurosigma	0.89	3.1	0.91	3.12	0.86	2.97	0.81	2.84	0.94	3.3	0.94	3.24
Polykrikos	0.73	2.5	1	3.40	0.64	2.20	0.58	2.06	0.69	2.4	0.75	2.57
Pseudo-nitzschia	0.92	3.2	0.78	2.65	0.72	2.49	0.94	3.33	0.97	3.4	1.22	4.19
Rhizosolenia	1.13	3.9	1.06	3.59	1.28	4.40	1.03	3.62	1.25	4.3	1.06	3.62
Skeletonema	3.82	13.2	3.56	12.1	3.9	13.6	3.75	13.2	3.92	13.5	3.92	13.4
Stephanopyxis	0.21	0.73	0.19	0.66	0.22	0.77	0.19	0.69	0.22	0.77	0.22	0.77
Surirella	0.02	0.08	0	0	0.08	0.29	0.03	0.1	0	0	0	0
Tetradron	0.23	0.81	0.28	0.95	0.25	0.86	0.28	0.98	0.17	0.58	0.19	0.67
Triceratium	0.01	0.02	0	0	0.03	0.1	0	0	0	0	0	0
Thalassiosira	0.39	1.36	0.36	1.23	0.31	1.05	0.53	1.86	0.42	1.44	0.36	1.24
Thalassionema	0.14	0.5	0.31	1.04	0.08	0.29	0.14	0.49	0.03	0.1	0.17	0.57
Thalassiothrix	0.03	0.12	0	0	0.03	0.1	0.03	0.1	0.11	0.39	0	0
Tropidoneis	0.01	0.02	0	0	0	0	0.03	0.1	0	0	0	0
Total												
Bacillariophyceae	19.49	67.3	19.3	65.5	19.53	67.3	19.4	68.4	19.3	66.8	20	68.5
Chlorella	0.02	0.06	0.03	0.1	0.03	0.1	0.03	0.1	0	0	0	0
Dunaliella	0.04	0.15	0	0	0.06	0.19	0.11	0.39	0.06	0.19	0	0
Oscillatoria	0.37	1.27	0.47	1.61	0.44	1.53	0.28	0.98	0.28	0.96	0.36	1.24
Spirogyra	0.55	1.9	0.58	1.98	0.5	1.72	0.53	1.87	0.58	2.02	0.56	1.90
Ulothrix	0.41	1.4	0.47	1.61	0.39	1.34	0.42	1.47	0.39	1.34	0.36	1.24
Total Chlorophyceae	1.38	4.78	1.56	5.29	1.42	4.88	1.36	4.8	1.31	4.51	1.28	4.38
Anabaena	0.31	1.05	0.44	1.51	0.28	0.96	0.28	0.98	0.17	0.58	0.36	1.24

Phytoplankton	Mean count (×10^3 cells/L	%	S1	S1 (%)	S2	S2 (%)	S3	S3 (%)	S4	S4 (%)	S5	S5 (%)
Ananaenopsis	0.01	0.04	0.03	0.09	0	0	0.03	0.1	0	0	0	0
Aphanizomenon	0.13	0.46	0.11	0.38	0.19	0.67	0.17	0.59	0.08	0.29	0.11	0.38
Aphanocapsa	0.71	2.45	0.86	2.93	0.69	2.39	0.69	2.45	0.67	2.3	0.64	2.19
Nostoc	0.04	0.15	0.06	0.19	0.11	0.39	0.08	0.1	0	0	0.03	0.1
Total Cyanophyceae	1.2	4.14	1.5	5.10	1.25	4.31	1.19	4.21	0.92	3.17	1.14	3.9
Amphidinium	0.02	0.06	0.06	0.19	0.03	0.1	0	0	0	0	0	0
Boreadinium	0.11	0.38	0.14	0.47	0.06	0.19	0.11	0.39	0.11	0.38	0.14	0.48
Dinophysis	0.01	0.02	0	0	0	0	0	0	0.03	0.1	0	0
Entomoneis	0.06	0.21	0.19	0.66	0.03	0.1	0	0	0.03	0.1	0.06	0.19
Gymnodinium	0.01	0.02	0.03	0.09	0	0	0	0	0	0	0	0
Total Dinophyceae	0.2	0.69	0.42	1.42	0.11	0.38	0.11	0.39	0.17	0.58	0.19	0.67
Ceratium	0.13	0.46	0.11	0.38	0.14	0.48	0.11	0.39	0.17	0.58	0.14	0.48
Prorocentrum	0.07	0.25	0.08	0.28	0.08	0.29	0.09	0.29	0.08	0.29	0.03	0.1
Protoperidinium	2.21	7.61	2.5	8.50	2.28	7.85	2.17	7.64	2.14	7.39	1.94	6.67
Pyrocystis	0.01	0.02	0	0	0	0	0	0	0	0	0.03	0.1
Total Pyrrophyceae	2.42	8.33	2.7	9.17	2.5	8.61	2.36	8.3	2.39	8.25	2.14	7.33
Total Phytoplankton	24.56		25.5		24.83		23.6		24.1		24.7	

Table 13. Zooplankton composition in Maheshkhali Channel, Cox's Bazar

Zooplankton	Mean count (×10^3 cells/L)	%	S1	S1 (%)	S2	S2 (%)	S3	S3 (%)	S4	S4 (%)	S5	S5 (%)
Amphipoda	0.06	0.19	0.03	0.1	0.03	0.10	0.03	0.59	0.17	0.19	0.06	0.00
Copepoda	1.27	4.37	1.17	3.97	1.06	3.64	1.06	4.11	1.17	5.09	1.47	5.05
Crab zoea	0.06	0.19	0	0	0.03	0.1	0.03	0.59	0.17	0.19	0.06	0.1
Daphnia	0.03	0.12	0.03	0.09	0.03	0.1	0.03	0.2	0.06	0.1	0.03	0.1
Fish egg	1.24	4.29	1.11	3.78	1.31	4.5	1.31	4.21	1.19	4.32	1.25	4.67
Fish larvae	0.24	0.82	0.22	0.76	0.33	1.15	0.33	0.69	0.19	0.58	0.17	0.95
Isopoda	0.18	0.61	0.22	0.76	0.19	0.67	0.19	0.29	0.08	0.67	0.19	0.67
Lucifer	0.17	0.58	0.1	0.38	0.17	0.57	0.17	0.69	0.19	0.58	0.17	0.67
Moina	0.02	0.08	0.03	0.09	0.03	0.1	0.03	0.2	0.06	0	0	0
Mollusk larvae	0.03	0.12	0	0	0	0	0	0	0	0.48	0.14	0.1
Mysids	0.01	0.02	0.03	0.09	0	0	0	0	0	0	0	0
Rotifer	0.4	1.42	0.44	1.5	0.47	1.63	0.47	1.08	0.31	1.44	0.42	1.43
Shrimp larvae	0.07	0.25	0.11	0.38	0.03	0.1	0.03	0.2	0.06	0.29	0.08	0.29
Zooplankton	3.73	12.9	3.4	11.6	3.69	12.7	3.69	12.2	3.47	13.8	4	14
Total Plankton	29		29.39		29.1		29		28.4		28.9	

Table 14. Site suitability rating system for Maheshkhali Channel, Cox's Bazar

Parameter	Station 1		Station 2		Station 3		Station 4		Station 5	
	Rating	Score	Rating	Score	Rating	Score	Rating	Score	Rating	Score
Salinity	6	.9	7	1.05	6	.9	6	.9	6	.9
DO	9	1.35	9	1.35	9	1.35	9	1.35	9	1.35
pH	8	.8	8	.8	8	.8	8	.8	8	.8
Temperature	10	1.5	10	1.5	10	1.5	10	1.5	10	1.5
Chlorophyll-a	10	1.5	10	1.5	10	1.5	10	1.5	10	1.5
Water depth	6	.9	6	.9	6	.9	7	1.05	7	1.05
Current speed	6	.9	7	1.05	8	1.2	6	.9	6	.9
Weighted Category	7.85		8.15		8.15		8		8	
	Good		Good		Good		Good		Good	

Conclusion:

In terms of feasibility for Green mussel farming, environmental variables in all Stations were categorized as good. But the Stations 2 and 3 might be considered as best potential cultural site for Green mussel as they had higher total weighted value compared to the other stations. In the environmental variables chart, it was undoubtedly evident that the consistency of all the parameters over whole years and moderate salinity, water depth and current speed was the major issue that made Maheshkhali Channel mostly favorable for Green mussel culture and they are naturally abundant in the stations too.

11.1.3 Site suitability of The Naf River Estuary, Cox's Bazar

The temperature readings during the study time round vary from 27.88-33.05ºC. No major fluctuation in temperature was observed throughout the study time. No significant difference ($p > 0.05$) was observed in temperature throughout the Stations. Temperature in April and June months was significantly higher ($p < 0.05$) than that in other months. The value of transparency fluctuated from 20-53.07 cm. The transparency of Station 5 was significantly higher ($p < 0.05$) than that in other Stations. Transparency in March and June months was significantly higher ($p < 0.05$) than that in other months. The value of turbidity fluctuated from 18.46-137.4 NTU. No major fluctuation in turbidity was observed throughout the study time. The value of pH fluctuated from 7.34-8.4. No major fluctuation in pH was observed throughout the study time. But pH of Station 1 was significantly higher ($p < 0.05$) and Station 3 was significantly lower ($p < 0.05$) than that in other Stations. The dissolve oxygen readings during the year round vary from 7.01-12.08 mg/l. No major fluctuation in DO was observed throughout the study time. But DO of Station 3 was significantly higher ($p < 0.05$) than that in other Stations and Station 1 was significantly lower ($p < 0.05$) than that in other Stations. The value of salinity highly fluctuated from 8-30 ppt. But this fluctuation was observed in the monthly variation. In September (2017) salinity decreased significantly ($p < 0.05$). The depth readings during the year round vary from 1.02-4.96m. Station 5 depth was significantly higher ($p < 0.05$) than other Stations.

The value of alkalinity fluctuated from 86.8-335.8 ppm. Wide range of fluctuation was observed throughout the study time. But No significant difference ($p > 0.05$) was observed in alkalinity among the different Stations. The value of nitrate fluctuated from 0.02-0.2 ppm. No major fluctuation was observed throughout the study time. The value of nitrite fluctuated from 0.002-0.2 ppm. No major fluctuation was observed throughout the study time. Also, no significant difference ($p > 0.05$) was observed in nitrite among the different Stations. During the study period, phosphate concentrations fluctuated widely from 0.007-1.04 ppm. Highly significant difference ($p < 0.05$) was also observed in phosphate. The phosphate of Station 1 was significantly higher ($p < 0.05$) than that in other Stations. Highly significant difference ($p < 0.05$) of phosphate was also observed in the monthly variation. However, phosphate in March month was significantly higher ($p < 0.05$) than that in other months. The value of ammonia fluctuated from .012-0.44 ppm. The ammonia of Station 3 and 5 was significantly higher ($p < 0.05$) than that in Station 1, 2 and 4. The value of ammonium fluctuated from 0.05-1.5ppm. No significant difference ($p > 0.05$) was observed in ammonium among the different Stations. The value of chlorophyll a fluctuated from 1.7-6.4. Wide range of fluctuation was observed throughout the study time. Highly significant difference ($p < 0.05$) was also observed in chlorophyll a. Chlorophyll a of Station 3 was significantly higher ($p < 0.05$) than that in other Stations.

