Figure 4. Preliminary success on Kain Magur breeding

Discussion and conclusion.

Five commercially important fish species was selected under this project to study the feeding and breeding biology, found out the peak breeding season, domestication under captive condition and finally breeding of the fish under hatchery conditions. Majority of the fish are carnivorous in nature except the Mugil cephalus. Domestication of carnivorous fish is really very hard. However, the research team has already collected some brood fish of each species except the Taposi. The Taposi is a very sensitive fish and the researchers are unable to collect good quality live fish. A few live fish has been collected but died during transportation or immediately after stocking. However, fish species are under domestication protocol and more fish will be collected simultaneously. Breeding trial will be conducted if any fish is ready for breeding.

Development of culture technique of microalgae isolated from brackishwater for shrimp and fish larvae feed (Comp.- B)

Researchers

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Objectives.

● To identify and isolate commercially important microalgae from the coastal region

● To investigate the nutritional status of identified microalgae

● To utilize the isolated microalgae as a live feed supplements for fish/shellfish larvae culture

Achievement

Experiment 1. Identified and isolated commercially important microalgae from the coastal region

Firstly, plankton samples were collected fortnightly in a month from pelagic waters of the open sea estuary and river. Sixty litters of pelagic water from 6 different parts (after 300 meter interval) of the areas were passed through the plankton net with the help of a plastic bucket of 10 liter capacity. The water was passed down through the net and the plankton condensed at the lower end of the plankton net then it was collected into a sampling bottle. After collection, one part was preserved by 5% formalin for identification and another part used for Isolation. This year total of 16 species has been identified (Fig1). Meanwhile, 3 species has been isolated from the samples.

Experiment 2. Cultured of isolated micro-algae

The Zarrouk media (ZM), Kosaric media (KM) and local derived bangla media (BM) was used for Spirulina culture. Meanwhile, compost media was used for Skeletonema culture. Whereas, Gillard and Ryther's Modified f/2 media was tested for other micro-algae culture.

Culture of *Spirulina platensis*

In this experiment, total three tpyes of media were used for the culture of spirulina and the culture period was 12 days. In this periods regular growrth and water quality parameters have been observed. Among 3 media, in Zarrouk's media spirulina growth rate was always higher whereas in Bangla media growth rate was always low. After six days the spirulina growth was highest among 3 types media. Then it was harvested, dried and analyzed for nutritional value.
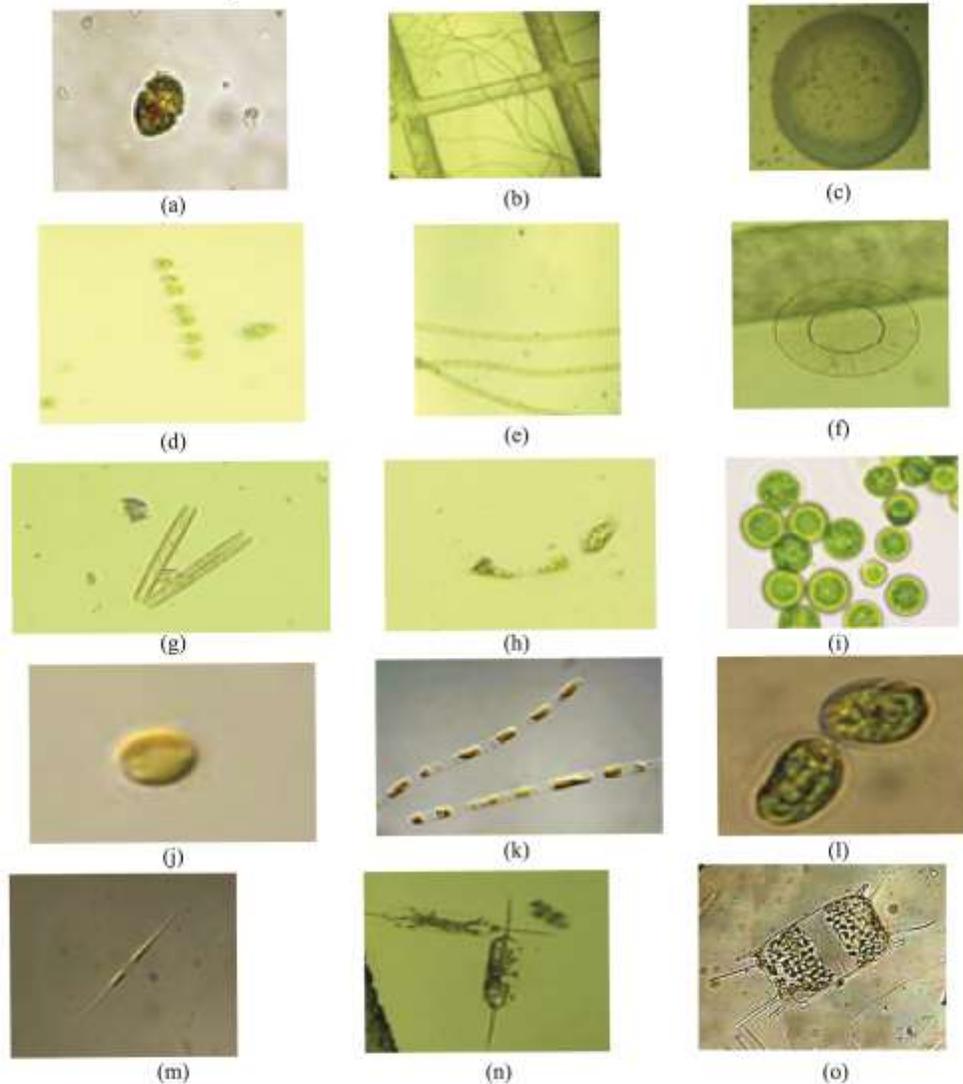
Identified micro-algae

Figure 1 : (a) *Gymnodinium*, (b) *Spirulina platensis*, (c) *Coscinodiscus*, (d) *Chaetoceros*, (e) *Spirogyra*, (f) *Planteoniella*, (g) *Thalassionema*, (h) *Thalassiosira*, (i) *Nanochloropsis oculata*, (j) *Isochrisis*, (k) *Skeletonema costatum*, (l) *Tetraselmis*, (m) *Cylindrotheca*, (n) *Ditylum* (o) *Biddulphia*

Daily Growth performance of KM, ZM and BM media

Table 1. Daily growth pattern of Spirulina under different seected media.

Date	KM (cell/ml)		ZM (cell/ml)		BM (cell/ml)	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
09.03.22	1000-2000	1500±500	8000-20000	14000±6000	3000-600	4667±1247
10.03.22	2000-3000	2500±500	12000-24000	18000±6000	4500-7000	6167±1178
11.03.22	5600-18000	11800±6200	15000-35000	25000±10000	5000-8000	6333±1247
12.03.22	10000-26000	18000±8000	27000-37000	32000±5000	5500-9000	7167±1433
13.03.22	19000-29000	24000±5000	30000-40000	35000±5000	7000-13000	9667±2494
14.03.22	28000-35000	31500±3500	32000-41000	36500±4500	8000-17000	13667±4027
15.03.22	5000-22000	13500±8500	4000-5000	4500±500	9000-14000	11000±2160
16.03.22	6000-21000	13500±7500	5000-7000	6000±1000	9500-14000	11333±2160
17.03.22	10000-18000	14000±4000	11000-12000	11500±500	6000-7000	6667±471
18.03.22	12000-17000	14500±2500	15000-20000	17500±2500	5000-7000	5667±942
19.03.22	15000-15500	15250±250	21000-25000	23000±2000	5000-6000	5333±471
20.03.22	15000-17000	16000±1000	27000-3000	28500±1500	3000-4000	3333±471

In this experiment, total three tpyes of media were used for the culture of spirulina and the culture period was 12 days. Among 3 medias, in Zarrouk's media spirulina growth rate was always high whereas in Bangla media growth rate was always low. After six days spirulina growth was highest in 3 types of media. On that day Mean±SD value of KM, ZM, BM were respectively 31500±3500, 36500±4500, 13667±4027. Then there was a harvesting of 3 media. For that reason, growth rate went slow down. That's mean that production circle being continuous by harvest and culture after 7 days.
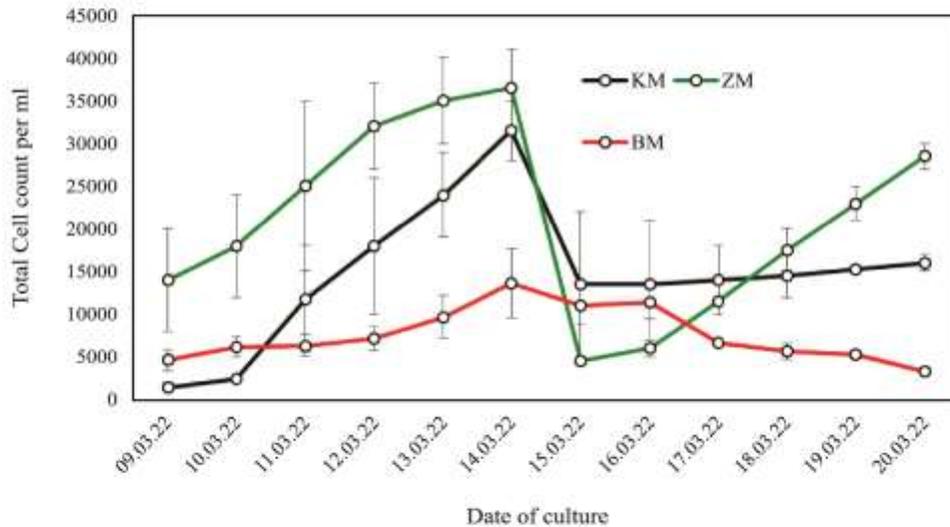

Figure 3. Spirulina growth comparison of BM, KM and ZM media.

Among three types media Zarrouk's media performance is comparatively higher. Here maximum growth rate of Zarrouk's media was 36500 cell/ml whereas in Koseric media and Bangla media were 31500 and 13666 cell/ml, respectively. In addition, lowest growth rate was 4500 cell/ml, 1500 cell/ml and 3333 cell/ml in ZM, KM and BM, respectively. After six days spirulina, production rate was high in every media and end of cropping caused 1st harvest within 7days create collapsd spirulina production. Spirulina growth doubling time was after four days for every media. Because of harvesting at seven days, production went down and then production was up gradually.

Culture of *Nannochloropsis oculata*

Nannochloropsis oculata was cultured by using Gillard and Ryther's Modified f/2 medium after isolated by serial dilution. For microalgae culture in the laboratory, 200 mL of microalgae inoculated into 2 L sterile culture water of 30 ppt salinity, which contained 2 mL of Gillard and Ryther's Modified f/2 media. Culture conditions were maintained as water temperature 24 to 25°C, pH 7.5 to 8.5, salinity 30ppt, and light 2000-4000 lx.