Table 15. Water quality parameters (min-max) of five stations in The Naf River (Station wise variation)

Parameters	Station 1	Station 2	Station 3	Station 4	Station 5	Sig.
Depth (m)	3.1±1.56 (.9-5.5)	3.08±1.50 (.9-5.2)	3.1±1.58 (.8-5.5)	3.04±1.44 (0.8-5.1)	3.14±1.5 (.8-5.3)	NS
Temperature (˚C)	28.9±1.2 (27-31.2)	29.44±2.14 (27-34.1)	29.67±1.88 (27.9-33.7)	29.22±1.99 (26-33.2)	29.72±1.98 (28-34.3)	NS
Transparency (cm)	36±9.21 (20-51)	38.9±11.45 (20-54)	39.76±10.48 (20-53)	40±11.45 (20-57)	40.71±11.5 (20-58)	NS
Turbidity (NTU)	55.01±43.5 (18.3-140)	51.9±44.2 (17.6-152)	50.3±43.6 (15.2-150)	47.58±33.3 (16.4-111)	51.4±37.8 (19.3-136)	**
pH	7.86±.44 (7.3-8.7)	7.9±.39 (7.4-8.5)	7.9±.39 (7.3-8.3)	7.9±.34 (7.4-8.4)	7.9±.43 (7.3-8.6)	NS
DO (mg/l)	8.95±1.4 (6.3-10.6)	10.27±1.62 (8.2-12.7)	10.24±1.76 (7.2-12.8)	9.9±1.4 (6.6-11.2)	10.11±2.35 (6.1-14.8)	NS
Salinity(ppt)	26.43±7.78 (8-30)	26.43±7.78 (8-30)	26.29±7.77 (8-30)	26.00±7.73 (8-30)	25.57±7.86 (8-30)	NS
Alkalinity (mg/l)	219.4±97.1 (89-341)	224.1±97.5 (73-356)	212.5±97.03 (76-328)	213.1±76.73 (86-320)	223.6±101.1 (102-357)	*
Nitrite(ppm)	.051±.079 (.002-.3)	.035±.054 (.002-.2)	.047±.078 (.002-.2)	.038±.054 (.002-.2)	.036±.052 (.002-.2)	NS
Nitrate(ppm)	.2±.00 (.2-.2)	.2±.00 (.2-.2)	.2±.00 (.2-.2)	.2±.00 (.2-.2)	.2±.00 (.2-.2)	NS
Phosphate (ppm)	.4±.54 (.007-1.6)	.37±.55 (.007-1.5)	.28±.38 (.007-.9)	.27±.36 (.007-.9)	.22±.30 (.007-.815)	NS
Ammonia (ppm)	.14±.16 (.013-.42)	.12±.15 (.013-.42)	.07±.08 (.012-.22)	.16±.14 (.013-.44)	.09±.09 (.013-.24)	NS
Ammonium (ppm)	1.5±.00 (1.5-1.5)	1.5±.00 (1.5-1.5)	1.5±.00 (1.5-1.5)	1.5±.00 (1.5-1.5)	1.5±.00 (1.5-1.5)	NS
Chlorophyll a	3.57±1.55 (2.10-5.9)	3.8±1.32 (1.9-5.7)	3.84±1.24 (2.5-6.4)	3.68±1.28 (2.5-6.3)	3.7±1.37 (1.7-5.7)	**

Here, "* " indicates the level of significance,

* → <0.05; ** → <0.01; *** → <0.001; NS → Not Significant

Table 16. Water quality parameters (mean±SD) in The Naf River (Month wise variation)

Parameter	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Sig. level**
Depth (m)	1.02±.16	2±.2	1.8±.5	4.96±.25	4.96±.26	2.96±.26	3.94±.28	NS
Temperature(ºC)	28.88±.38	33.05±1.34	27.88±.43	30.24±.30	27.74±.92	29.2±.78	28.7±.78	**
Transparency(cm)	53.07±2.99	30.93±1.67	38.67±7.79	44.27±4.03	20.0±.00	43.0±4.9	43.6±3.29	NS
Turbidity (NTU)	18.46±2.12	18.74±1.55	49.44±3.98	42.56±5.69	137.4±15.86	67.92±11.61	24.14±5.1	NS
pH	7.7±.05	7.6±.07	7.34±.05	8.28±.08	8.4±.32	7.82±.18	8.08±.30	NS
DO(mg/l)	9.44±.91	7.01±.82	9.6±1.3	10.2±.88	10.8±1.04	12.08±1.6	10.14±.45	*
Salinity (ppt)	30±.00	30±.00	25.4±1.9	30±.00	8±.00	29.6±.51	30±.00291	NS
Alkalinity	291.6±40.84	316.4±13.08	335.8±12.71	144.13±21.55	86.8±10.94	148.4±13.66	206.8±16.14	NS
Nitrite(ppm)	.008±.002	.043±.071	.066±.009	.169±.031	.002±.00	.002±.00	.002±.00	NS
Nitrate(ppm)	.2±.00	.2±.00	.2±.00	.2±.00	.2±.00	.2±.00	.2±.00	NS
Phosphate	1.04±.422	.21±.06	.82±.00	.064±.01	.007±.00	.007±.00	.007±.00	NS
Ammonia	.19±.03	.052±.077	.039±.015	.2±.074	.307±.171	.013±.00	.013±.00	**
Ammonium	1.5±.00	1.5±.00	1.5±.00	1.5±.00	1.5±.00	1.5±.00	1.5±.00	NS
Chlorophyll a	3.20±.58	3.91±.68	3.18±.37	2.46±.24	2.24±.34	5.38±.6	5.62±.51	**

Here, "* " indicates the level of significance,

* → <0.05; ** → <0.01; *** → <0.001; NS → Not Significant

In The Naf river both phytoplankton and zooplankton were observed. A total of 30 phytoplankton genera, representatives of five classes were identified. In The Naf river 11 genera of zooplankton were identified. The contribution of phytoplankton was 83.7% of the total count of plankton. The observed five class of phytoplankton was Bacillariophyceae, Chlorophyceae, Cyanophyceae, Dinophyceae and Pyrrophyceae. The phytoplankton community in The Naf river was dominated by the class Bacillariophyceae (78% of the total count) consisting of 30 genera. *Pseudo-nitzschia* and *Coscinodiscus* were the most dominant genus of Bacillariophyceae. No significant difference ($p > 0.05$) was observed in Bacillariophyceae among the different Stations. But highly significant difference ($p < 0.05$) was observed in Bacillariophyceae in the monthly variation. The contribution of class Chlorophyceae consist of 3 genera (*Spirogyra, Ulothrix* and *Oscillatoria*). The contribution of class Pyrrophyceae consisted of 2 genera (*Ceratium, Prorocentrum*). The zooplankton community in The Naf river was dominated by the species Copepod and Rotifer.

Table 17. Phytoplankton composition in the The Naf river, Cox's Bazar

Phytoplankton	Mean count (×10^3cells/L)	%	S1	%	S2	%	S3	%	S4	%	S5	%
Asterionella	0.0476	3.18	0.0	3.18	0.0	3.18	0.0	3.18	0.0	3.18	47.6	3.18
Biddulphia	0.219	0.19	238.1	0.19	380.9	0.19	95.2	0.19	190.48	0.19	190.48	0.19
Chaetoceros	.333	3.39	190.48	3.39	809.52	3.39	285.71	3.39	190.48	3.39	190.48	3.39
Coscinodiscus	1.838	2.49	2095.95	2.49	2095.95	2.49	1857.14	2.49	1666.76	2.49	1476.19	2.49
Cyclotella	0.1524	1.50	142.86	1.50	47.62	1.50	285.71	1.50	190.48	1.50	95.24	1.50
Diploneis	.00952	0.15	0.0	0.15	0.0	0.15	47.62	0.15	0.0	0.15	0.0	0.15
Ditylum	0.752	2.42	952.38	2.42	1047.62	2.42	333.33	2.42	619.05	2.42	809.52	2.42
Eucampia	.01142	0.84	47.62	0.84	190.48	0.84	95.24	0.84	142.86	0.84	95.24	0.84
Golenkinia	0.00286	0.09	0.0	0.09	142.86	0.09	0.0	0.09	0.0	0.09	0.0	0.09
Hemiaulus	0.371	7.80	380.95	7.80	333.33	7.80	333.33	7.80	190.48	7.80	619.05	7.80
Leptocylindricus	0.0095	0.97	47.62	0.97	0.0	0.97	0.0	0.97	0.0	0.97	0.0	0.97
Melosira	0.0952	0.36	142.86	0.36	0.0	0.36	95.24	0.36	190.48	0.36	47.62	0.36
Nitzschia	0.0286	0.28	47.62	0.28	47.62	0.28	47.62	0.28	0.0	0.28	0.0	0.28
Odontella	0.0191	0.04	0.0	0.04	0.0	0.04	95.24	0.04	0.0	0.04	0.0	0.04
Pleurosigma	0.4191	0.54	619.05	0.54	476.19	0.54	380.95	0.54	285.71	0.54	333.33	0.54
Pseudo-nitzschia	16.342	2.00	0.0	2.00	13571.43	2.00	7476.19	2.00	34141.86	2.00	26523.81	2.00
Rhizosolenia	0.4191	4.70	190.48	4.70	428.57	4.70	285.71	4.70	952.38	4.70	238.10	4.70
Skeletonema	0.524	0.13	333.33	0.13	333.33	0.13	333.33	0.13	857.14	0.13	761/90	0.13
Tetradron	0.1142	0.11	95.24	0.11	95.24	0.11	190.48	0.11	95.24	0.11	95.24	0.11
Triceratium	0.1238	0.28	238.10	0.28	95.24	0.28	95.24	0.28	142.86	0.28	47.62	0.28
Thalassiosira	0.01905	6.35	47.62	6.35	47.62	6.35	0.0	6.35	0.0	6.35	0.0	6.35
Thalassionema	0.01905	1.20	0.0	1.20	47.62	1.20	0.0	1.20	0.0	1.20	47.62	1.20
Thalassiothrix	0.11429	5.49	95.24	5.49	95.24	5.49	238.10	5.49	47.62	5.49	95.24	5.49
Total Bacillariophyceae	22.07619	4.08	5857.14	4.08	20190.48	4.08	12571.43	4.08	40047.62	4.08	31714.29	4.08
Spirogyra	0.01905	0.21	95.24	0.21	0.0	0.21	0.0	0.21	0.0	0.21	0.0	0.21
Ulothrix	10.2	0.04	11714.29	0.04	9619.05	0.04	9047.62	0.04	10904.76	0.04	9714.29	0.04
Oscillatoria	0.04762	0.17	0.0	0.17	0.0	0.17	190.	0.17	0.0	0.17	47.62	0.17

							48					
Total Chlorophyceae	10.17143	3.95	11333.33	3.95	9619.05	3.95	9238.1	3.95	10904.76	3.95	9761.9	3.95
Cerataulina	0.04762	1.63	47.62	1.63	47.62	1.63	47.62	1.63	0.0	1.63	95.24	1.63
Total Dinophyceae	0.04762	1.78	47.62	1.78	47.62	1.78	47.62	1.78	0.0	1.78	95.24	1.78
Ceratium	0.14286	5.60	95.24	5.60	238.10	5.60	190.48	5.60	47.62	5.60	142.86	5.60
Prorocentrum	0.01905	8.20	0.0	8.20	47.62	8.20	0.0	8.20	0.0	8.20	47.62	8.20
Total Pyrrophyceae	0.16190	0.54	95.24	0.54	285.72	0.54	190.48	0.54	47.62	0.54	190.48	0.54
Total Phytoplankton	32.50476	0.24	17857.14	0.24	30095.24	0.24	21857.14	0.24	50952.38	0.24	41761.90s	0.24

Table 18. Zooplankton composition in the The Naf River, Cox's Bazar

Zooplankton	**Mean count (×10^3 cells/L)**	**%**	**S1**	**%**	**S2**	**%**	**S3**	**%**	**S4**	**%**	**S5**	**%**
Acetes	0.00952	0.3	47.62	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0	0.3
Amphipoda	0.02857	3.11	47.62	3.11	0.0	3.11	47.62	3.11	0.0	3.11	47.62	3.11
Copepoda	1.8381	0.15	952.38	0.15	1523.81	0.15	2095.24	0.15	2285.71	0.15	2333.33	0.15
Crab zoea	0.24762	26.4	142.86	26.4	428.57	26.4	95.24	26.4	380.95	26.4	190.48	26.4
Daphnia	0.00952	5.39	0.0	5.39	47.62	5.39	0.0	5.39	0.0	5.39	0.0	5.39
Fish egg	0.28571	1.35	285.71	1.35	285.71	1.35	285.71	1.35	523.81	1.35	47.62	1.35
Isopoda	0.01905	11.4	0.0	11.4	0.0	11.4	95.24	11.4	0.0	11.4	0.0	11.4
Lucifer	0.00952	24.6	0.0	24.6	0.0	24.6	0.0	24.6	0.0	24.6	47.62	24.6
Mollusc larvae	0.01905	4.2	0.0	4.2	0.0	4.2	47.62	4.2	47.62	4.2	0.0	4.2
Rotifer	0.40952	0.9	380.95	0.9	476.19	0.9	523.81	0.9	380.95	0.9	285.71	0.9
Shrimp larvae	0.2	6.59	47.62	6.59	285.71	6.59	238.10	6.59	333.33	6.59	95.24	6.59
Total zooplankton	3.06667	2.85	1904.76	2.85	3047.62	2.85	3428.57	2.85	3904.76	2.85	3047.62	2.85
Tolal Plankton	35.57143	1.8	19761.90	1.8	33142.86	1.8	25285.71	1.8	54857.14	1.8	44809.52	1.8

Table 19. Site suitability rating system for The Naf river, Cox's Bazar

Parameter	Station 1		Station 2		Station 3		Station 4		Station 5	
	Rating	Score	Rating	Score	Rating	Score	Rating	Score	Rating	Score
Salinity	10	1	10	1	10	1	10	1	10	1
DO	10	1	10	1	10	1	10	1	10	1
pH	10	1	10	1	10	1	10	1	10	1
Temperature	9	1.35	9	1.35	9	1.35	9	1.35	9	1.35
Chlorophyll-a	10	1.5	10	1.5	10	1.5	10	1.5	10	1.5
Water depth	6	0.9	6	0.9	6	0.9	6	0.9	6	0.9
Weighted	7.65		7.65		7.65		7.65		7.65	
Category	Good		Good		Good		Good		Good	

Conclusion:

Based on the ranking mentioned above, the parameters indicate that all the stations in the The Naf River were good for Green mussel culture.

Site suitability of Green mussel culture in the coastal areas of Bangladesh

In order to determine the site suitability of Green mussel culture in the coastal areas like Rezu Khal, Maheshkhali Channel and The Naff River Estuary by using the site capability rating system to fulfil our objective-1. We extensively studied the year-round variation of physico-chemical factors and plankton abundance in these regions. Based on our result, it is concluded that Rezu Khal is not that much good site for Green mussel culture but it is capable and moderately suitable for Green mussel farming. It was clearly shown that the water depth and salinity fluctuation was the major issue that made Rezu Khal unfavorable for Green mussel culture. In addition, the plankton abundance was also low to support the viable and profitable growth of Green mussel in this Khal. The feasibility of Green mussel culture in Maheshkhali channel is categorized as good. In the environmental variables chart, it was undoubtedly evident that the consistency of all the parameters over whole years and moderate salinity, water depth and current speed was the major issue that made Maheshkhali Channel mostly favorable for Green mussel culture and they are naturally abundant in the stations too. Based on the ranking, the parameters indicate that all the stations in The Naf River are also good for Green mussel culture. However, the salinity is the big issue in some month that goes to very low due to heavy rainfall during monsoon season.

11.2. Study on the breeding biology of Green mussels

11.2.1 Developmental stages of gonad

From the primary morphological identification of development stages based on gonad coloration in female it is found that development stages were yellow to orange, dark orange to reddish for mature stage and pale orange for spawning stage. In case of male color were creamy white difficult to identify different stages. Gonad development stages of *Perna viridis* were identified by the histological view following Al-Barwani 2011 based on oocyte and spermatocyte condition.

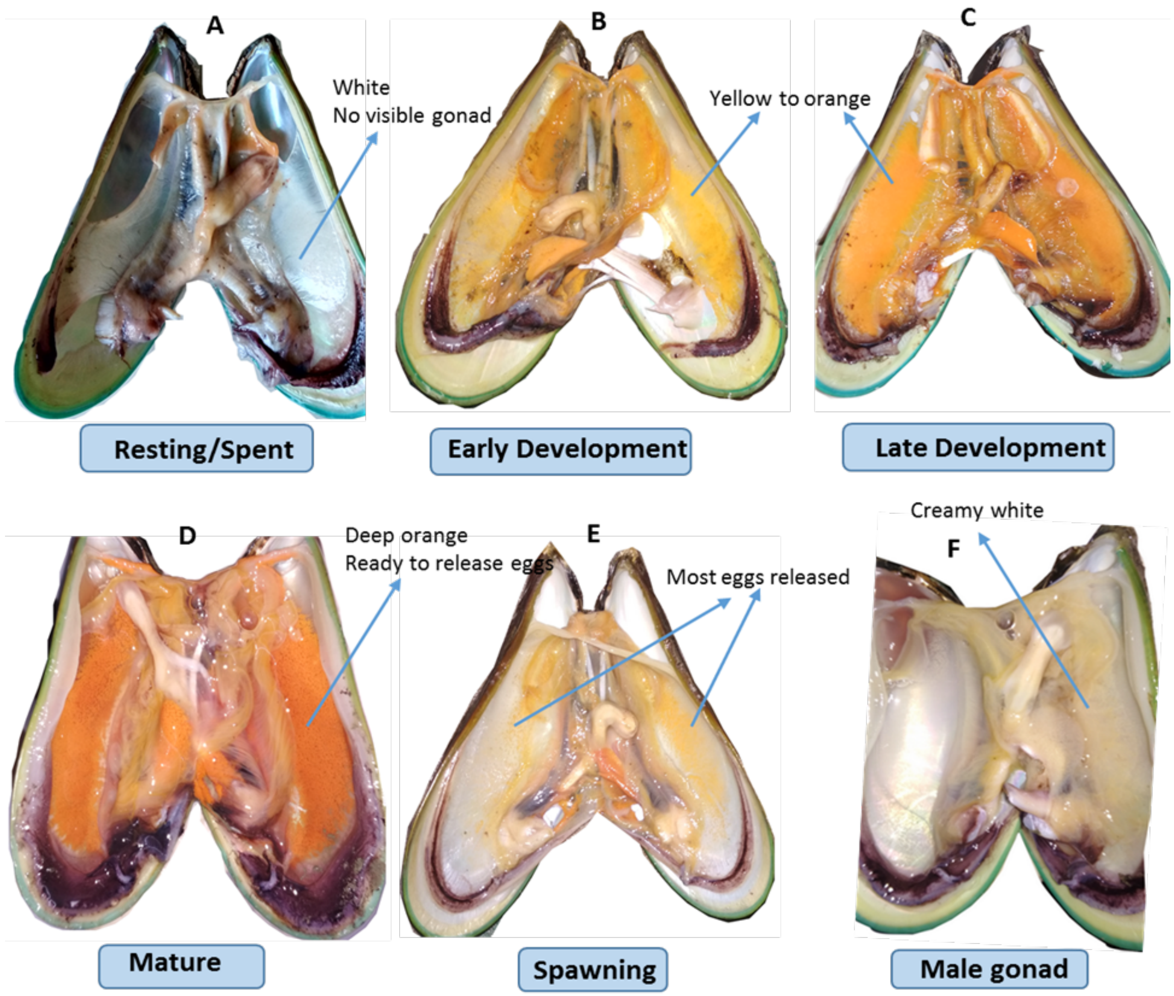


Fig 42. Macroscopic temporal variation of gonad development of Green mussel *Perna viridis* collected from the coastal waters of Bangladesh during November 2017 to October 2018. Female gonads color varied from white (resting) to yellow (early developmental) to deep orange (mature) stage. Males gonads are creamy white color during development stages but pictorial views are difficult to separate at different gonad development stages

Resting- No evidence of gonad is present in the mantle lobe. Male and female undistinguishable; mostly completely spawned or individuals with rudiment sexuality (Figure 42A).

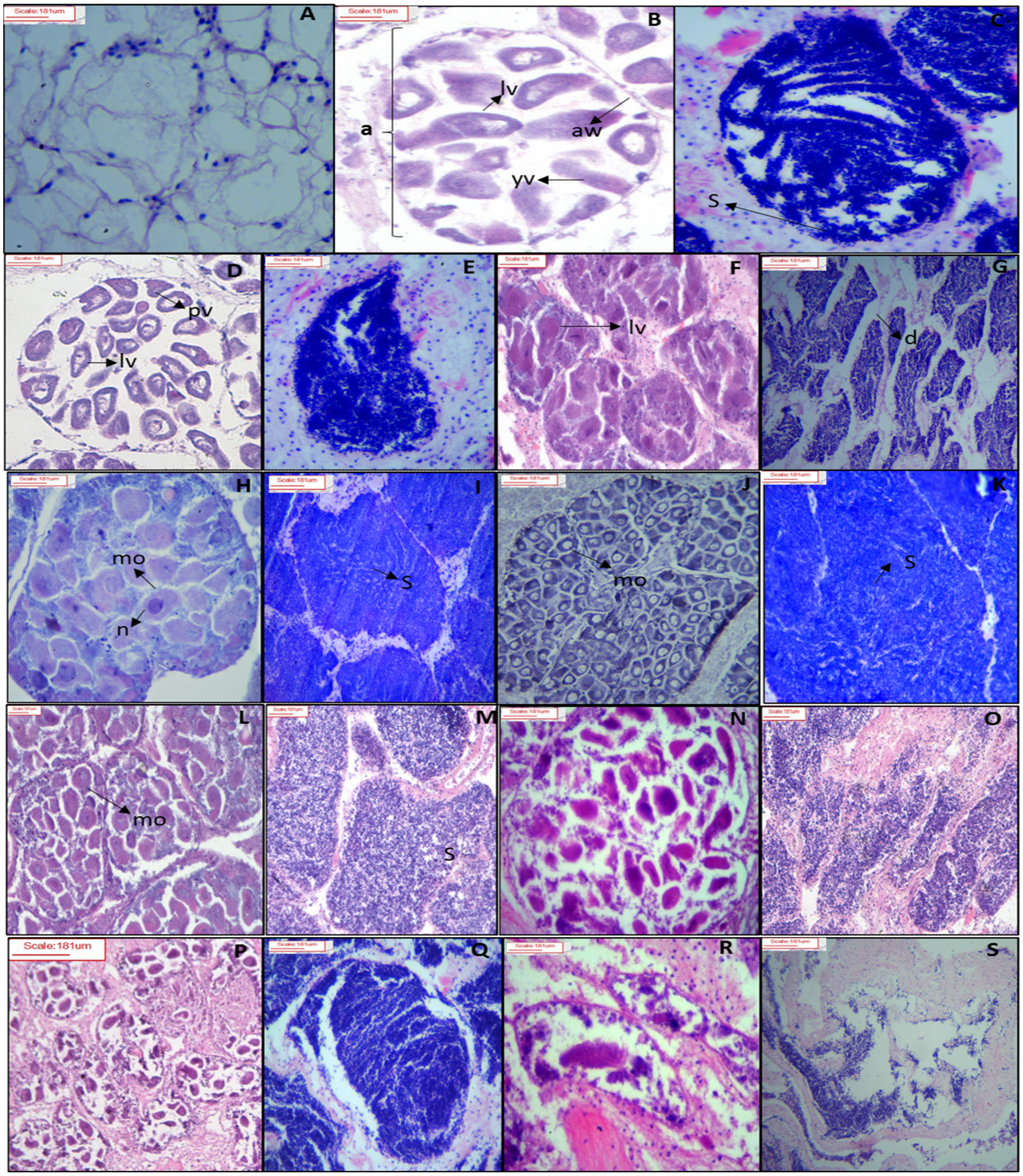


Fig. 42: Transverse histological section of female and male gonads of Green mussel *Perna viridis* collected from the coastal water of Bangladesh during November 2017 to October 2018. Resting stages (A), development stage A (B-female, C-male), development stage B (D-female, E-male), development stage C (F-female, G-male), development stage D (H-female, I-male), mature (J-female, K-male), spawning stage A (L-female, M-male), spawning stage B (N-female, O-male), spawning stage C (P-female, Q-male), and spent (R-female, S-male) are shown. Scale Bar-181 µm. a, acinus wall; yv, young vitellogenic oocyte; lv, late vitellogenic oocyte; S, spermatozoa; pv, previtellogenic oocyte; lv, late vitellogenic oocyte; mo, mature oocyte; n, nucleus.

Development A - Acinus with early vitellogenic and late vitellogenic oocyte had been developed though very few in number (Figure 1B). In male spermatophore was had started to form (Figure 42C).

Development B - First appearance of mature oocyte was recorded and the number of early and late vitellogenic oocyte increased. Mature oocyte comprises 30% and 70% of the acini composed of developing oocyte (Figure 42D). In male spermatophore concentration increased, first appearance of spermatid was found (Figure 42E).

Development C - Acini size inceased and mature gametes covers 50% of the acini, others were developing gamete (Figure 42F, G).

Development D - 70% gamete reached maturity in both male (Fig. 42I) and female (Fig. 42H).
Mature- Round fully ripe mature ova of polygonal shape were predominant with reduction in developing oocyte in female (Fig. 42J). Ripe spermatozoa were dispersed densely with very narrow duct space in male (Fig 42K).

Spawning A - Gamete discharge started with reduced density in gamete for male and female (Fig. 42L). Partial empty space was observed in male (Fig. 42M).

Spawning B - 50% empty space in the acini was seen (Fig 1N). Apparent evacuating duct resulted from the releasing of spermatozoa with no boundary (Fig. 42O).

Spawning C - Acini was 70% empty (Fig 1P). Reformation of sperm lamellae in the acinus was seen (Fig 42Q).

Spent- Reabsorption of oocyte with collapsed acinus was noticed (Fig. 42R). Sperm broke down (Fig. 42S).

11.2.2 Sex ratio:

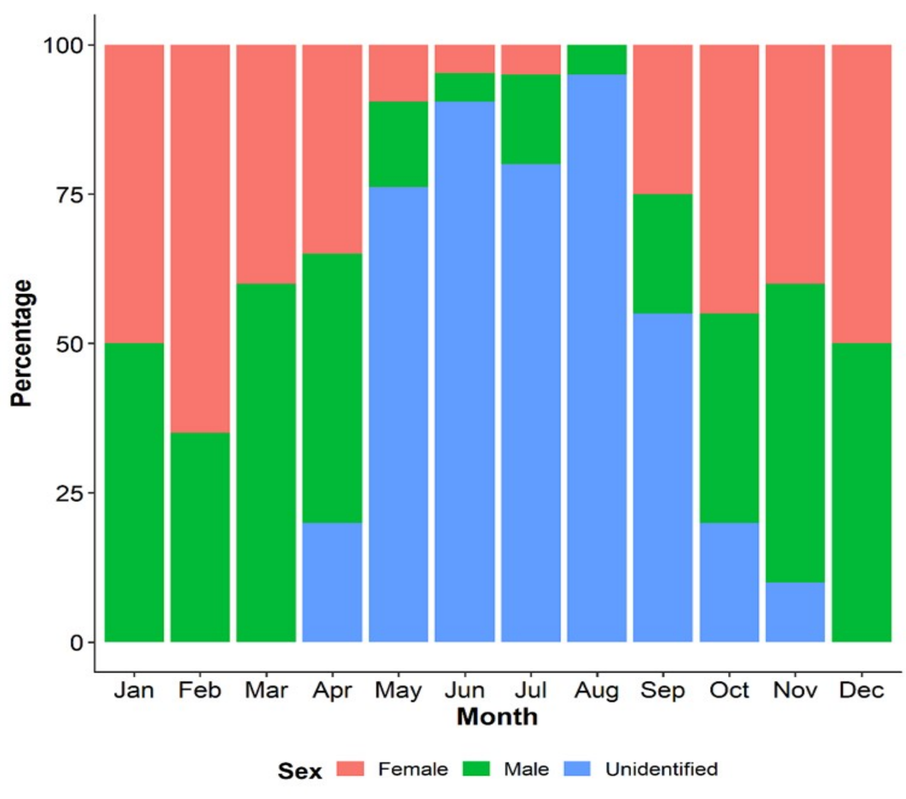


Fig. 43: Proportion (%) of male, female and undifferentiated individuals of *Perna viridis* collected from the coastal water of Bangladesh from November 2017 to October 2018.