Nannochloropsis oculata growth performance

Growth of *Nannochloropsis oculata* was observed for 14 days. The growth increased for the 1st 7 days then the growth fallen. The growth increased further as the media was changed (Figure 5).
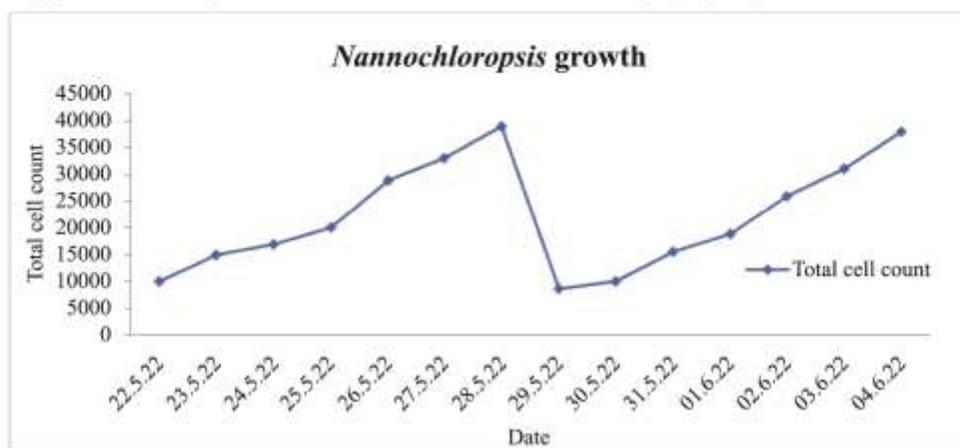

Figure 4. Growth Performance during culture periods.

Culture of *Skeletonema costatum*

Skeletonema was cultured in 25 ppt saline water using the nutrients *viz.*, compost, EDTA, Iron, Vitamin B complex and Sodium silicate.

Experiment 3. Nutritional status of Dried Spirulina

Proximate composition of Spirulina showed that the microalgae is rich with crude protein (46.05%) followed by moisture (18.6%), ash content (9.29%) and crude lipid content (2.33%).
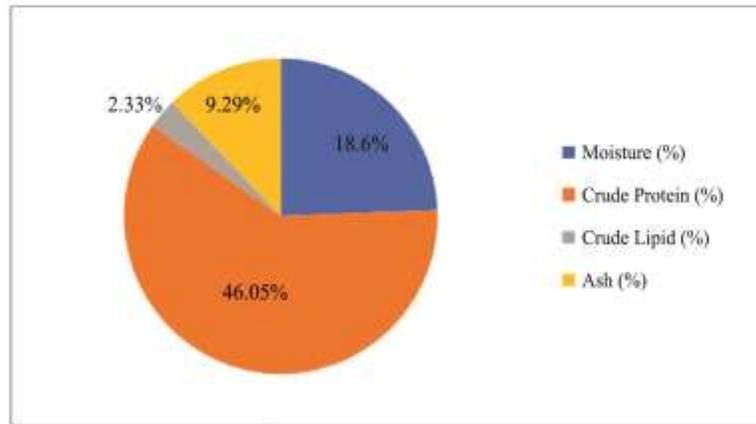
Figure 7. Proximate composition status of Spirulina.

Experiment 4. Feeding Regime of Rotifer (*Brachionus* sp.) Culture

Three different diets were used as feed for rotifers to observe the effects on growth of rotifers. Treatment 1 contains Spirulina powder; Treatment 2 contains live *Nannochloropsis* and Treatment 3 contains Baker's yeast. Initial stocking density rotifer was 10ind/ml. The trial was conducted for 8 consecutive days.

Growth Performance of Rotifer

This experiment showed that increasing of culture period (in days), the number of rotifers (ind/ml) gradually increased. The highest growth of rotifer was observed at the end of the culture period (on the 8th day) fed live *Nannochloropsis* sp. (114 ind/ml), which was considerably higher compared to the Baker's yeast and spirulina powder (Figure 8). On the other hand, Baker's yeast fed group had higher population growth compared to the spirulina powder fed group.
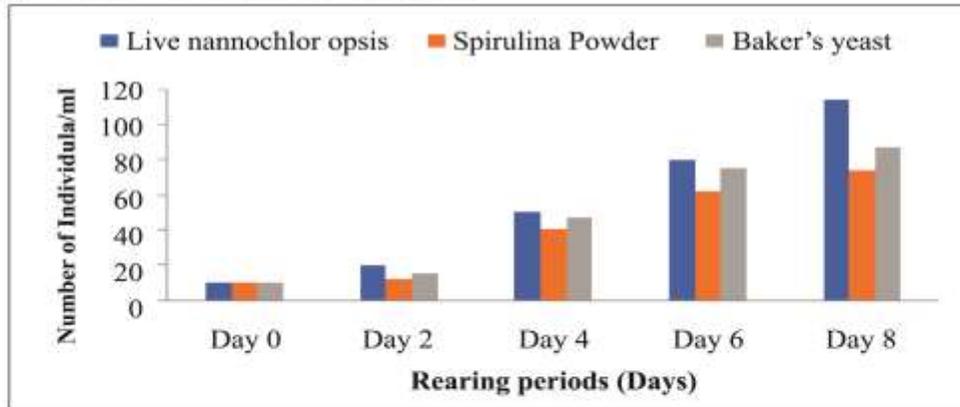

Figure 8. Growth Performance of three live feed (microalgae).

Discussion and conclusion

Phytoplankton ranges between 20 and 200 μm according to the scaling nomenclature of Sieburth et al. (1978). From collected samples sixteen micro-algae in brackishwater region were identified and at least three micro-algae were isolated perfectly and cultivated according to proper protocol using suitable media. The isolated micro-algae were *Spirulina platensis*, *Nannochloropsis oculata* and *Skeletonema costatum*.

First isolated micro-algae spirulina was cultured in three different medias. Among these three medias, Zarrouk's media showed best cell growth performance than Kosaric media and locally established Bangla media. In third day, cell growth of all media got double than starting day and at the 6th day cell growth was in peak. Regularly every crop was harvested at 7th day and re-cultured again by the supply of new supplemented nutrients. Highest cell counting 36,500±4500 was found in ZM media, whereas 31,500±3500 was found in KM and 13,667±4027 was found in BM media. *Nannochloropsis oculata* was cultured in f/2 media where 30ppt salinity was maintained. *Nannochloropsis oculata* cell growth showed gradual upgrade on first week and at seventh day the algae growth went downward. After harvesting, re-supply of media was done to increase the cell growth of *Nannochloropsis oculata*. *Skeletonema costatum* was cultured in compost media and 25 ppt salinity was maintained during experimental period.

The protein is an essential component of diet. The greatest single problem in the world today is the shortage of quality food protein. With the current system of production, agriculture cannot be relied upon to feed an ever-increasing world population. Hence, there is an urgent need to find out other protein sources. The best potential is seen in microbial protein or single cell protein (SCP), a new source of protein independent of agriculture. SCP are characterized by; fast growth rate; high protein content (43-85%) compared to field crops; require less water, land and independent of climate; grow even in wastewater; can be genetically modified for desirable characters such as amino acid composition and temperature tolerance (Tri-Panji and Suharyanto, 2011) Spirulina has been used as a complementary dietary ingredient of feed for fish, shrimp and poultry and increasingly as a protein and vitamin supplement to aqua feeds (Orio Ciferri and OrsolaTinoni, 1985). In this study, spirulina contains 46.5% crude protein, 18.6% moisture, 9.29% ash and 2.33 % lipid. It was similar to Po Chung et al. (1978) where the alga contained 45 to 61% crude protein. Ansuya Devi et al. (1981) reported the characteristics of the protein of freshwater mass cultures *Spirulina platensis*. The total protein was 50-65% of which nearly 9.9% was non-protein nitrogen.

The highest mean rotifers were observed in the live *Nannochloropsis* fed group (114 individual/ml) at the end of the rearing period, which was significantly higher than that of Baker's Yeast and powdered Spirulina fed group. Many studies have shown that the production of rotifers is greatly influenced by their food (Lubzens, 1987; Lubzens, 2001). The growth of many rotifers species mostly influenced by the types of food they ingested and feeding rations (Sarma and Rao, 1987). Moreover, the size and shape of food particles, mobility, digestibility, and nutritional composition are also responsible for the growth of rotifer. Our result was similar with Jewel Das et al. 2020, rotifers fed live *Nannochloropsis* sp. showed significantly better population growth and instantaneous growth rate compared to Baker's yeast and powder spirulina.

A micro alga is an important source of nutrition and is used widely in the aquaculture of other organisms, either directly or as an added source of basic nutrients. Aquaculture farms rearing larvae of molluscs, echinoderms, crustaceans, and fish used microalgae as a source of nutrition. Low bacteria and high micro algal biomass are a crucial food source for shellfish aquaculture.

Effect of *Najas* sp. on Physico-chemical Parameters of Soil, Water and Immunogenic Properties in Shrimp (*P. monodon*) Farming

Researchers

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Objectives

- To assess the primary productivity and soil, water quality of pond

- Comparative study on microbial community and shrimp health status

- To assess the bioactivity of *Najas* sp

Achievements

Study-1. Assessment of Primary Productivity and Physico-chemical parameter of water and Soil

Experimental design

The experiment was conducted in six ponds on station according to the following design.

Table 1. Design of the experiment.

Treatment	Vegetation	Replication
T_1	With weed 20%	
T_2	Wi th weed 40%	2
Control	Without weed	

The ponds were prepared by drying, liming (quick lime. dolomite 2.1) at 250 kg/ha of soil and then filled with the tidal water up to a depth of 1m. Water was treated with chlorine at 20 ppm to disinfect water and kill all animalcules. Fermented molasses was applied to the pond water to develop color of water to prevent penetration of sunlight and then fertilized with urea and TSP at 25 and 30 kg/ha, respectively for quick development of color of water and production of plankton. After production of sufficient plankton required quantity of PCR tested PL was acclimatized with the pond water. *Najas* sp. was stocked in treatment ponds by covering 20% and 40% water area. In all the ponds stocked PL were fed with Quality feed.

Figure 1. Preparation of ponds.   **Figure 2.** Feeding in culture ponds.

The water of the ponds was treated with dolomite at 15 ppm on monthly basis and fertilized with inorganic fertilizer whenever necessary. Feeding behavior and well-being of shrimp was checked twice daily by setting check tray. After 90 days of culture, shrimps of ponds were harvested by complete dewatering.