Among 242 observed slides 67 were male, 62 were female, and 113 were undifferentiated. The sex ratio was not significantly different from 1:1 ($\chi 2$, $P > 0.05$). No hermaphrodites were observed during the research. Male contributes 27.69% and 25.62% were identified as female based on their histological sex differentiation. Overall sex ratio of male and female was found to be 1:0.93 but considering the monthly ratio in some months male was dominant (March, April, July and November) and in others female dominance (February, October) was established.

11.2.3 Seasonal Variation in Condition Index:

Condition index can be a good indicator of reproductive biology as it is related with the body mass content and food availability. Figure 12 shows the seasonal variation in the condition index. A total of 90 individuals were studied size ranges from 6.6 to 10.9 cm where mean size was 7.9 cm. The graph shows that the value of CI was highest during the month of February and June and the female CI (gm/cm^3) value was relatively higher than male. Highest value of male (11.7942) and female (13.8375) was observed in the month of February and June respectively.

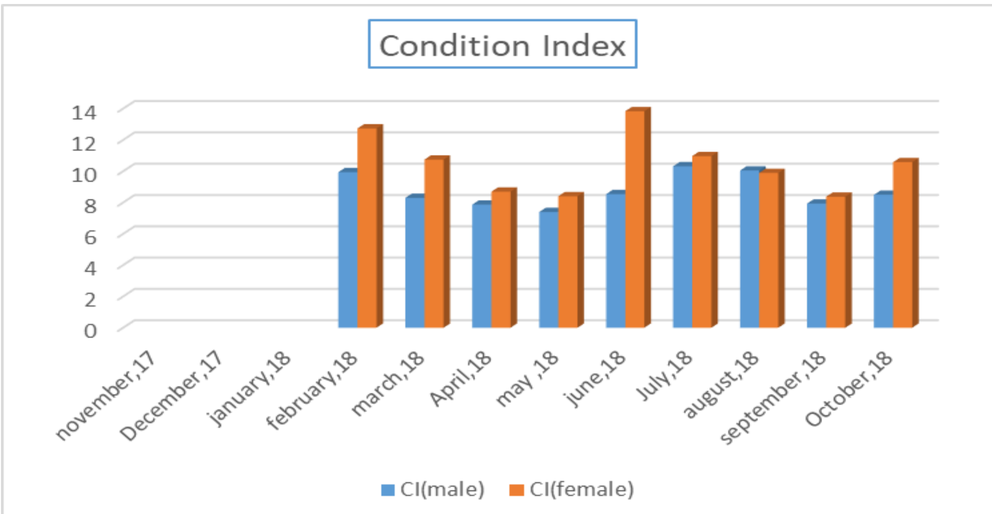


Fig. 44: Seasonal variation in condition index of male and female (dry weight basis)

11.2.4 Seasonal variations in gametogenesis:

Active gametogenesis of *Perna viridis* in Moheskhali Channel was observed annually after a clear inactive or resting stage. Mass gonad development for both male and female started from September after a long resting stage. First evidence of mature gonad was recorded in November (30%). Male started spawning prior to female in December (10% spawning, 60% mature, others development). Rapid spawning for both the sexes occurred from January to May during late winter to early spring. Undifferentiated gonad evident from April indicating resting phase before redevelopment. Resting phase continued till rapid gonad development in late autumn and early winter. Presence of spent gonad was evident for both male and female from February to June. Only 10% gonad development was noted in June and July of 2018 indicating a minor development.

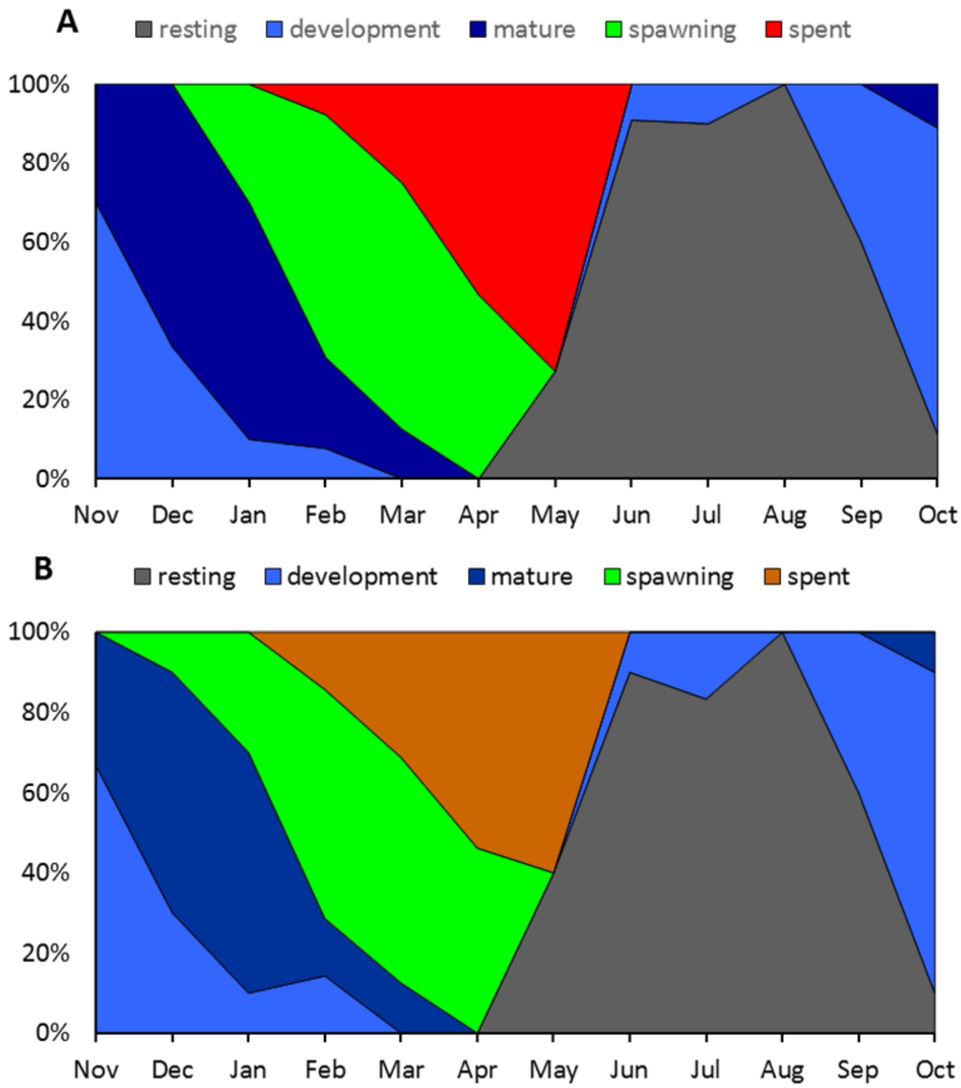


Fig. 45: Monthly variations of gonadal maturity stages (%) in female (A) and male (B) *Perna viridis* collected from the coastal water of Bangladesh during November 2017 to October 2018.

11.2.5 Gonadosomatic Index (GSI):

Seasonal variation in gonadosomatic index (GSI) was used to confirm the result of monthly histological stage distribution. Highest and lowest GSI value was found in January and July. An increasing shift stipulating gonad development was observed after July that reached its peak in January. Following January decreasing inclination of GSI value was interpreted indicating spawning. Male possessed higher GSI value than female though there were no significant differences in mean.

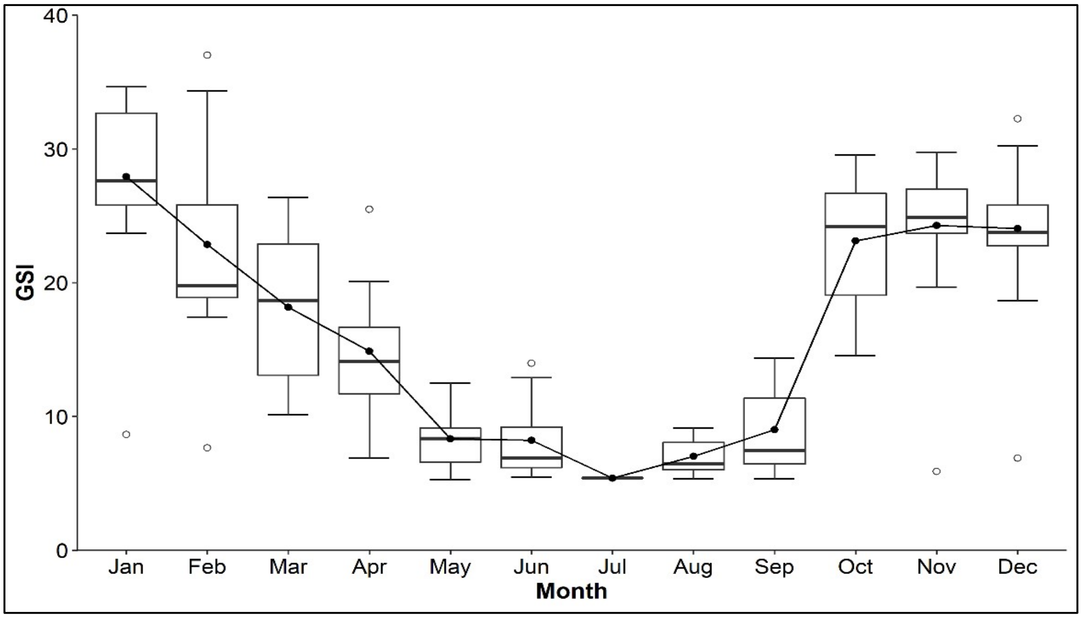


Fig. 46: Box and Whiskers plot of monthly variation of gonado-somatic index (GSI) of Green mussel *Perna viridis* collected from the coastal water of Bangladesh during November 2017 to October 2018.

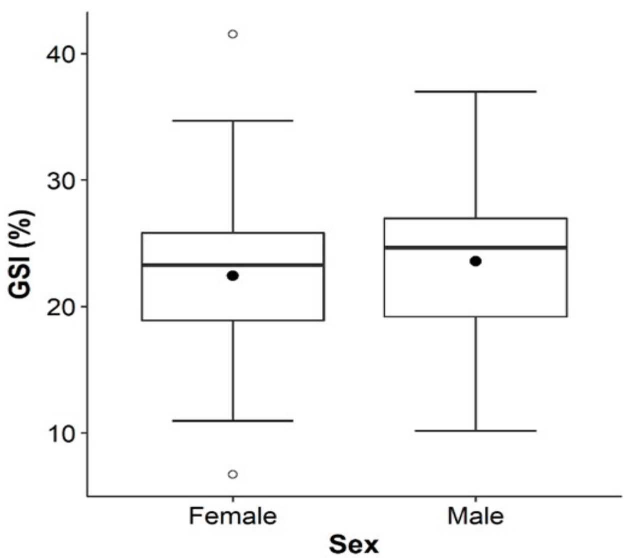


Fig. 47: Variation of GSI between male and female Green mussel *Perna viridis*

11.2.6 Conclusion:

Spawning of Green mussel (*Perna viridis*) in Maheshkhali Channel, Cox's Bazar Chittagong was observed once started from December to April and one minor spawning was observed during the month June and July. It can also be concluded that winter and early spring season was the most preferable seasons as the temperature fluctuation occurs. Hermaphrodite was present in the natural population of Green mussel in Bangladesh.

11.3. Study on the feeding biology of Green mussel

11.3.1 Gut plankton composition according to different length and weight

Length and weight of Green mussel were found to be highly correlated with each other. It was found that larger sized Green mussel consumed higher amount of total plankton (both phytoplankton and zooplankton).