Water Quality

The water quality variables *viz.*, temperature, depth, transparency, salinity, pH and total alkalinity were monitored at seven days interval following standard methods. Effect of *Najas* sp. on water quality was found. Some parameters were showed variation some were decrease like salinity, alkalinity, turbidity and total dissolved solid in other hand some were increased like pH, DO than control pond. The recorded average water quality variables are shown in Table 2.

Table 2. Water quality parameters in different culture ponds.

Physicochemical characteristics of water	Control pond	20% *Najas* sp.	40% *Najas* sp.
Temperature (^{o}C)	$31.28\pm.26^a$	31.36 ± 0.43^a	31.14 ± 0.13^a
pH	8.2 ± 0.58^a	8.57 ± 0.60^b	8.84 ± 0.39^b
Salinity (ppt)	7.4 ± 0.88^a	5.9 ± 0.54^b	5.83 ± 0.63^b
Alkalinity (mg/l)	115.5 ± 55.05^a	96.5 ± 12.75^b	90.5 ± 23.34^b
Ammonia (mg/l)	0.5	0.5	0.5
Dissolved Oxygen (mg/l)	7.8 ± 1.54^a	9.54 ± 1.13^b	9.17 ± 0.97^c
Conductivity (mS/cm)	7.89 ± 1.47^a	6.58 ± 1.03^b	6.55 ± 1.18^b
Total Dissolved Solids (g/l)	7.13 ± 0.78^a	5.54 ± 0.53^b	5.2 ± 0.65^b
Turbidity (cm)	50 ± 2	--	--

Different lowercase letters in each row indicate significant differences ($p<0.05$)

Physico-chemical characteristics of soil

Physico-chemical characteristics of soil was analyzed in the laboratory of SRDI, Khulna.

Table 3. Soil quality parameters in different culture ponds.

Soil characteristics	Control		20% *Najas*		40% *Najas*	
	Before culture	After culture	Before culture	After culture	Before culture	After culture
Soil type	Sandy clay loam					
Salinity	5	6.5	4	7	4.3	8.3
pH	7.7	8	8	8	8	8.3
Organic carbon (%)	0.722	2	0.842	1.6	0.882	0.4
Total nitrogen (%)	0.068	0.168	0.072	0.164	0.074	0.178
Phosphorus (μg/gm)	13.90	16.52	16.87	15.23	15.13	15.68
Potassium (mg/100g)	0.68	0.80	0.63	0.88	0.84	0.85

Plankton study

Phytoplankton and zooplankton were lower in *Najas sp.* containing ponds than control pond.

Table 4. Plankton on Najas sp. containing pond.

Treatments	Phytoplankton (Cells/L)	Phytoplankton sp.	Zooplankton (Cells/L)	Zooplankton
40% Najas	0.4×10^5	*Anabaena, Lyngbya*	1.75×10^3	*Nauplius, Helidiaptomus, Diaptomus, Cyclops, Moina*
20% Najas	0.6×10^3		2.1×10^3	
Control pond	0.8×10^3		2.4×10^3	

Comparative study on microbial community and shrimp growth performance

Vibrio bacterial load in control pond was higher than *Najas sp.* containing pond found after three months of culture.
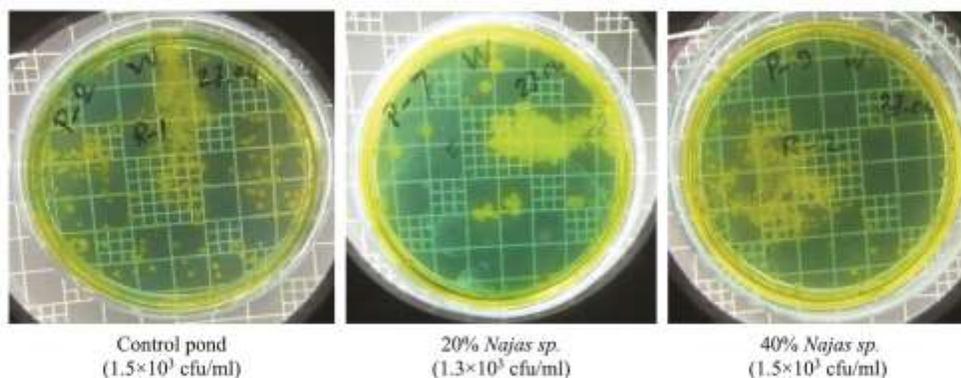

<div align="center">

Control pond   20% *Najas sp.*   40% *Najas sp.*
$(1.5 \times 10^3$ cfu/ml$)$ $(1.3 \times 10^3$ cfu/ml$)$ $(1.5 \times 10^3$ cfu/ml$)$

</div>

Figure 3. *Vibrio* bacterial load counting

Gut content analysis

Gut content analysis has been done to identify whereas shrimp take *Najas* sp. or not. Shrimp samples were taken from all of research ponds. Intestine has been removed and taken on slides for microscopic observation. A distinct difference in color has been found between *Najas* sp. containing pond and control pond. Further analysis is continuing to identify that they take periphyton or *Najas* sp. directly.
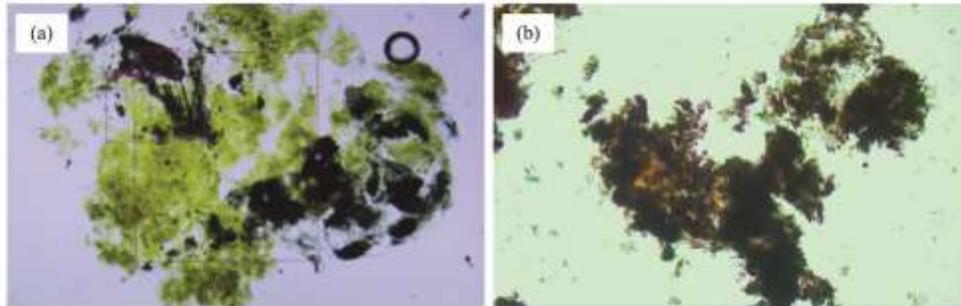

Figure 4. Gut contain analysis (a) *Najas* sp. containing pond (b) control pond.

Growth performance

Weight gain

Najas sp. containing pond showed better result than control pond. Best result showed in 20% *Najas* sp. containing pond. The average weight of shrimp was 17.38 ± 2.087 g, 30.44 ± 2.01 and 26.21 ± 1.89 in control, 20% *Najas* sp. and 40% *Najas* containing pond respectively after 90 days culture period.
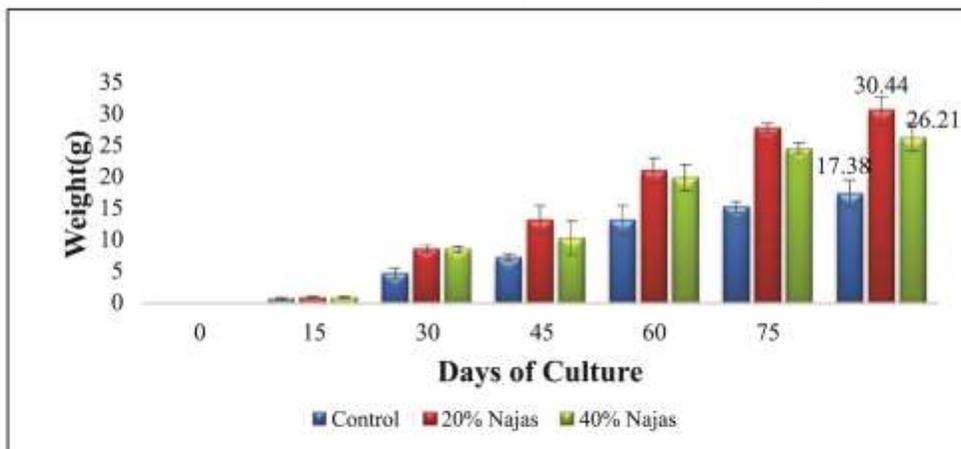

Figure 5. Weight gain of shrimp in different treatment.

Specific growth rate

The SGR was higher in 20% *Najas* sp. containing pond. SGR in control, 20% *Najas* sp. and 40% *Najas* sp. containing pond was 3.17 % /day, 3.79 % /day and 3.62 % / day in control, 20% *Najas* sp.and 40% *Najas* sp. containing pond, respectively.

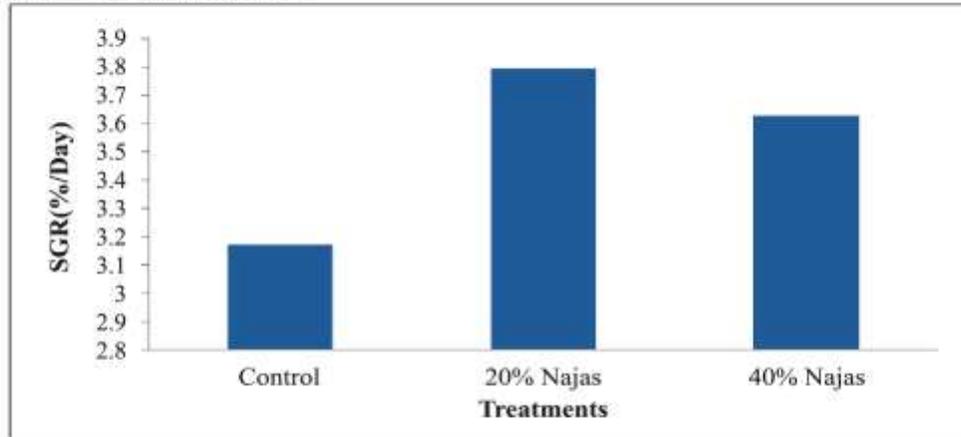**Figure 6.** SGR of shrimp in various treatments.

We might conclude from the preceding figure that there were higher SGR in 20% *Najas* sp. containing pond. This discrepancy is caused by shrimp molting across different treatments, which may be brought on by changes in water quality parameters and other pond biological circumstances.

Figure 5. Lab visit by honorable Secretary Dr. Mohammad Yamin Chowdhury

Table 5. Production performance of shrimp in different treatments.

Treatments	Stocking densities (No/m²)	Culture period (Days)	ABW (g)	Survival (%)	Production (kg/dec)	SGR (%/day)	FCR
T₁			17.38	75	1.6	3.17	1.58
T₂	4	90	30.44	70	3.9	3.79	1.15
Control			26.21	58	3.0	3	1.18

Figure 7. Shrimp harvested from *Najas sp.* containing pond.

The overall production and growth performance containing Najas sp. was satisfactory. This production scenario implies that, production rate (kg/dec) has been increased manifolds than the traditional culture practice. Indeed, further study is needed to validate the present findings before planning for extension to the farmers.

Study-2. Bioactive compound and micronutrient analysis of Najas sp. extracts

Proximate analysis

The proximate composition of dried *Najas sp.* was analyzed in shrimp feed and nutrition laboratory (SFNL) of SRS, Bagerhat, BFRI which was showed in the following Table:

Table 6. Proximate analysis (dry basis) of *Najas sp.*

Components	Percentage (%)
Protein	12.9
Lipid	4
Moisture	5.6
Ash	10
Fiber	8

Extraction of *Najas* sp. using different solvent

Experimental materials and chemical

Najas sp. was collected from treatment pond. All other reagents used in this study were of analytical or high-performance liquid chromatography (HPLC) grade.

Sample preparation

After collection of *Najas* sp. from pond it has been sun dried. Dried *Najas* sp. was grounded by a mechanical blender and sieved with a 335 mm mesh size sieve and the powder passed through the sieve was collected and stored at −80°C until needed for further analysis.

Solvent extraction

For comparison, the extraction of the *Najas* sp. samples was extracted using various conventional organic solvents such as ethanol, hexane, acetone, and water as a polar solvent mixture. Exactly, 50 g of *Najas* sp. powder was placed in 500 ml beaker followed by 300 ml solvent and the extraction was performed at 50°C on a hot plate under constant stirring (200 rpm) for 12 h. Then, the extract with solvent was separated from the residue using filtration. Finally, the extract was separated from the solvent using a rotary vacuum evaporator at 65°C. The extract was kept at 4°C until needed for further analysis. Different extraction yield was found from different solvent, maximum found in 50% MeOH it was 24.4% and minimum in water 12.78%.
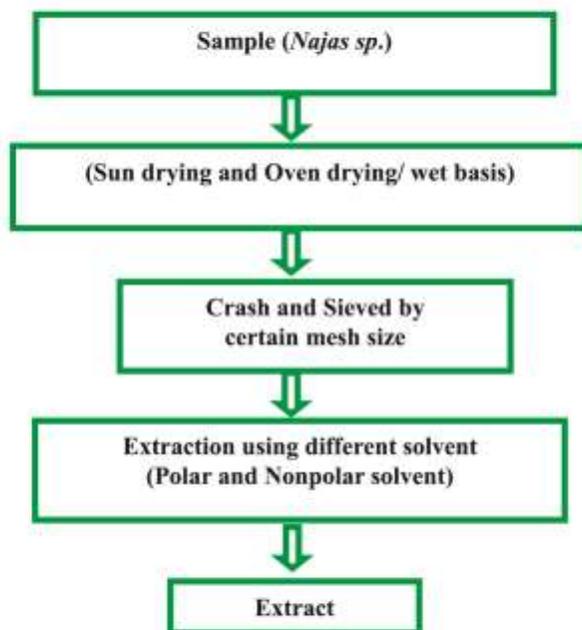

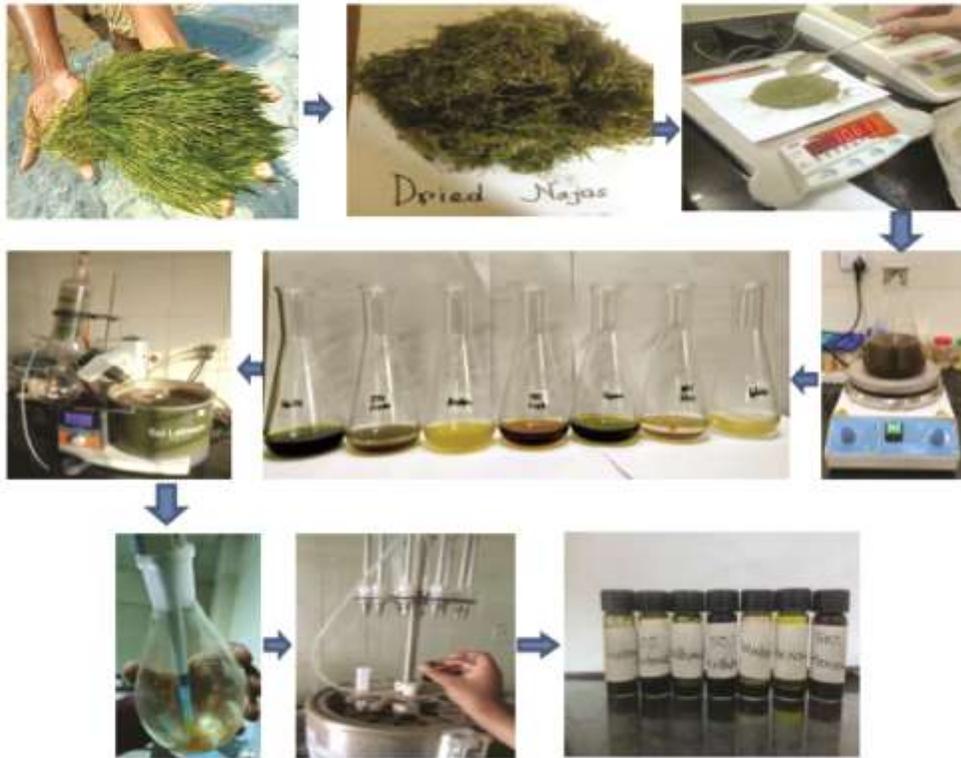**Figure 8.** Flow diagram *Najas* sp. extraction procedure using different solvent.

Figure 9. Pictorial view of *Najas* sp. extraction using different solvent.
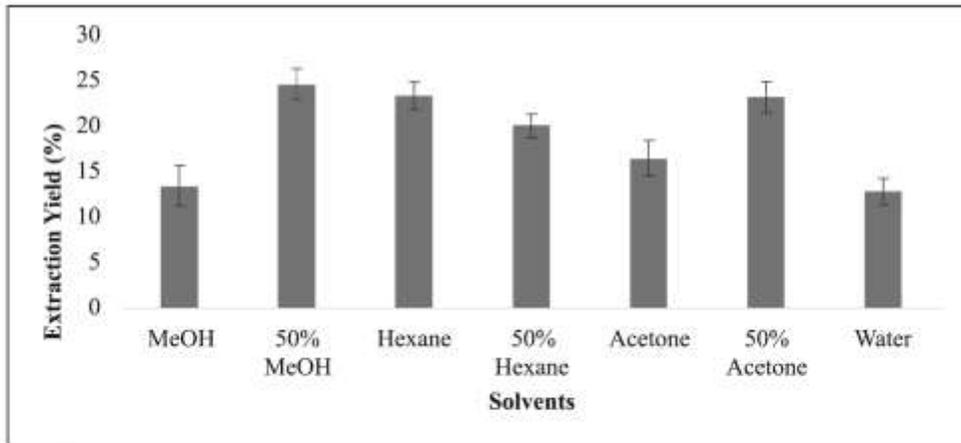

Figure 10. Extraction yield using different solvent.

Primary observation of bioactive compound by UHPLC

To determine which bioactive compounds are available in *Najas* sp. extracted sample was run by UHPLC machine. Different high peak area was observed. Analysis is going on to identify the bioactive compounds.

Figure 11. Chromatograph by UHPLC.

Phytochemical screening of *Najas* sp. extracts

Extracts were prepared using previously described method. Seven extracts using various solvents (Methanol, 50% methanol, acetone, 50% acetone, Water, Hexane and 50% hexane) were used.

Alkaloids

One half of a gram of the extract was dissolved in 1% HCl (5 ml) on a steam bath. The filtrate (1 ml) was treated with few drop of Dragendorff's reagent. Turbidity or precipitaion was taken as indicative of the presence of alkaloid.

Amino Acid

Xanthoprotein test

1 ml of extract and 1 ml of Conc. Nitric Acid are added (white precipitate is formed) it is heated for 2-3 minutes and cooled. Then 1 ml of 20% of NaOH is added. Appearance of orange colour indicates the presence of Aromatic amino acid. (Prashant Tiwari et al., 2011).

Phenol

FeCl$_3$ test

1 ml of the extract and 1 ml of 5% ferric chloride are added. Appearance of dark green colour/reddish brown / blue / violet / purple indicates the presence of phenol. (Ashok Kumar et al., 2012).

Flavonoids

Alkaline reagent test

1 ml of the extract and 1 ml of the 10% of sodium hydroxide are added. Appearance of yellow fluorescence indicates the presence of flavonoids. (Ashok Kumar et al., 2012).

Tannins

FeCl$_3$ test 2 ml of the extract and 2 ml of the 5% ferric chloride are added. Appearance of green colour indicates the presence of Tannins (Yogeshwari et al., 2017).

Saponin Foam test

2 ml of the extract and 2 ml of the Dis. H_2O are added and shaken vigorously. Formation of stable foam indicates the presence of Saponins (Rimjhim Sheel et al., 2014).

Terpenoids

Salkowskis test

One ml of the extract and 2 ml of the chloroform and 3 ml of the conc.H_2SO_4 are added. Appearance of yellow/reddish brown colour indicates the presence of Terpenoid's (Ashok Kumar et al., 2012).

Phlobatanins

1% Hydrochloric acid test 2 ml of the extract and 2 ml of the 1% HCL test are added and heated in boiling water bath. Appearance of red colour indicates the presence of Phlobatanins (N. Lata et al., 2010).

Quinones

Hydrochloric Acid test 1 ml of the extract and 1 ml of the conc. HCL are added. Appearance of yellow colour indicates the presence of Quinone's (R Dhivya et al., 2013).

Coumarin

Sodium hydroxide test 1 ml of the extract and 1 ml of 10% sodium hydroxide are added. Appearance of yellow colour indicates the presence of Coumerin (Yogeshwari et al., 2017).

Anthocyanin

Sulphuric acid test

1 ml of the extract and 1 ml of concentrated sulphuric acid are added. Appearance of yellowish orange colour indicates the presence of Anthocyanin (Seema Firdouse et al., 2011).

(a)   (b)   (c)

(d)   (e)   (f)
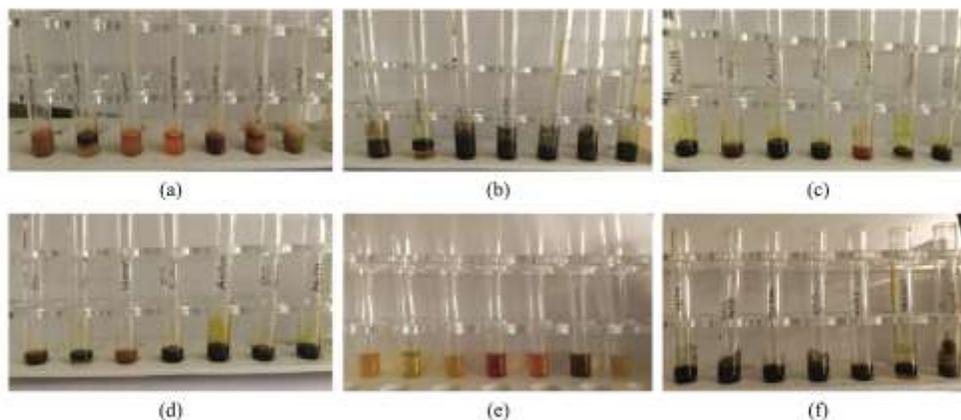
Figure 12. Some picture of phytochemical screening (a) Amino acid test (b) Tanin test (c) Quinon test (d) Flavonoids (e) Alkaloid test (f) Phenol test.