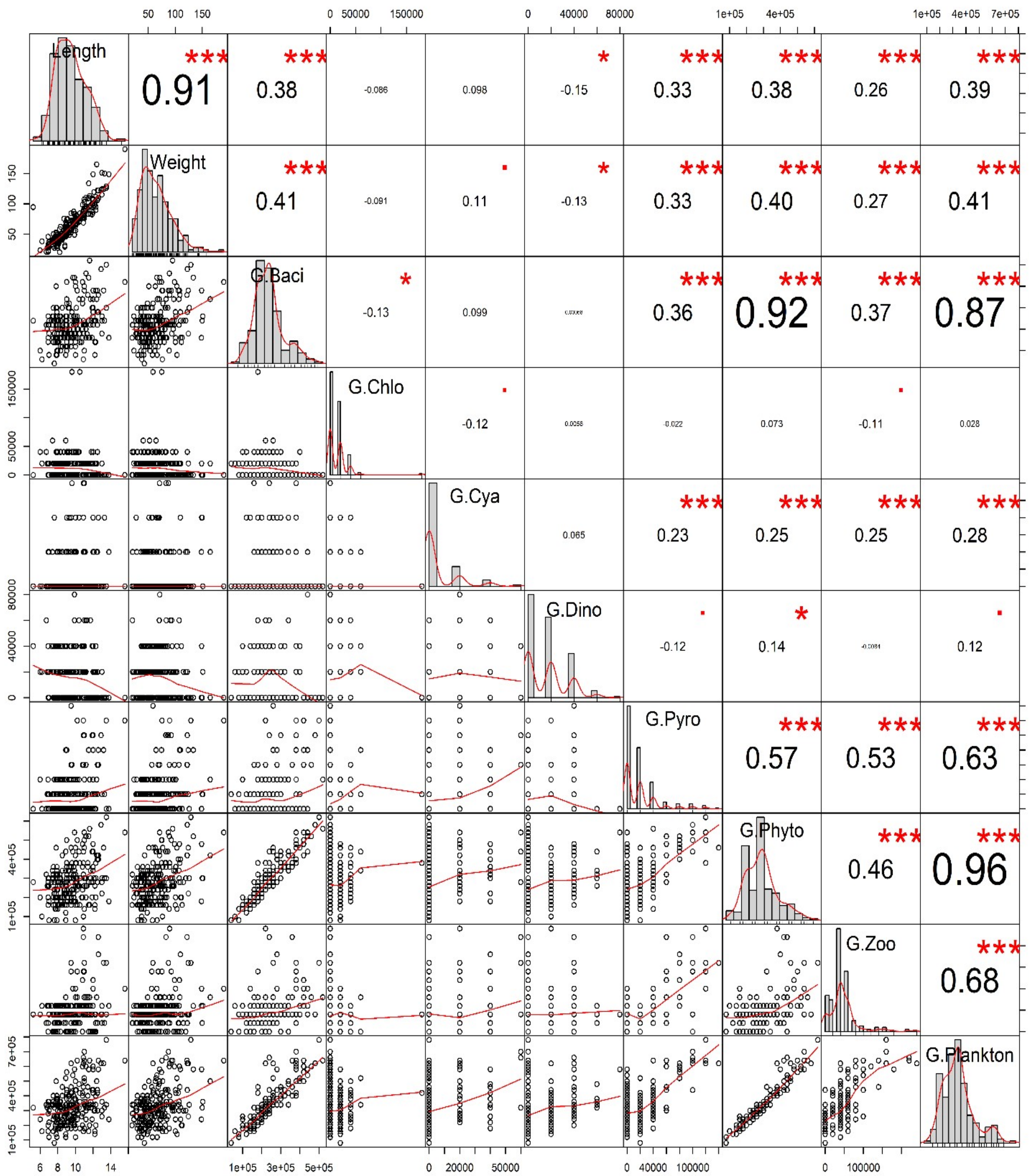


Fig. 48: Interrelation among the size (length and weight) of Green mussel and the major groups of gut plankton ingestion by the Green mussel *Perna viridis* collected from the coastal water of Bangladesh during November 2017 to October 2018. Correlation values and their significance level were indicated for different length- weight of the Green mussel and the different groups of the ingested gut plankton. *significant correlation, ** highly significant correlation, *** very highly significant correlation.

11.3.2 Gut plankton composition

The total gut plankton was found highly correlated with total zooplankton, total phytoplankton, Pyrrophyceae, bacillariophyceae. These values were highly correlated with some water quality parameters ($p^{H)}$ as well. However, negative correlation was found with temperature.

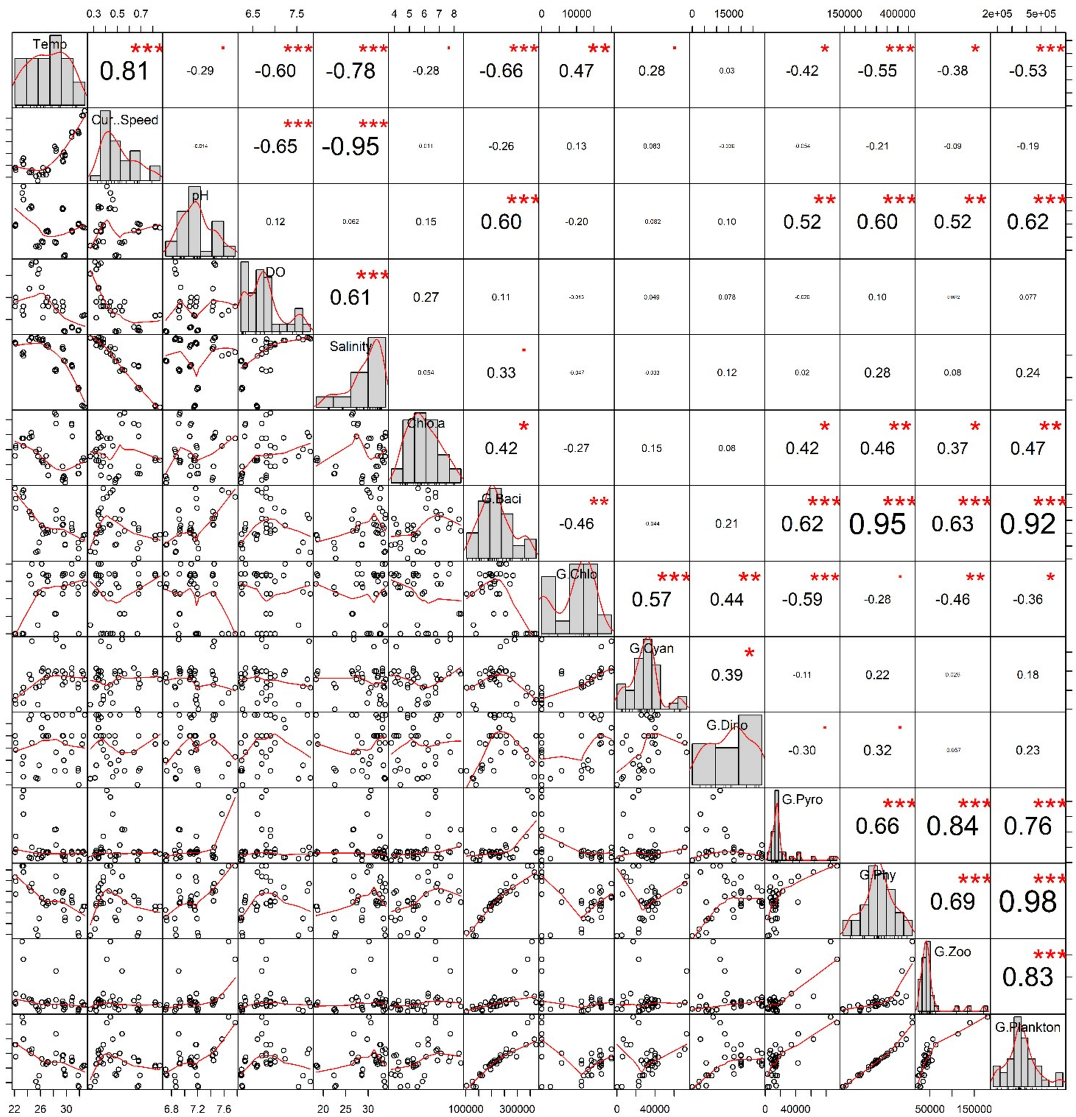


Figure 49. Interrelation among the different physico-chemical water quality parameters and the gut plankton ingestion by the Green mussel *Perna viridis* collected from the coastal water of Bangladesh during November 2017 to October 2018. Correlation values and their significance level were indicated for each water quality parameters and different groups of ingested gut plankton. *significant correlation, ** highly significant correlation, *** very highly significant correlation.

11.3.3: Plankton composition according to breeding season

According to different types of breeding seasons, the stomach content was also analyzed. The breeding seasons were grouped into three consecutive classes (Development=1, Spawning=2 and Resting=3. When it is in developmental season, it takes a greater number of plankton than spawning and resting season. During the spawning season, it takes fewer number of planktons. Highly significant variations were found among each group of plankton in the developmental stage (Table no. 5). During the whole breeding season, *Perna viridis* prefer mainly the phytoplankton of *Navicula*, *Coscinodiscus*, *Rhizosolenia*, *Nitzschia*, *Skeletonema* and *Tropidoneis* from the group Bacillariophyceae, *Microcystis* and *Anabaena* from Cyanophyceae, *Amphidinium*, *Alexandrium* and *Dinophysis* from Dinophyceae, *Ceratium*, *Prorocentrum*, *Pyrocystis* and *Protoperidinium* from Pyrrophyceae. Several types of zooplankton like fish larvae, crab zoea, copepod etc. are also found.

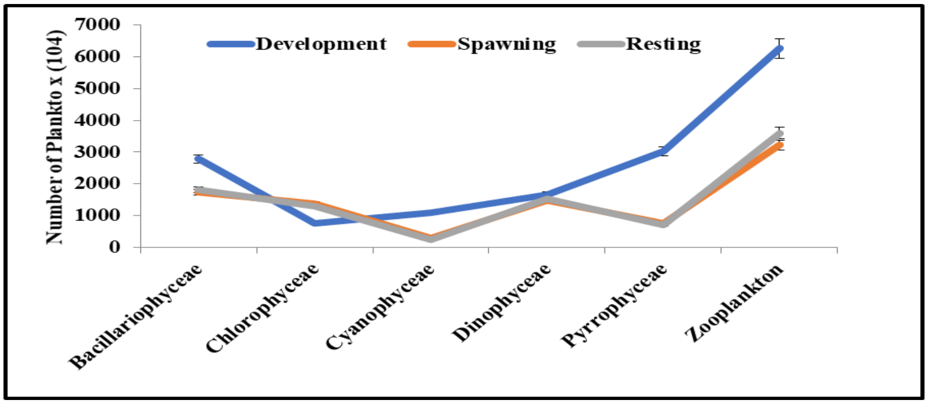


Fig. 50: Plankton variation according to breeding season

11.3.4: Monthly variation of gut plankton composition

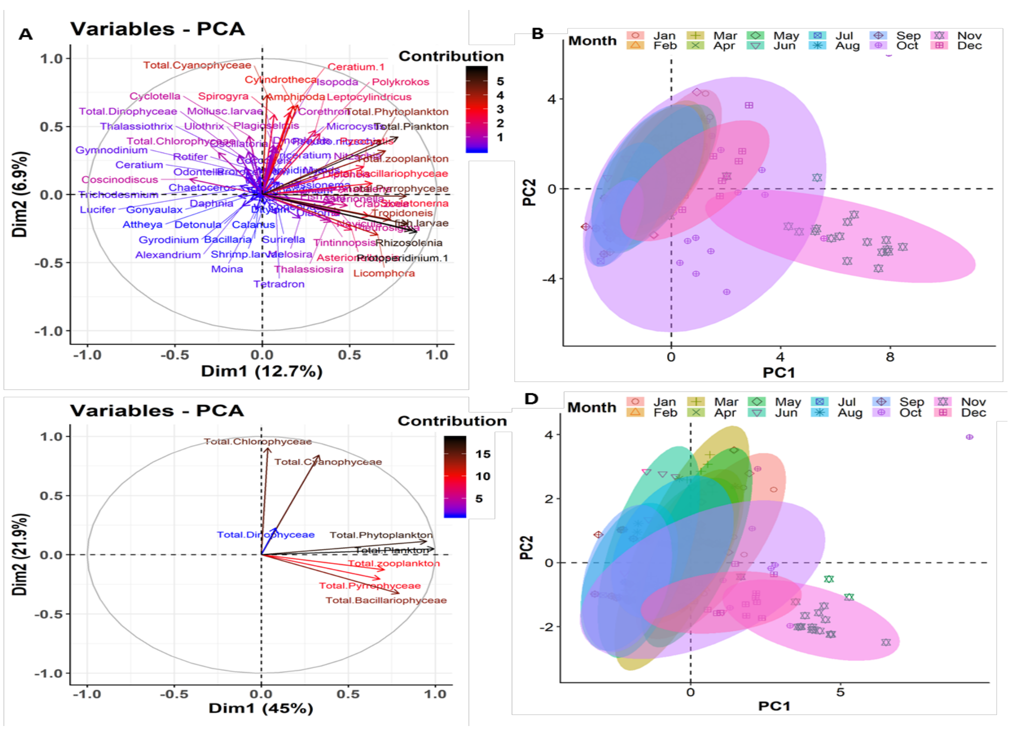


Fig. 51: Biplot of principal component analysis (PCA) of the ingested gut plankton at different months of Green mussel *Perna viridis* collected from the coastal water of Bangladesh during November 2017 to October 2018. A-B: PCA analysis based on the all genus and major groups of the ingested gut plankton at different months; C-D: PCA analysis based on the only major groups of the ingested gut plankton at different months. The outcomes of the analyses are based on the twenty replicated Green mussels for each month (n=240).