Table 5. Results of phytochemical analysis.

Sl. No.	Phyto-chemicals	Test Names	Methanol	50% Methanol	Acetone	50% Acetone	Water	Hexane	50% Hexane
1.	Alkaloids	Alkaloids	-	+	-	+	+	-	+
2.	Amino Acid	Xanthoprotein	-	+	+	+	+	+	+
3.	Phenol	FeCl3 test	+	-	+	-	+	+	+
4.	Flavonoids	Alkaline reagent	+	+	-	+	-	+	-
5.	Tannins	FeCl$_3$ test	+	-	+	-	-	+	-
6.	Saponin	Foam test	+	-	-	+	+	-	-
7.	Terpenoids	Salkowskis test	-	+	-	+	+	-	-
8.	Phlobatanins	1% HCl acid test	+	-	+	-	-	-	+
9	Quinones	Hydrochloric acid	-	+	+	+	+	+	-
10	Coumarin	Sodium hydroxide test	-	-	+	-	+	-	+
11	Anthocyanins	Sulphuric acid	+	+	+	+	+	-	+

Presence (+)   Absence (-)

Refinement of Existing Organic Shrimp (*Penaeus Monodon*) Farming Using Eco-Friendly Management Protocol in Southwest Region of Bangladesh

Researchers
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Objectives
- To assess the present status of organic shrimp (*P. monodon*) farming compare to standard protocol
- To mitigate the gaps of existing culture practices according to the standard protocols

Achievements

Study-1. Assessment of the present culture status of organic shrimp (P. monodon) farming in southwest region of Bangladesh

In this year, preliminary survey site has been selected in Bagerhat, Khulna and 5 potential upazilas of Satkhira district comprising organic shrimp farming. Prior to do the survey work, a questionnaire has been developed regarding organic shrimp farming status. Survey have been conducted using the structured

questionnaire in respective upazilas of each district. Production practices including application of different inputs by the farmers are observed closely and recorded. Water source and water exchange technique were observed and data were recorded and growth of organic shrimp are monitored. Data on total production rate (kg/ha) in organic shrimp in different occasions including production cost and return achieved are being collected. The percentage of organic shrimp farming that were observed in survey till date were 70% in Satkhira, 20% in Khulna and 10% in Bagerhat districts.
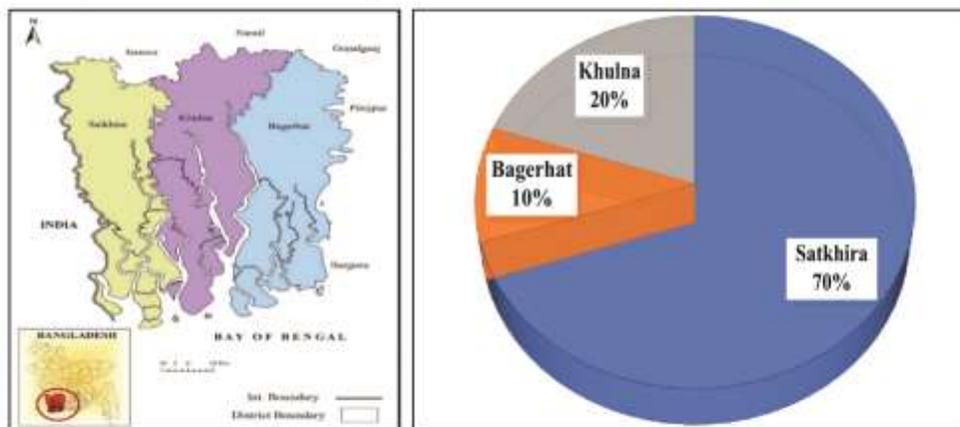

Figure 1. Study map and Percentage of organic shrimp practices in the study area.

Table 1. List of organic shrimp farm area in the surveyed.

District s	Upazila	Sampling points
Satkhira	Sadar	Ellar char
	Ashashuni,	Rotonpur
	Shamnogor,	Nurnogor
	Kaligonj	Fultoli,Sekandar nagar,Roghurampur
	Debhata	Bengdaha,Hijoldanga
Khulna	Chalna	Boro Khalisa, Pankhali
	Rupsha	Anandonogor
	Dumuria	Arazi sazaria
	Batiaghata	Batiaghata, Hatbati,
	Terokhada	Azopora, Shekhpura,
	Digholia	Brahmogati, Barakpur
Bagerhat	Kachua	Char khatalia,
	Mongla	Kanainogor, Uttar Kanainogor, Burirdanga, Vuirdanga
	Rampal	Jhonjhonia, Boughata, Bagha, Bastola
	Fakirhat	Sadar, Pilgonj

Difference between traditional organic shrimp farming practices in the survey area and standard organic shrimp farming are described in the following Table.

Items	Standard organic shrimp farming	Traditional organic shrimp farming
Water sources	Water sources should be without risk from hazardous substances and other contamination.	Farms were established nearby industry like Jute mills, Metal industry, Automatic rice mill, Electrical industry, Engineering workshop etc.
Fry stocking	Stocking density of fry should not exceed 15 fry/m³.	Stocking density of fry were more than 15 fry/m³.
	Production pond should be equipped with aerator to provide suitable condition for shrimp.	No aerator was used for the production of organic shrimp.
Farm ecosystem	Biodiversity should be maintained in farm by appropriate plantation or natural vegetation.	Biodiversity was not maintained in farm by destruction of trees in farm area.
	In case of farm location nearby mangrove area, conservation and rehabilitation should be planned.	No plan was design for conservation and rehabilitation of mangrove area.
General health management	Shrimp health and water quality in production pond should be regularly checked.	Shrimp health and water quality in production pond were not regularly checked.
Shrimp feed Production	The following substances and materials are prohibited in aquatic animal feeds. All chemotherapeutants and antibiotics, urea, pure amino acid, synthetic appetizers, materials and product derived from genetically modification organisms, substances or materials that prohibit for aquatic animal feeds as notify in Animal Feed Quality Control regulation.	Urea, antibiotics, dye in the survey area were used in shrimp feed production in the survey area. For *gher* preparation, organic inputs like liming during pond drying, dolomite and zeolite whenever necessary were found in the survey.
	Feed should be produced from natural raw or organic materials. Ingredients used in shrimp feed shall be from those inappropriate for human as much as possible.	Compost of different local tree leaves e.g. Neem, Juice of Mehagani fruits and mustard oilcake was used as feed of organic shrimp in these areas. Sometimes dried cowdung were used as feed supplement. Fermented juice rice bran, molasses and yeast powder were used as organic ingredients.
Feed storage	Feed storage shall be separated from other buildings. The storage area should be dry, clean, and with a proper condition to maintain feed quality, temperature, cleanliness and prevent the disease-carriers such as rats, birds and others.	Feed were stored nearby farm shed in dirty, unsanitary environment with spades, hoes, baskets, pipes and other materials.
Harvesting	Substances used during harvesting and post harvesting should be from natural sources. Harvester shall be healthy and not infected with any contagious disease. Water and ice shall be in hygienic condition. Organic shrimp should be transported separately from conventional one.	Organic shrimp are not separated from non-organic shrimp during transportation. All equipment was not cleaned. Dehydration occurred in shrimp lack of clean, safe water and ice. Control temperature more than 5⁰C.

Study-2. Analysis of the nutritional status of shrimp produced from organic and in-organic farming

Achievements

The proximate composition of in-organic and organic shrimp was analyzed in shrimp feed and nutrition laboratory (SFNL), of SRS, BFRI. Analyses were done using methods recommended by the Association of Analytical Chemists (AOAC).

Percentage of protein content was high in Organic shrimp (24.27 ±0.82) compared to inorganic shrimp (17.43±1.47). Moisture content in organic and inorganic shrimp were 68.36±1.39 and 73.06±1.26, respectively. The highest lipid content was present in organic shrimp (4.87±0.94) while the lowest value was shown by inorganic shrimp (4.53±1.62). Comparatively, the percentage of total carbohydrate content is the highest in inorganic shrimp than organic shrimp.
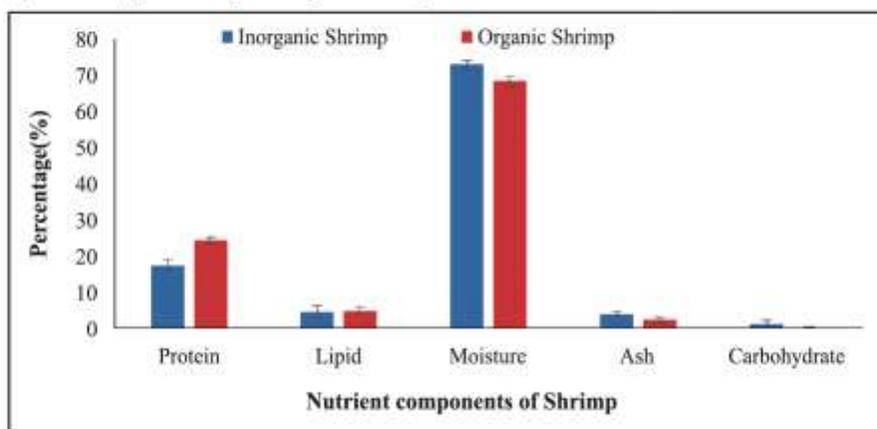

Figure 3. Proximate composition of in-organic and organic shrimp.

Study-3. Intervention for increasing production of organic shrimp (Penaeus monodon) in station ponds

Achievements

Experimental design
The study was conducted in the pond complex of Bangladesh Fisheries Institute (BFRI), Shrimp Research station, Bagerhat with the following experimental design:

	Major organic inputs as feed	Stoking density	Culture duration
Treatment	Formulated organic feed		
Control	Popular inputs used by farmer (Mixture of Mastered oil cake, Rice bran and Mola sses)	5-10/m²	120 days

Each treatment with 2 replications

Culture management of pond
Bottom sludge from all ponds was removed and sun dried completely. Monk and dike of the ponds were repaired. Soils of ponds were treated with lime (CaCO$_3$) at 250 kg/ha. Partition was done for the purpose of replication of each treatment.

All the ponds area was encircled with blue net for ensuring biosecurity. All ponds were filled with tidal water from Dharatana river and was treated with rotenone. Finally, the required quantity of organic PL from Cox-bazar was acclimatized and stocked in the pond according to the design in 18th April, 2021. The initial weight of PL was found .0035g. Growth (Length and weight) performance is being monitored fortnightly.