Total plankton, fish larvae, *Rhizosolenia* and *Protoperidinium* were the major loading variables for monthly variation in plankton composition. (Figure 51 A). Very overlapping characteristics were shown almost all months except October, November and December (Figure 51 B, D). During the plankton group variation, except zooplankton and Pyrrophyceae all groups were equally contributed (Figure 51 C).

11.3.5: Electivity Indices

11.3.5.1: Bacillariophyceae

There were differences in electivity indices for different plankton and among the different plankton groups. Among 77 genera of plankton available in sea water, Green mussel (*P. viridis*) preferred *Coscinodiscus* (+0.66) followed by *Thalassiothrix* (+0.63), *Cyclotella* (+0.55), *Detonula* (+0.47), *Nitzchia* (+0.39), *Melosira* (+0.35), *Leptocylindricus* (+0.27), *Pleurosigma* (+0.27), *Asteriopnellopsis* (+0.16), *Licomphora* (+0.04), *Thalassiosira* (+0.04), in the Bacillariophyceae group. The main genera for those Green mussel (*P. viridis*) showed avoidance from the group Bacillariophyceae are *Pseudonitzchia* (-0.07), *Tropidoneis* (-0.11), *Navicula* (-0.14), *Odontella* (-0.34), *Rhizosolenia* (-0.43), *Ditylum* (-0.49), *Skeletonema* (-0.69), *Skeletonema* (-0.69), *Diatoma* (-0.70), *Polykrikros* (-0.93), *Surirella* (-0.93).

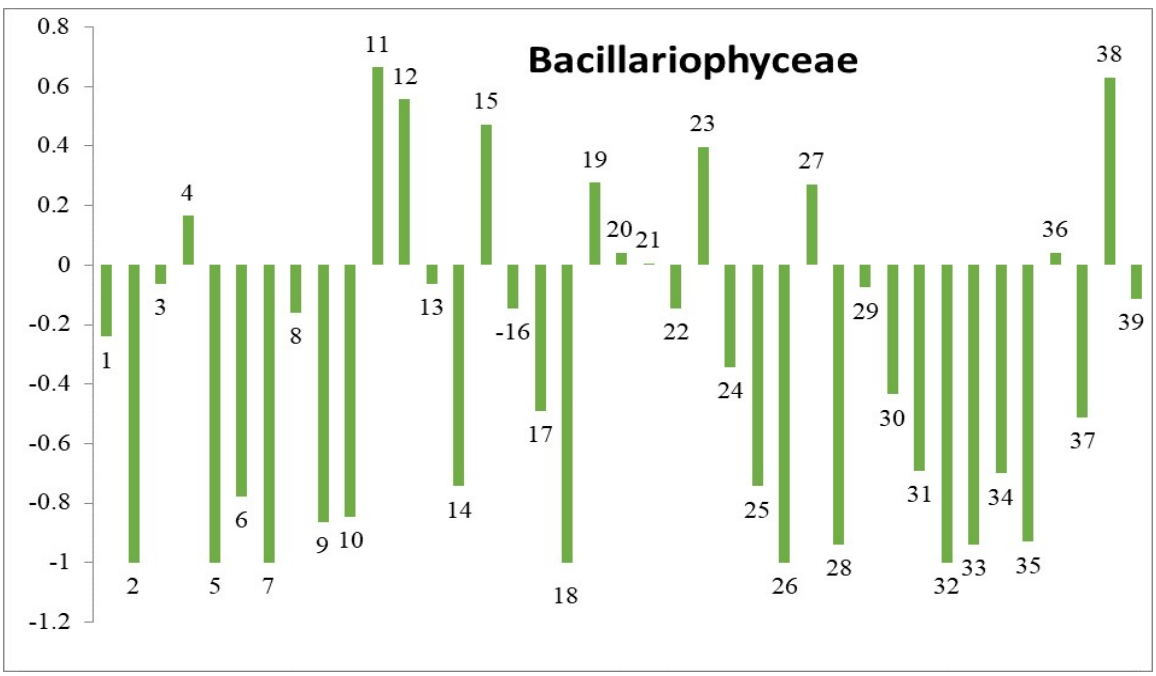


Fig. 52: Graphical representation of selectivity index of Bacillariophyceae

1. *Attheya* 2. *Amphora* 3. *Asterionella* 4. *Asterionellopsis* 5. *Biddulphia* 6. *Bacillaria* 7. *Bacteriastrum* 8. *Chaetoceros* 9. *Cocconeis* 10. *Corethron* 11. *Coscinodiscus* 12. *Cyclotella* 13. *Cylindrotheca* 14. *Diatoma* 15. *Detonula* 16. *Diploneis* 17. *Ditylum* 18. *Fragilaria* 19. *Leptocylindricus* 20. *Licomphora* 21. *Melosira* 22. *Navicula* 23. *Nitzschia* 24. *Odontella* 25. *Plagioselmis* 26. *Planktoniella* 27. *Pleurosigma* 28. *Polykrikos* 29. *Pseudo-nitzschia* 30. *Rhizosolenia* 31. *Skeletonema* 32. *Stephanopyxis* 33. *Surirella* 34. *Tetradron* 35. *Triceratium* 36. *Thalassiosira* 37. *Thalassionema* 38. *Thalassiothrix* 39. *Tropidoneis.*

11.3.5.2: Chlorophyceae

In case of the group of Chlorophyceae mainly selective species was *Spirogyra* (+0.17) and showed strong avoidance for the species of *Chlorella* (-0.1), O*scillotoria* (-0.007) and *Ulotrix* (-0.25) from the equivalent group.

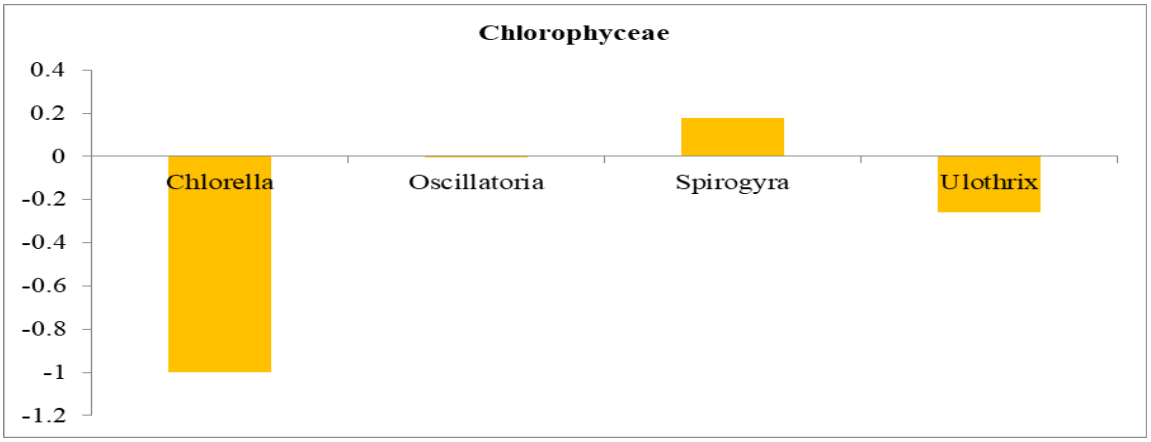


Fig. 53: Graphical representation of selectivity index of Chlorophyceae

11.3.5.3: Cyanophyceae

Though some species of Cyanophyceae group as *Anabaena*, *Trichodesmium*, *Microcystis* etc. were found in the gut of the *P. viridis* but when the electivity indices was analyzed it showed that they were not actively or strongly selective in the diet of the Green mussel even these species were strongly avoided by Green mussel. But these species were found in the water sample. *Anabaena* (-0.45), *Microcystis* (-0.59), *Trichodesmium* (-0.45) and *Nostoc* (-0.1) from the group Cyanophyceae were actively avoided.

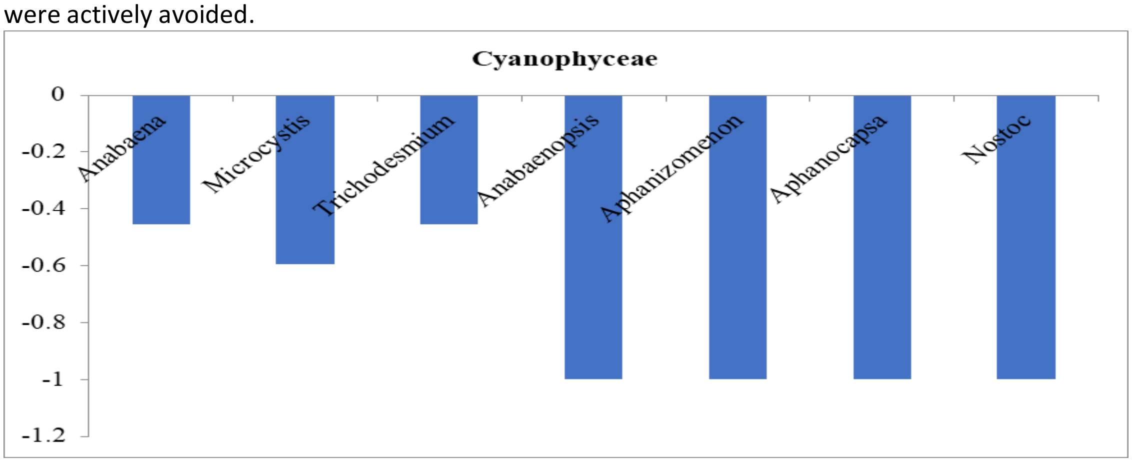


Fig. 54: Graphical representation of selectivity index of Cyanophyceae

11.3.5.4: Dinophyceae

In the group of Dinophyceae, *Dinophysis* (+0.19) followed by *Gymnodinium* (+0.08) are actively selected by *P. viridis* but other species as *Alexandrium, Gyrodinium*, *Amphidinium* were also found in the gut content of the Green mussel but they were not actively selected when electivity indices was analyzed and the values are *Amphinidium* (-0.72), *Gyrodinium* (-0.06), *Gonyaulux* (-0.68) and *Alexandrium* (-0.42).

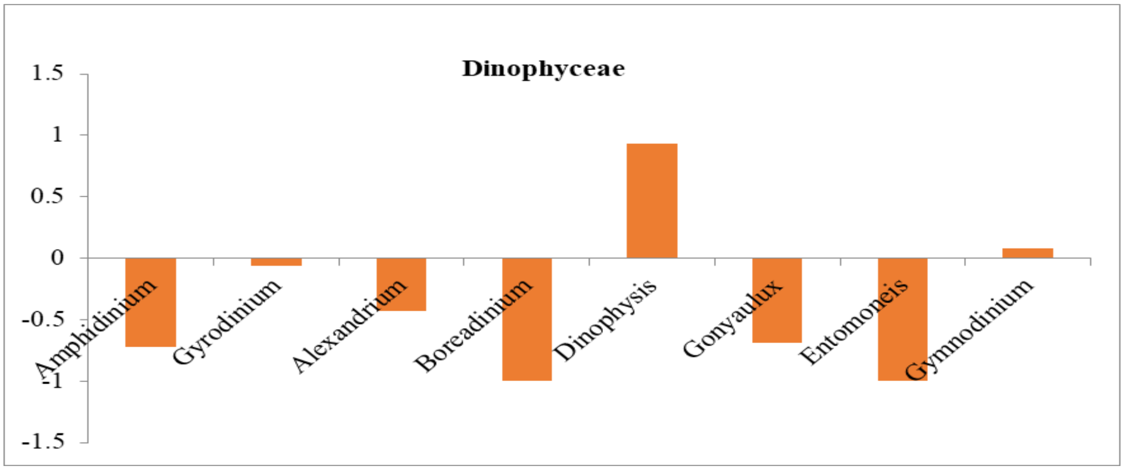


Fig. 55: Graphical representation of selectivity index of Dinophyceae

11.3.5.6: Pyrrophyceae

When electivity analysis was done for the group of Pyrrophyceae then it showed that *Pyrocystis* (+0.22) followed by *Ceratium* (+0.09) and *Prorocentrum* (+0.08) and was strongly selected by *P. viridis*. *Protoperidinium* (-0.56) from the group of Pyrrophyceae was actively avoided.