Figure 4. Pond preparation and stocking of shrimp.

Physicochemical characteristics of Soil

Physicochemical characteristics of soil (Texture, salinity, pH, organic carbon, total nitrogen, phosphorus and potassium) was analyzed in the laboratory of SRDI Khulna throughout the culture period. All the parameters were found almost suitable in both treatment and control pond.

Table 3. Soil characteristics of organic shrimp ponds.

Physicochemical characteristics of soil	T	C
Salinity	4.7	6.9
pH	8.3	8.1
Organic carbon (%)	0.842	0.762
Total nitrogen (%)	0.072	0.071
Phosphorus	14.64	13.15
Potassium	0.64	0.88

## Water quality management and measurements

Water quality parameters such as water temperature, pH, dissolved oxygen (DO), salinity, alkalinity, ammonia and iron were measured regularly at 7 days interval throughout the experimental period. All the parameters were found almost suitable in both treatment and control in every sampling date without showing considerable difference (Table 4).

Table 4. Water quality parameters of organic shrimp ponds.

Parameters	Results (Mean± SD)		Standard Value
	Treatment	Control	
Temp (°C)	30.41±1.85	30±1.62	28-31
DO (mg/l)	6.20±1.17	7.85±1.36	5-12
pH	7.9±0.607	8.15±0.672	7.5-8.5
Salinity (ppt)	5.71+1.33	5.45+1.32	5-30
Alkalinity (mg/l)	160.33+16.22	144.44+23.12	60-180
Ammonia (mg/l)	0.55+0.22	0.51+0.24	<0.1
Iron (mg/l)	0.1+0.29	0.4+0.51	0.05-0.5

Qualitative and quantative plankton analysis

Several phytoplankton and zooplankton groups in treatment ponds that largely dominated over control pond in organic shrimp farming system. Among the zooplankton groups, Euglenophyceae, Rotifers, Copepods, Crustaceans and among the phytoplankton groups Bacillariophyceae, Cyanophyceae, Chlorophyceae are available in treatment pond. Higher quantities of zooplankton were recorded in treatment ponds due to availability of nutrients and favorable water quality parameters. Lowest phytoplankton counts were found in control (2.52×10^3 cells/l) and highest in treatment ponds (10×10^3 cells/l). Similarly, Zooplankton counts were found (1.2×10^3 cells/L) in control ponds and (3.75×10^3 cells/L) in treatment ponds.

Fig: *Nostoc* **Fig: *Oscillatoria*** **Fig: *Nauplius***
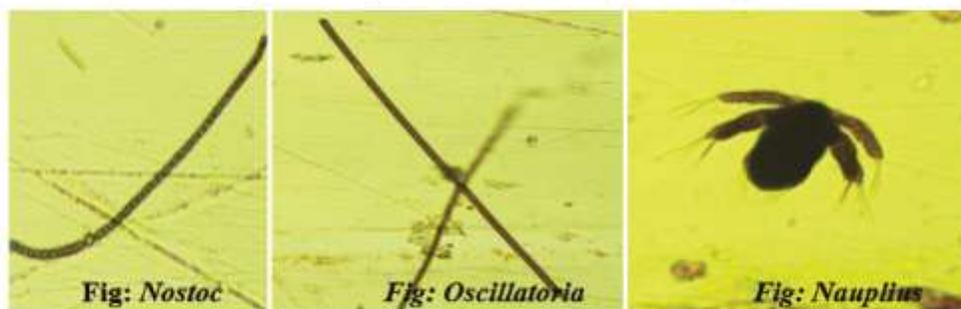
Feeds and feeding

An organic feed was prepared following the formulation chart given in project proposal which proximate analysis are given below:

Table 5. Proximate composition of Formulated organic feed.

Parameter	Calculated value (%)
Protein	31.0
Lipid	3.0
Moisture	7.0
Ash	13.0
Fiber	8.5

Fig 5 : formulation of organic feed

Shrimps were fed twice daily with formulated organic feed at a rate of 3-5% body weight. To increase natural productivity of water a mixture of organic ingredients *viz*. molasses, yeast and rice bran (MYR) at 40 kg/ha; 120 g/ha; 25 kg/ha were applied.

Growth Performance of Organic shrimp

After 120 days of culture, all shrimps were harvested, and production were estimated. Higher growth was observed in treatment pond compared to control after 120 days culture period. The mean weight in control pond and treatment ponds were 17.83±1.46 g and 26.21±1.05 g, respectively. Compared to control, treatment pond shows good result. After 120 days of culture average length in control pond and treatment pond 12.24± 0.57 and 18.21±0.95 cm, respectively. The survival rate (%) of organic shrimp in treatment pond and control was found 71.17±2.00, 62.92±1.22, respectively. Total production (kg/ha) was found 1823.59±12.43, 1056.31±22.53, respectively. Growth performance of organic shrimp in control and treatment ponds are shown the following Figure.
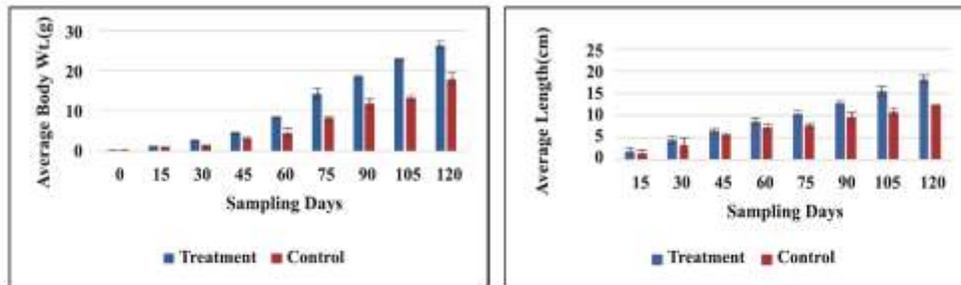

Figure 5. Growth performance of organic shrimp.

In 2021-22, the remarkable achievement was no disease outbreaks among the experimental ponds. Highest body weight gain, survival and production was achieved under the treatment using formulated organic feed. It indicated its superiority over the control. Validation through repitition of this formulated feed, optimization of the stocking density of organic shrimp needed to be tested to maximize the profit level before extension to farmer's field.

Figure 6. Organic shrimp after 4-month rearing.

Bioaccumulation of Hazardous Organochlorine Pesticides in Shrimp and its Risk Assessment on Human Health

Researchers

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Objectives

- To assess bioaccumulation of pesticides in cultured and wild shrimp/prawn
- To assess the risk of pesticides residues on human health

Methodology

Experiment-1. Assessment of bioaccumulation of pesticides in cultured and wild shrimp/prawn

Research programme was carried out in 8 Upazillas of Bagerhat throughout the project duration such as Kochua, Chitolmari, fakirhat, Mollahat, Sarankhola, Mongla, Morelganj and Bagerhat Sadar upazilla. Shrimp/prawn samples were collected randomly from the wild and cultured areas from the sampling sites for assessment of pesticidal residues in wild and cultured shrimp/prawn.
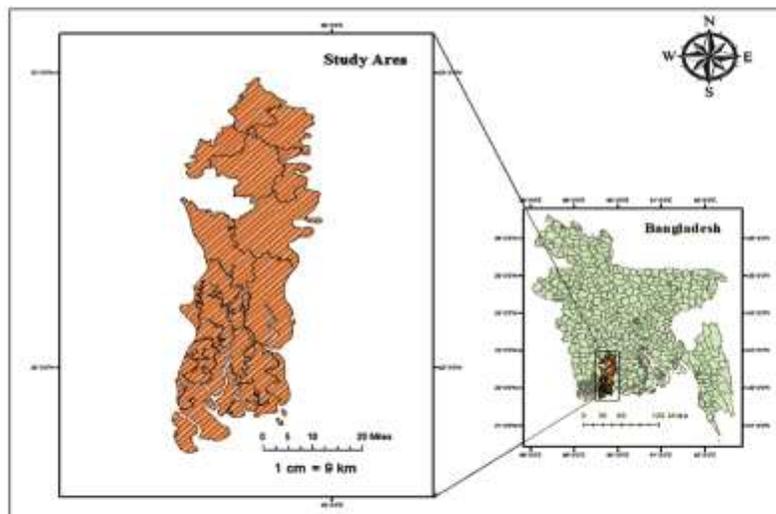

Figure 1. Study area for the Assessment of bioaccumulation of pesticides.

Sampling area was different in every year of the project. The experiment was designed as follows.

Table 1. Experimental design.

Sampling Area	Mollahat	Chitalmari	Kochua	Sarankhola	Mongla	Fakirhat	Morelganj	Bagerhat Sadar
No of sampling site for cultured shrimp/prawn	2	2	2	2	2	2	2	2
No of sampling site for wild shrimp/prawn	1	1	1	1	1	1	1	1
Sampling interval	30 days	30 days	30 days	30 days	30 days	30 days	30 days	30 days

Equipments

Mincer fish chopper (Weisser No. 81 K), round bottomed flask (500 and 100 mL), volumetric flask (50 and 10 mL), Homogeniger IKAR T25 digital ULTRA-Turrax, Nitrogen evaporator(N-EVAPTM111), SPE Cartidge (C18-REC 300 mg/3 mL) Magnetic Starrier, Gas Chromatograph (GC-2010, Shimadzu).

Chemicals and Reagents

Reagents Methanol, n-Hexane, Ethyl acetate/Acetonitrile, Primary Secondary Amine (all were with high purity 99.99%, HPLC grade) and anhydrous sodium sulphate, anhydrous magnesium sulphate was purchased from Merk Company (Germany). DDT, Dieldrin, Heptachlor and Endrin reference standards were obtained from Sigma Aldrich Chemicals (USA).
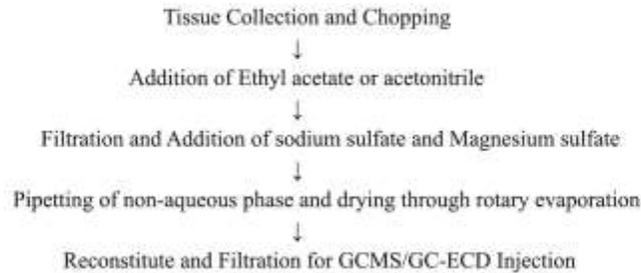
Extraction and clean-up procedure for pesticides residue analysis using GC-MS/MS

The extraction has carried out according to the procedure described by QUEschERS Method and necessary modification has also adopted for extraction, separation and clean-up sample. Ten gram of shrimp/prawn sample is taken in a Teflon tube. Twenty ml Ethyl-acetate/Aceto-nitrile is added and have to hand shaken for 1 min. Then it was shaken with vortex mixture for 2 min. About 1.5 g NaCl and 6g anhydrous $MgSO_4$ is added and then shaken in the hand for 1 min. Then, shaken it with vortex mixture for 1/2 min. Then it was filtered with 20 g $NaSO_4$ and 10 ml anhydrous Ethyl-acetate. The mixture was centrifuge at 5000 rpm for 5 minutes.

Then 10 ml supernatant was taken in a round bottom flask. The supernatant is evaporated with rotary evaporator with keeping temperature not more than 400C. About 5mL n-Hexane was added in a round bottom flask. Then 2 ml n-Hexane solution was taken in a test tube.

For cleaning-up

About 2 ml H_2SO_4 is added with that 2 ml n-Hexane soln. Then it was vortexed for 1 min. After vortexing, the mixture was centrifuged at 4000 rpm for 3 minutes. After that the supernatant was taken in a tube and filtered with 0.45 µg syringe filter in a vial. Then finally 2mL sample was taken in a vial for GC-ECD or GC-FID analysis.

Tissue Collection and Chopping
↓
Addition of Ethyl acetate or acetonitrile
↓
Filtration and Addition of sodium sulfate and Magnesium sulfate
↓
Pipetting of non-aqueous phase and drying through rotary evaporation
↓
Reconstitute and Filtration for GCMS/GC-ECD Injection

Flow chart of sample extraction for Pesticides (QUEschERS Method, Anastassiades et al.,2003)

Experiment 02. Impact assessment of the pesticide's residues on human healthRisk Assessment of the pesticides

Risk assessment was conducted based on local and country-wide consumption rates for key species. Data was analyzed individually for each of the shrimp/prawn species collected. An associated cumulative risk assessment was carried out for each contaminant (pesticides). Risk assessment was conducted based on local and country-wide consumption rates for key species (DoF 2013). To drive advisory consumption recommendations for the fish species analyzed, two equations developed by USEPA * (2000) was applied.

$$CR_{lim} = \frac{ARL \times BW}{CSF \times Cm}$$

Where CR_{lim} is the maximum allowable fish consumption rate (kg/day), ARL the maximum acceptable individual lifetime risk level (10^{-5}), BW is the consumer body weight (55kg), Cm is the measured concentration of contaminant m in a given species of fish (mg/kg or ppm), and CSF is the cancer slop factor [mg/kg/day)]

$$CRmm = \frac{CR\,lim \times Tap}{Ms}$$

Where CRmm is the maximum allowable fish consumption rate (meals per year). CR_{lim} is the maximum allowable fish consumption rate (kg/d). Tap is the time averaging period (365.25 days per year), and MS is the meal (0.227kg fish/meal)

Results

A total of 260 shrimp and prawn samples were collected throughout the project period from the wild and cultured areas of Morelganj, Sarankhola, Mongla, Kochua,Chitalmari, Fakirhat, Mollahat and Bagerhat sadar upazilla of Bagerhat district for pesticidal residue analysis. Then the samples were analyzed for detection of DDT, Dieldrin, Endrin and Heptachlor by GC-MS Machine using standard analysis protocol.

Table 2. Accumulation of pesticides residues in shrimp of different sampling sites of Bagerhat.

| Samples	Targeted pesticides	Study area (Upazillas in Bagerhat Zilla)							
		Kachua	Chitolmari	Fakirhat	Mollahat	Mongla	Sarankhola	Morrelganj	Bagerhat Sadar
Cultured Shrimp	Heptachlor (ppm)	0.003±0.002	0.002±0.001	0.002±0.001	0.004±0.001	0.001±0.001	0.001±0.002	0.003±0.002	0.01±0.01
	Dieldrin (ppm)	0.002±0.001	0.001±0.001	0.002±0.001	0.001±0.001	0.002±0.001	0.002±0.001	0.002±0.001	0.004±0.02
	Endrin (ppm)	0.004±0.001	0.002±0.001	0.001±0.001	0.002±0.001	0.004±0.001	0.004±0.001	0.004±0.001	0.0085±0.005
	DDT (ppm)	0.002±0.001	0.001±0.001	0.002±0.001	0.001±0.001	0.00	0.00	0.00	0.00
Wild Source	Heptachlor (ppm)	0.002±0.001	0.003±0.001	0.002±0.001	0.004±0.001	0.003±0.001	0.002±0.001	0.001±0.001	0.002±0.001
	Dieldrin (ppm)	0.005±0.002	0.002±0.002	0.003±0.002	0.002±0.002	0.002±0.002	0.004±0.002	0.002±0.002	0.005±0.002
	Endrin (ppm)	0.0002±0.001	0.002±0.001	0.001±0.001	0.0002±0.002	0.0002±0.001	0.0002±0.001	0.0002±0.001	0.0002±0.001
	DDT (ppm)	0.003±0.001	0.002±0.001	0.001±0.001	0.0023±0.001	0.00	0.00	0.00	0.00
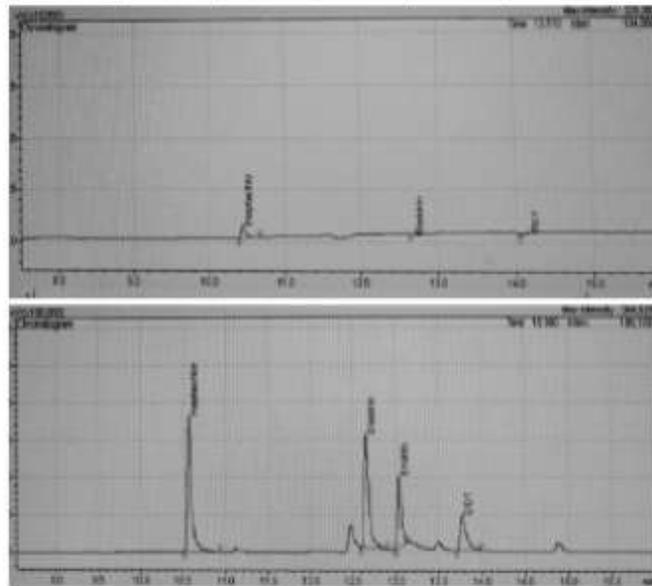
Figure 2. Chromatogram peaks obtained during real time analysis of standard and sample. From the analysis, it was found thatpesticide residues were found in different samples from 8 Upazillas of Bagerhat but in low concentrations. None of the samples exceed the Maximum residiual level. Among our four targeted pesticides, Heptachlor was frequently found.
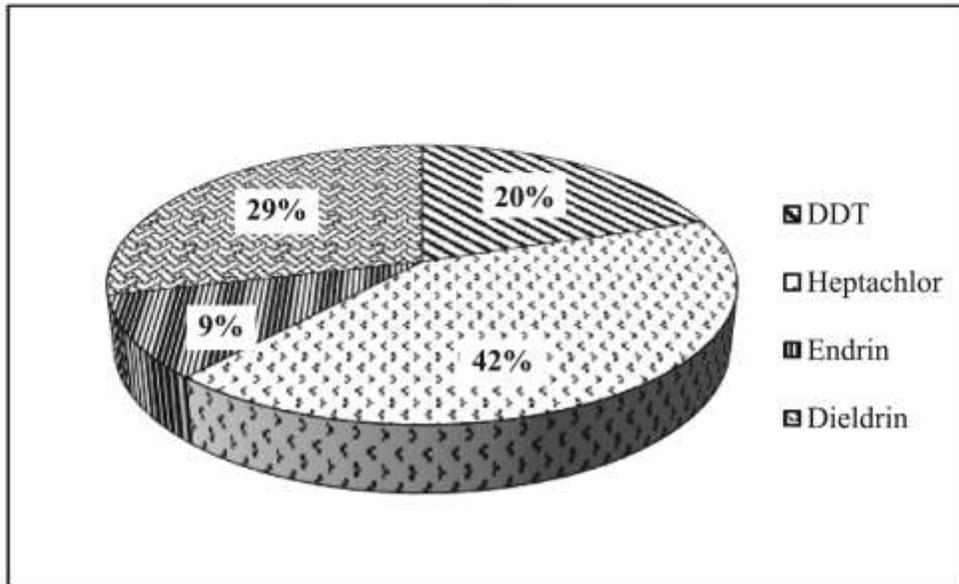
Figure 3. Ratios of available pesticides (below Maximum residual level) in our study area.

In this study, we also found that pesticide residues were frequently found (didn't exceed the acceptable limit) during the colder months and less available during the warmer months of the year.
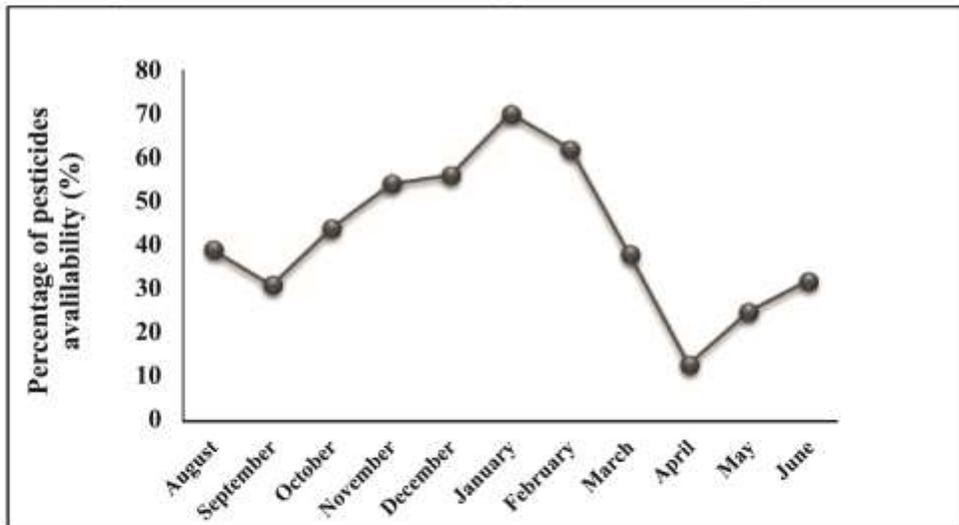

Figure 4. Monthly Scenario of available targeted pesticides in our study area.