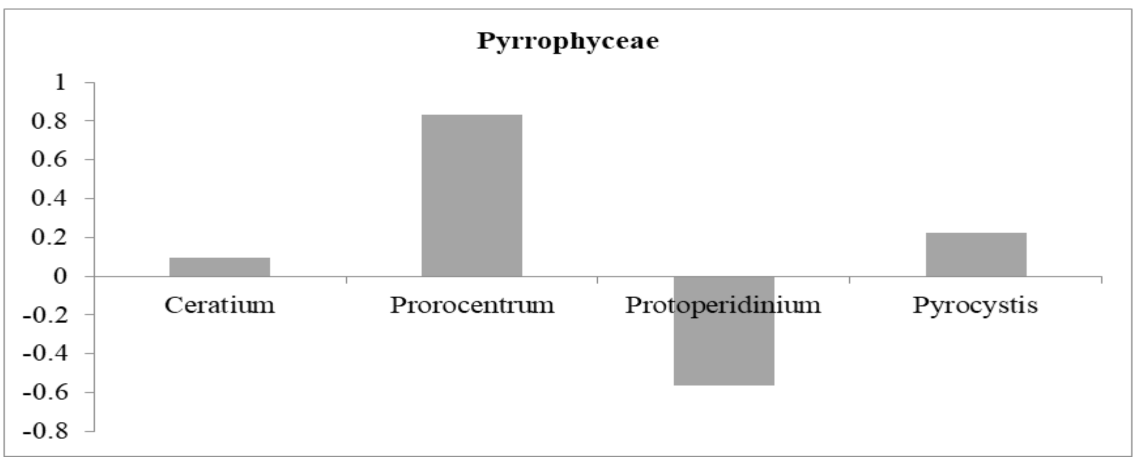


Fig. 56: Graphical representation of selectivity index of Pyrrophyceae

11.3.5.7: Zooplankton

Rotifer (+0.67) was the preferred zooplankton followed by Daphnia (+0.49), Fish larvae (+0.13), Lucifer (+0.06), Mollusk larvae (+0.31) and Moina (+0.46) which were actively selected by Green mussel. Another types of species of zooplankton Copepoda followed by Crab zoea, Fish eggs, Isopoda, Shrimp larvae etc. were also found in the gut of the *P.viridis*. But these species were not actively selected by *P. viridis* and that's why they showed negative values and the values are Amphipoda (-0.87), Copepoda (-0.15), Calanus (-0.06), Crab zoea (-0.51), Fish egg (-0.11), Isopoda (-0.92), Mysids (-0.93) amd Shrimp larvae (-0.19).

Fig. 57: Graphical representation of selectivity index of Zooplankton

The Green mussel (*P. viridis*) preferred diatoms (Bacillariophyceae) and Dinophyceae and Zooplankton, whereas showed avoidance for Chlorophyceae, Cyanophyceae and Pyrrophyceae. *Coscinodiscus* spp. is the selective phytoplankton by *P. viridis* which has previously been reported (Sivalingam, 1977), but the reason was not yet understood. Another study showed that, the selective ingestion of *Coscinodiscus* sp. was corresponded to the highest gametogenesis period (Al-Barwani *et al.,* 2013). From the being study, the *Coscinodiscus* spp. was found selectively increased over other species during all the factors described. A recent current study showed that outstanding selectivity for *Thalassionema* was noticed in the gut of *P. viridis* (Erika *et al.,* 2006). But this attendant work showed the opposite result though this species found in the gut of the *P. viridis*, but when it about selectivity of species it shows the avoidance.

On the other hand, *P. viridis* can also selectively ingest more diverse groups of phytoplankton such as *Pleurosigma* spp., *Lauderia* spp., *Leptocylindrus* spp., *Coscinodiscus* spp., *Rhizosolenia* spp., and *Nitzchia* spp., while still selectively rejecting few groups of phytoplankton mainly *Chaetoceros* spp. and *Bacteriastrum* spp. (Erika *et al.,* 2006). *Cheatoceros* spp. was also found at January and March months in Maheshkhali channel but *P. viridis* never consume this species while *Coscinodiscus* was low to be detected in water (Tan and Ransangan, 2017). The same result also found in case of the completed study in the region of Bangladesh. Gut of Green mussel is enriched with Coscinodiscus spp. (Tan and Ransangan, 2017). Consequently, larger phytoplankton species particularly Coscinodiscus spp. dominating in the nutrient enriched coastal water such as Bay of Bengal (Sarkar *et al.,* 2006) and Sepanggar Bay (Sidik *et al*., 2008).

The Selective ingestion of *P. viridis* is induced by high phytoplankton cell density, where Coscinodiscus spp. is selectively ingested, while *Chaetoceros* spp. and *Bacteriastrum* spp. are selectively rejected. The selective feeding mechanism is phytoplankton specific and could be related to nutritional value of the phytoplankton. Current study also showed that zooplankton constituted an important alternative food source of P. viridis, where copepods and bivalve larvae are the major prey. The other main selective zooplanktons are Daphnia, Lucifer, Moina, Rotifer, Mollusc larvae etc. The relatively higher abundance of copepods in stomach could be explained by the higher composition of copepods in the water.

11.4 Spat settlement of Green mussel

Mussel settlement patterns on natural and artificial substrates are highly variable in space and time (King *et al.,* 1990, Alfaro and Jeffs, 2003). This variability has been attributed to the timing and magnitude of larval supplies, and the physical conditions of the environment such as coastal current, wind patterns, and water temperature (Petraitis, 1991). Seed and Suchanek (1992) have defined the term settlement, as the process whereby individuals come in contact with, and permanently attach to the substratum, a process that includes metamorphosis of the Mussel larvae. Settlement process in Mussels occurs in two phases, a primary settlement phase and secondary settlement phase. The primary settlement phase initially occurs on filamentous algae, which allows a period of grow-out and development free from competition with adult Mussels (Snodden and Roberts, 1997). This phase takes place at shell length of ~ 260 microns in *Mytilus edulis*, although it can be delayed to shell length of 350-400 microns (King *et al.,* 1990). The secondary settlement phase usually occurs after a period of migration, which may last up to three months (Lane *et al.,* 1985). At this phase, the Mussel spat settle permanently onto adult Mussel beds or a suitable, previously uncolonized site (Snodden and Roberts, 1997). The majority of secondary settling Mussels size range were recorded between 0.9-1.5 mm (King *et al.,* 1990). King *et al.* (1990) have shown evidence that some populations of *M. edulis* may settle directly onto adult beds, without an initial growth phase on filamentous substrata.

Commercial Mussel farming depends heavily on reliable seed supply from natural beds. A minimum 5000/m^2 spat density is required for commercial operation (Cheung and Lee, 1984). Efficient spat collection requires accurate forecasting of the time of spat fall, the right substrate, and the right depth. Mussel larvae are commonly found in the plankton of most coastal waters for most of the year, and there are probably some settlement events throughout the year (Walter, 1982). However, there are seasonal peaks of settlement which vary annually and locally. Timing of those seasonal peaks of settlement is highly critical since setting the collector earlier than the spawning period.

Table 20. Monthly variation of Green mussel spat settlement in ropes settled in different ways in Maheshkhali Channel, Cox's Bazar

Months	**Hanging rope**		**Hanging rope net**		**Pole-rope**	
	<2 mm size	>2mm size	<2 mm size	>2mm size	<2 mm size	>2mm size
December	65±18	0±0	74±14	0±0	42±8	0±0
January	380±64	164±22	342±46	155±24	260±46	118±32
February	360±42	440±48	280±34	360±32	240±42	250±45
March	80±12	120±28	64±12	140±36	48±12	80±42
April	0±0	40±14	0±0	25±8	0±0	15±48
May	0±0	0±0	0±0	0±0	0±0	0±0
June	0±0	0±0	0±0	0±0	0±0	0±0
July	18±4	0±0	26±8	0±0	12±6	0±0
August	36±7	10±2	42±7	14±3	24±5	8±3
September	0±0	42±6	0±0	36±6	0±0	26±5
October	0±0	0±0	0±0	0±0	0±0	0±0

Larval occurrence noticed from the December and spat settlement was noticed from the December in Maheskhali channel of Cox's Bazar. At December, comparatively little number of Green mussel spats were settled. January and February were the pick months of spat settlement in the Maheshkhali Channel of Bangladesh. From March, spat settlements were decreased rapidly, and at April a few spats only larger size was noticed. At May and June, spat settlements were observed at all. However, a minor settlement of Green mussel was also observed from the month of July to September. Among the three systems, both the hanging rope and hanging rope-net showed better settlement compare to the pole culture.

11.5 Growth performance of Green mussel in pilot-scale culture system

Three pilot-scale culture sites were established in the Maheskhali Channel. Each culture sites consists of 4 different culture systems such as hanging rope culture, hanging rope-net culture, cage culture and pole culture systems. In the case of cage culture, we established cages at three depth to investigate the effects of depth on the settlement of Green mussel. We observed a large number of Green mussels spat settlement, however many of these spats were eaten by the barnacles. The density of Green mussel settlement was observed as follows:

Table 21. Monthly variation of Green mussel density on different culture system in Maheshkhali channel

Culture Methods	March	April	June	July	August	September
Hanging Rope (In/feet)	84±8	153±14	172±8	170±12	182±12	190±16
Pole culture (In/feet)	80±12	120±16	122±16	128±14	134±21	138±24
Rope net culture (In/feet)	127±18	157±14	158±12	164±18	168±14	174±18
Cage culture (In/feet^2)	160±26	189±24	188±18	192±26	212±24	224±26

Our results initially suggest that Green mussel can be cultured in hanging rope, rope net and cage culture. The number of Green mussels increase over the month is mainly due to the continuous settlement over the time. The calculated density of Green mussel in the actual culture system was much lower than spat settlement data might be due to the predation by barnacles and we calculated average settlement that includes many non-settlement areas in the ropes

Fig. 58: Green mussel grown in our culture system at Maheshkhali channel

We observed the monthly growth performance of Green mussel over the time. The data showed the satisfactory growth performances of the Green mussel. Growth performance data also showed that cage culture and rope culture might be the best options of Green mussel culture.

Table 22. Monthly growth performances (in gram) of Green mussel in different culture system in Maheshkhali channel

Culture Methods	April	May	June	July	August	September
Hanging Rope	0.54	3.23	5.86	8.96	13.42	18.24
Pole culture	0.45	2.42	5.42	7.54	10.84	14.34
Rope net culture	0.50	2.73	4.92	8.54	11.25	16.26
Cage culture	0.52	2.98	5.94	9.46	12.84	16.58

11.6 Seasonal variation of nutritional profile and heavy metal content of Green mussel

11.6.1 Monthly variation of proximate composition

The proximate composition analysis was done all the year round according to different seasons. In case of moisture content of Green mussel, the highest amount was found during autumn and lowest amount was found during winter season. Protein percentage was found in highest amount during the season of winter and it was 19.88% and when it is autumn, it shows the least percentage. Lipid content was found in the highest amount during winter season and lowest was during autumn. Then, when the ash content analysis was done, the amount was maximum during the spring season and minimum percentage was found during late autumn. Analysis was also done for Nitrogen free extract and the maximum and minimum amount was found during winter and autumn. In a whole, Green mussel contain highest amount of moisture and protein.

Table 23. Monthly variation in proximate composition (on wet basis) is given below:

Parameters	**Summer**	**Rainy season**	**Autumn**	**Late autumn**	**Winter**	**Spring**
Moisture (%)	72.24±3.24	76.28±3.14	76.94±2.86	74.48±2.24	70.12±2.84	71.24±1.98
Protein (%)	18.46±1.28	16.28±0.84	16.24±0.84	18.02±0.64	19.88±0.41	18.94±0.28
Lipid (%)	2.84±0.22	2.12±0.28	2.02±0.22	2.44±0.18	2.94±0.24	2.90±0.32
Ash (%)	2.56±0.18	2.24±0.26	2.14±0.18	2.01±0.24	2.26±0.32	2.85±0.12
NFE (%)	3.90±0.22	3.08±0.28	2.66±0.44	3.05±0.21	4.8±0.42	4.07±0.33

11.6.2 Heavy metals analysis:

The heavy metal content was also analyzed for Green mussel according to different seasons and the analysis unit was ppm. The main considered heavy metal parameters were Lead (Pb), Cadmium (Cd), Mercury (Hg) and Chromium (Cr). During rainy season Lead and cadmium was found 0.51 ppm and 0.15 ppm. These are the maximal content for Lead and Cadmium. Mercury was found constant throughout the year and it was **<**0.10 ppm**.** In case of Chromium, rainy season again showed the greater amount and it was 0.6515 ppm. So, for heavy metal, during rainy season, all heavy metals showed the maximum content though mercury was same throughout the year.

Table 24. Season wise variation of heavy metals in Green mussel meat

Parameters	**Summer**	**Rainy season**	**Autumn**	**Late autumn**	**Winter**	**Spring**
Lead Pb (ppm)	0.41	0.51	0.45	0.36	0.17	0.28
Cadmium Cd (ppm)	0.05	0.15	0.12	0.1053	0.088	0.11
Mercury Hg (ppm)	<0.10	<0.10	<0.10	<0.100	<0.10	<0.10
Chromium Cr (ppm)	0.359	0.6515	0.389	0.239	0.220	0.243

11.6.3 Essential amino acid and Essential fatty acid profile:

The fifteen different types of fatty acids were found in the lipid content of Green mussel (*P. viridis*). Saturated fatty acids were found in highest amount during summer and lowest during late autumn but Myristic acids were not found during summer but highest amount was found during rainy season. Palmitic acids and Stearic acid both are found in maximum content during summer season and minimal during winter and late autumn. In case of Unsaturated fatty acids and Monounsaturated fatty acids, they are found in higher amount during winter and rainy season. Palmitoleic acid and Oleic acid were found in highest amount during rainy season and spring season but Oleic acid was not found at all during summer season. Polyunsaturated fatty acid was also found in Green mussel and it was in maximum amount during winter and lowest during rainy season. Linoleic acid and Linolenic acid were found in higher amount during winter and spring. Arachidonic acid, Docosahexaenoic acid and Eicosapentaenoic acid were also analyzed and Green mussel contain these fatty acids too. Arachidonic acid is highest during summer and lowest during spring. Docosahexaenoic acid and Eicosapentaenoic acid were both lowest during spring and highest during winter.

Table 25. Variation of fatty acid content in different seasons

Parameters	**Summer**	**Rainy season**	**Autumn**	**Late autumn**	**Winter**	**Spring**
Saturated fatty acids	46.21±3.21	45.35±2.86	44.45±2.54	39.25±3.42	38.24±3.24	43.24±3.44
Myristic acids (C14:0)	--	8.88±1.15	6.23±0.87	5.88±1.12	6.12±0.78	8.21±1.24
Palmitic acids (C16:0)	39.17±2.84	30.35±2.38	31.64±2.52	30.63±3.12	28.12±2.65	29.94±3.14
Stearic acid (C18:0)	7.04±.0.89	6.12±0.54	6.58±0.46	2.74±0.24	4.01±0.46	5.09±0.86
Unsaturated fatty acids	53.79±4.21	54.65±3.64	55.55±4.86	60.75±4.22	61.76±4.22	56.76±3.84
Monounsaturated fatty acids	14.75±1.12	20.48±1.26	18.24±0.86	16.68±0.96	15.24±0.84	20.26±1.34
Myristoleic acid (C14:1)	0.72±0.06	0.96±0.12	1.14±0.16	0.88±0.12	1.24±0.14	1.36±0.22
Palmitoleic acid (C16:1)	14.02±1.12	14.62±1.06	12.24±1.24	10.26±1.46	8.9±0.84	12.98±1.34

Oleic acid (C18:1)	-	4.89±0.22	4.76±0.46	5.54±0.24	5.02±0.42	5.92±0.24
Polyunsaturated fatty acids	39.03±3.48	34.17±2.88	37.31±3.24	44.07±4.12	46.52±4.20	36.5±3.12
Linoleic acid (C18:2)	2.20±0.14	1.22±0.12	2.24±0.22	1.88±0.08	2.54±0.12	2.12±0.42
Linolenic acid (C18:3)	4.00±0.48	1.04±0.08	2.58±0.44	3.24±0.48	2.34±0.12	4.44±0.48
Arachidonic acid (C20:4)	13.32±1.24	2.84±0.84	2.12±0.26	3.12±0.38	2.36±0.24	1.96±0.24
Eicosapentaenoic acid (C20:5)	10.18±2.12	15.80±2.14	14.68±2.24	16.24±3.82	19.04±4.08	12.54±2.44
Docosahexaenoic acid (C22:6)	9.33±1.14	13.27±1.68	15.69±3.12	19.59±4.06	20.24±3.94	15.44±3.14

Different types of amino acids are also contained by Green mussel (*P. viridis*) and they are varied according to different seasons. 14 types of amino acids were found in Green mussel. From all of these amino acids, Glutamic acid showed the highest amount and it was summer. Aspartic acid was maximum during late autumn and Threonine and Serine were during winter. They were minimal in amount during winter, late autumn and rainy season. Glycine was higher during summer and lower during autumn and winter. Alanine and Valine were highest during spring and lowest during summer and autumn. Methionine was found lowest during summer and highest during winter. In case of Isoleucine and Leucine, maximum amount was found in the season of winter. Tyrosine, Histidine, Lysine and Arginine were also found in Green mussel meat and Lysine was found at a greater amount than Histidine and Arginine.

Table 26. Seasonal variation of amino acid content

Parameters	Summer	Rainy season	Autumn	Late autumn	Winter	Spring
Aspartic acid	3.91	3.24	3.68	3.98	2.88	3.25
Threonine	2.65	2.24	2.34	1.98	2.78	2.56
Serine	3.09	2.56	2.78	3.24	3.12	2.85
Glutamic acid	5.96	4.32	4.94	4.78	4.12	4.26
Glycine	3.02	2.68	2.54	2.88	2.54	2.96
Alanine	2.61	2.48	2.66	2.74	2.88	3.12
Valine	2.35	2.12	1.98	2.34	2.78	2.84
Methionine	0.87	1.12	1.24	1.88	2.34	2.14
Isoleucine	1.88	1.76	1.86	2.12	2.46	2.24
Leucine	3.04	2.86	2.78	3.24	3.28	2.88
Tyrosine	2.17	2.25	2.43	2.02	1.86	2.04
Histidine	1.69	1.34	1.68	1.88	1.94	1.66
Lysine	3.66	3.88	3.46	3.52	3.96	3.44
Arginine	2.98	2.55	2.96	2.64	2.78	2.68

12. Research highlight/findings:

- Site capability rating system based on the biophysical variables confirmed that Rezu khal is not much good for Green mussel culture. However, Maheshkhali Channel and The Naf River is mostly suitable for Green mussel culture in the coastal areas of Bangladesh. Maheshkhali Channel has the best suitability among all the sites.

- Spawning of Green mussel (Perna *viridis*) in Maheshkhali Channel, Cox's Bazar Chittagong occurs twice. Major spawning season started from Novermber to March and minor spawning during the month of June and July. Winter and early spring season was the most preferable seasons as the temperature fluctuation occurs during that time.

- Green mussel selectively fed on the plankton. Phytoplankton contributes the major portion of their diet. Green mussel is highly selective and preferentially fed on *Coscinodiscus* and *Thalassionema*. Feeding habit of Green mussel are highly influenced by the season, sex, breeding season and size of the Green mussel.

- Larval occurrence and spat settlements were noticed from the December in Maheskhali channel of Cox's Bazar. January and February were the pick months of spat settlement in the Maheshkhali Channel of Bangladesh. At May and June, spat settlements were not observed at all. However, a minor settlement of Green mussel was also observed from the month of July to September.

- Pilot scale culture systems further confirmed the feasibility of Green mussel culture in Bangladesh. Hanging rope culture, rope net culture and cage culture, all have the high potentiality for Green mussel culture. However, our experience showed that hanging rope from a floating raft would be the best options of Green mussel culture in the context of Bangladesh.

- Nutritional profiling data showed that Mussel meat is enriched with high protein and lipid. The protein contents vary with the season, the lipid of Green mussel contain higher percentages of unsaturated and polyunsaturated fatty acids along with DHA and EPA, and the highest amount of these nutrients are found during spring and summer in Mussels. The amino acid content in Mussel meat is very high during spring and summer and the highest content of glutamic acid is found in Mussels.

- Heavy metal analysis data showed that the heavy metal content of Green mussel were within the safe limit for human consumption.

- In conclusion, it can be stated that there is huge potential for the culture of Green mussel in the coastal areas of Bangladesh.

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	Office furniture, computer and computer chair and table	128,000	Office furniture, computer and computer chair and table	128000	All office equipment purchased
(b) Lab &field equipment	Refractometer, Mutli-parameter, water sampler set, WTW photoplex, Plankton net, Laboratory	990,000	Refractomer, mutliparameter, water sampler, WTW photoplex, plankton net,	984000	All field and laboratory equipment purchased successfully

	freeze, Current meter		laboratory freeze, current meter		
(c) Other capital items	-	-	-	-	-

2. Establishment/renovation facilities: N/A

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
Electricity supply of research desk of the Fisheries Oceanography laboratory			To upgrade the electricity facility in the research desk	We successfully upgraded it	

3. Training/study tour/ seminar/workshop/conference organized: N/A

Description	Number of participants			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training					
(b) Workshop					

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	431991	428373	428373	0.0	100%	-
B. Field research/lab expenses and supplies	2410229	2336275	2409700	-73425	100%	-
C. Operating expenses	581880	581864	581862	2.0	100%	-
D. Vehicle hire and fuel, oil & maintenance	216000	204452	216000	-11548	100%	-
E.Training/workshop/seminar etc.	0	0	0	0	-	-
F. Publications and printing	135000	0	135000	-135000	100%	-
G. Miscellaneous	110000	99760	93954	-5806	100%	-
H. Capital expenses	1114900	1088740	1114500	-25760	100%	-

D. Achievement of Sub-project by objectives:

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
To apply site	Five stations were selected from	The data confirmed	Suitable

capability rating system for fast and effective evaluation of potential farming sites	each of the Rezu khal, The Naf river and Maheshkhali channel area. Water quality and plankton dynamic were monitor year-round. Site capability rating system were applied	that Maheshkhali channel and The Naf river are most suitable—as reported by three Master theses	culture sites for Green mussel culture were known
To obtain near real-time data on breeding biology of Green mussel	Year-round analysis of gonadal tissue was done by histochemistry. Beside this, length-weight relationship, condition index, gonad index and gonadosomatic index were documented	Two breeding seasons of Green mussel was identified –as reported by Master thesis	Breeding and spawning time of Green mussel was known
To obtain near real-time data on the feeding biology of the Green mussel	The gut contents were analyzed year-round. The relative contribution of phytoplankton and zooplankton were document and electivity index were calculated. All the values were further related with season, sex, size and breeding season	Feeding biology, food selectivity and electivity indices were document for Green mussel by Master thesis	Feeding biology of Green mussel was known
To obtain near real-time data on larval and spat occurrence of Green mussel	Larval and spat settlement occurrence were monitored monthly by establishing ropes and rope nets at Maheskhali channel	Peak season of larval occurrence and spat settlement was documented—soon to be published in a scientific article	Quantitative Spat availability in Maheshkhali channel was known
To establish pilot-scale culture system of Green mussel	Green mussel culture systems were established at three sites of Maheshkhali Channel. Each site consist of four different culture system namely ropes, ropes nets, poles and cage culture.	Growth performance and settlement density in different culture systems were document	Hanging rope culture and rope-net culture is most suitable culture system
To know the nutritional profiles and heavy metal content of Green mussel in different season	Proximate composition, heavy metal contents, fatty acid composition and amino acid profiling was conducted on seasonal basis	Heavy metal and nutritional value of Green mussels in terms of proximate composition, fatty acid and amino acid content was documented	Safety and nutritional value of Green mussel was known

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publications		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/	**1**	1	BFRI, Bangladesh

booklet/leaflet/flyer etc.			
Journal publication	4	2	Four paper is almost ready to publish in high quality journal
Conference publication	-	4	CVASU, BFRF
Information development	**-**	-	
Other publications, if any Daily Newspaper	**-**	6	Prothom-alo, Bdnews-24, Krishi Khamar, Sothik Barta and others
Master's thesis	**0**	6	Department of Marine Bioresource Science, CVASU

F. Technology/Knowledge generation/Policy Support:

i. **Generation of technology (Commodity & Non-commodity)**

Not Applicable

Generation of new knowledge that help in developing more technology in future

The project generates many new knowledges about the culture feasibility of Green mussel that would be helpful to develop different technology in future. For example, we determine the breeding and spawning biology and season that would be helpful to develop artificial breeding technology of Green mussel. Besides, we investigated many basic researches such as site suitability and feeding biology that would be also helpful to develop Green mussel culture technology in the coastal areas of Bangladesh. The pilot-scale culture experiments showed the feasibility of Green mussel culture in Bangladesh and the finding would be helpful to develop Green mussel culture technology in future.

ii. **Technology transferred that help increased agricultural productivity and farmers' income**

Not Applicable

iii. **Policy Support**

The findings of the present study would be helpful for the policy maker to take decision about the introduction of non-conventional Green mussel aquaculture system as an alternative income generating source of coastal fishermen and for boosting the blue economy of Bangladesh

G. Information regarding Desk Monitoring

i) Desk Monitoring:

1) CRG Sub- Project Implementation Progress Workshop/Seminar held in BARC, Farmgate Dhaka on 21 December 2017. Appreciated
2) CRG Sub-Project Final output Workshop held in BARC, Farmgate Dhaka on 19-20 September 2018. Expressed satisfaction

ii) Field Monitoring:

Duration of Field Visit: From 21th March 2018 to 24th March 2018. The monitoring was done by the Deputy Project Director of NATP. The Visiting team was highly satisfied with the progress of the project. They felt that we are doing the challenging work in the coastal high tidal areas in the seas. The team suggested for continuing this work and if possible, it would be better to develop the long-term research plan.

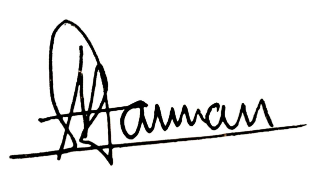
G. Lesson Learned:

i) There is a huge potential of Green mussel aquaculture in Bangladesh. The development of such aquaculture technology would boost the blue economy concept of Bangladesh and can provide alternative income and employment opportunity of the resource poor farmers in the coastal region of Bangladesh.
ii) The participatory approach of the research is needed to develop the culture technology of Green mussel in Bangladesh where the researcher would work with the direct and active participation of the coastal fishermen.

iii) Long-term research is needed to develop such technology with direct participation and collaboration of different government institution like DoF, research organization like BFRI and Universities and non-government organization like NGOs

H. Challenges:

- Poaching of the research materials and culture systems
- Conflicts with the other coastal fishermen who catches fish from the same habitat
- Short duration of the project implementation time
- Impulsiveness of the climatic condition
- The deep depressions causing local cautionary signals, sudden landslides, downpour flood, tidal gush and cyclonic storm



Signature of the Principal Investigator
Date ………………………….
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Counter signature of the Head of the organization/authorized representative
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