Among our study areas, the samples from the Kachua upazilla found to have pesticides in frequent times and less frequent pesticides were found in Saronkhola Upazilla. Although none of the samples exceed the alarming concentration of pesticides.
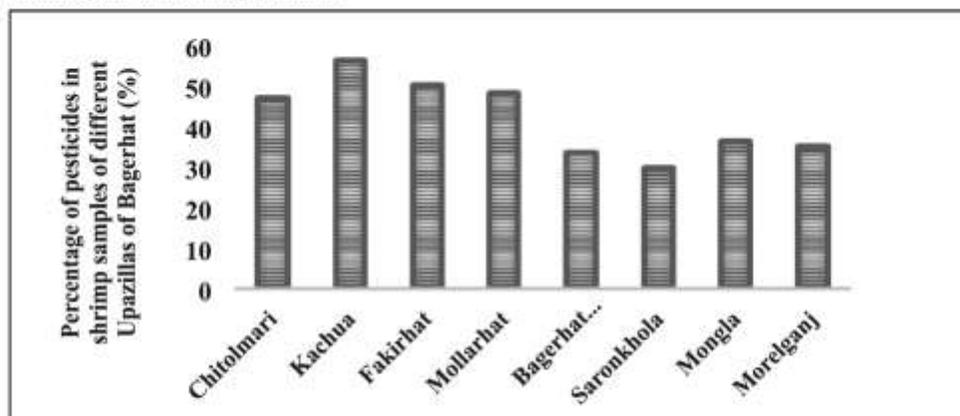

Figure 6. Scenario of available pesticides in different sampling sites.

Risk assessment of these accumulated pesticides residues on human health.

Risk assessment was done using these pesticide concentrations based on local and national consumption rates for important species (DoF, 2013). The result of risk assessment of accumulated pesticides in cultured and wild shrimp samples is presented below (Table 3 and 4).

Gas Chromatography Electron Capture Detector (GC-ECD) was used to examine samples of shrimp to determine the accumulation of DDT, Dieldrin, Heptachlor, and Endrin residues as well as the risk-based consumption rate for human health. Eight Upazillas of Bagerhat viz. Bagerhat Sadar, Kochua, Chitolmari, Mongla, Morelganj, Fakirhat , Mollahat and Saronkhola were selected to assess the bioaccumulation of residual concentration for DDT, Dieldrin, Heptachlor and Endrin. Among the obtained results of pesticides residues, none of the samples showed alarming concentration level according to the acceptable limit of European Union (EU). According to the EU, acceptable limit for Heptachlor and Endrin in shrimp/prawn is 0.01 ppm. Acceptable limit for Dieldrin in shrimp/prawn is 0.02 ppm and DDT in shrimp/prawn is 0.05 ppm (Islam et al., 2015). In this study, we found that all the pesticides concentration were very low in the shrimp samples. Using the two equations of (USEPA,2000). It was observed in Bagerhat Sadar Upazilla that if anyone consumes shrimp upto 159.43 kg/year, then he/she might accumulate 0.01 ppm Heptachlor residue (Table 3) in his/her body and may fall into serious health risk like cancer. But this is quite impossible to consume that amount of shrimp in a year which indicates that the pesticides accumulation in shrimp is very much low in concentrations and not alarming for human consumption. Like heptachlor, the concentration of other pesticides in cultured and wild shrimp of other 7 upazillas of Bagerhat were found in very low concentration (Table 3 and 4).

Although the concentration of pesticides found very much lower, the percentage of available Heptachlor were higher (41.7%) than other three targeted pesticides in shrimp samples and the lower percentage from the samples were found in endrin (9.6%). The reason behind this is that in south-west region of Bangladesh around 80% people are practicing Shrimp/Prawn culture with vegetables or rice as an integrated way for their living (Islam et al., 2015). With the growing demand for food, use of chemicals like fertilizer and pesticides in agricultural land has increased since 1954. Most of the organochlorine pesticides (OCPs) were banned in 1970s for their long persistence in the environment (Annonymous, 1979 and Annonymous, 1989). Due to negative impacts on human health and the environment, several pesticides, including DDT, have been prohibited in Bangladesh since 1995 after being used extensively for several decades (Matin et al., 1998). But because of their long persistence, OCPs are still detectable in low concentration in fish and shrimp from various waterways (Zhang et al., 2014; Prodhan et al., 2010; Prodhan et al., 2009; Kaur et al., 2008; Antunes and Gil 2004; Osuna-Flores and Riva, 2001; Chan et al., 1999; Berg et al., 1999; Sapozhnikova et al., 2004).

Table 3: Accumulation of pesticides residue concentration in cultured shrimp and its risk-based consumption rate for human

Sampling Sites	Pesticides	ARL	BW*	CSF	Pesticides concentration C_n (ppm)	CR_{lim} (kg/day)	CR_{am} (Kg/year)
Kachua	Heptachlor	0.00001	55	2	0.003	0.09	521.7
Chitolmari		0.00001	55	2	0.002	0.14	811.6
Fakirhat		0.00001	55	2	0.002	0.14	811.6
Mollahat		0.00001	55	2	0.004	0.07	405.8
Mongla		0.00001	55	2	0.001	0.275	1594.3
Saronkhola		0.00001	55	2	0.001	0.275	1594.3
Morelganj		0.00001	55	2	0.003	0.09	521.7
Bagerhat Sadar		0.00001	55	2	0.01	0.02	159.43
Kachua	Dieldrin	0.00001	55	2	0.0021	0.13	759.21
Chitolmari		0.00001	55	2	0.0011	0.25	1449.40
Fakirhat		0.00001	55	2	0.0023	0.12	693.19
Mollahat		0.00001	55	2	0.0011	0.25	1449.40
Mongla		0.00001	55	2	0.0024	0.11	664.31
Saronkhola		0.00001	55	2	0.002	0.14	797.17
Morelganj		0.00001	55	2	0.002	0.14	797.17
Bagerhat Sadar		0.00001	55	2	0.004	0.07	398.59
Kachua	Eldrin	0.00001	55	2	0.004	0.07	398.59
Chitolmari		0.00001	55	2	0.002	0.14	797.17
Fakirhat		0.00001	55	2	0.001	0.28	1594.35
Mollahat		0.00001	55	2	0.002	0.14	797.17
Mongla		0.00001	55	2	0.004	0.07	398.59
Saronkhola		0.00001	55	2	0.004	0.07	398.59
Morelganj		0.00001	55	2	0.004	0.07	398.59
Bagerhat Sadar		0.00001	55	2	0.0085	0.03	187.57
Kachua	DDT	0.00001	55	0.34	0.002	0.81	4689.25
Chitolmari		0.00001	55	0.34	0.001	1.62	9378.50
Fakirhat		0.00001	55	0.34	0.002	0.81	4689.25
Mollahat		0.00001	55	0.34	0.001	1.62	9378.50
Mongla		0.00001	55	0.34	0	-	-
Saronkhola		0.00001	55	0.34	0	-	-
Morelganj		0.00001	55	0.34	0	-	-
Bagerhat Sadar		0.00001	55	0.34	0	-	-

Table 4: Accumulation of pesticides residue concentration in wild shrimp and its risk-based consumption rate for human

Sampling Sites	Pesticides	ARL	BW*	CSF	Pesticides concentration C_n (ppm)	CR_{lim} (kg/day)	CR_{mm} (Kg/year)
Kachua	Heptachlor	0.00001	55	2	0.002	0.14	797.17
Chitolmari		0.00001	55	2	0.003	0.09	531.45
Fakirhat		0.00001	55	2	0.002	0.14	797.17
Mollahat		0.00001	55	2	0.004	0.07	398.59
Mongla		0.00001	55	2	0.003	0.09	531.45
Saronkhola		0.00001	55	2	0.002	0.14	797.17
Morelganj		0.00001	55	2	0.001	0.28	1594.35
Bagerhat Sadar		0.00001	55	2	0.002	0.14	797.17
Kachua	Dieldrin	0.00001	55	2	0.002	0.14	797.17
Chitolmari		0.00001	55	2	0.001	0.28	1594.35
Fakirhat		0.00001	55	2	0.002	0.14	797.17
Mollahat		0.00001	55	2	0.001	0.28	1594.35
Mongla		0.00001	55	2	0.002	0.14	797.17
Saronkhola		0.00001	55	2	0.002	0.14	797.17
Morelganj		0.00001	55	2	0.002	0.14	797.17
Bagerhat Sadar		0.00001	55	2	0.004	0.07	398.59
Kachua	Eldrin	0.00001	55	2	0.0002	1.38	7971.73
Chitolmari		0.00001	55	2	0.002	0.14	797.17
Fakirhat		0.00001	55	2	0.001	0.28	1594.35
Mollahat		0.00001	55	2	0.0002	1.38	7971.73
Mongla		0.00001	55	2	0.0002	1.38	7971.73
Saronkhola		0.00001	55	2	0.0002	1.38	7971.73
Morelganj		0.00001	55	2	0.0002	1.38	7971.73
Bagerhat Sadar		0.00001	55	2	0.0002	1.38	7971.73
Kachua	DDT	0.00001	55	0.34	0.003	0.54	3126.17
Chitolmari		0.00001	55	0.34	0.002	0.81	4689.25
Fakirhat		0.00001	55	0.34	0.001	1.62	9378.50
Mollahat		0.00001	55	0.34	0.0023	0.70	4077.61
Mongla		0.00001	55	0.34	0	-	-
Saronkhola		0.00001	55	0.34	0	-	-
Morelganj		0.00001	55	0.34	0	-	-
Bagerhat Sadar		0.00001	55	0.34	0	-	-

In case of monthly scenario of available pesticides, this study found that pesticides were more available or frequently found among samples in the month of November to January. These results are in agreement with the results obtained by Watterson (1991), Robertson et al. (2002), John et al. (2001) and Tsiplakou et al. (2010). They observed that organochlorine pesticide residues were found in higher percentage in samples during the colder months and lower during the warmer months of the year.

Among our sampling sites, the high percentage of available pesticides in shrimp sample was found in Kachua Upazila. This was happened due to the integrated vegetables farming and application of pesticides in that vegetable fields (Islam et al., 2017). Although some pesticides were banned, low concentration of that pesticides were found due to their longer persistence in the environment (Jayaraj et al., 2017).

Influencing Haemocytic Defense in Black Tiger Shrimp (*Penaeus monodon*) using Diversified Lipid A-core Oligosaccharides to Cope with WSSV Infection

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Objectives

- To identify suitable gram-negative bacteria as a better immunomodulators

- To develop effective administration methods of LPS to boost immunity

- To assess the efficacy of algal supplement on survival and metamorphosis of *Macrobrachium rosenbergii* larvae into Post Larvae (PL)

Experiment 1. Identification of suitable gram-negative bacteria as a better immunomodulators

Identification of bacterial candidate

Prior to identify suitable gram-negative bacteria specially by targeting *Vibrio* spp., hepatopancreas from shrimp affected with vibriosis symptoms, water and soil samples were collected from local environment. The samples were plated on selective media, Thiosulfate Citrate Bile-salts Sucrose (TCBS) agar by spreading and incubated at 37ºC overnight. Based on dominant and definite colony morphology, different Vibriospp. strains were initially separated and selected. The selected single colony from each initial plate was transferred to a new plate to obtain pure cultures by repeated streaking. A total of three purified strains of Vibriospp. were initially taken for experimental purpose and all the strains were stored in nutrient broth containing 50% glycerol at -80°C for further identification. One of the three strains was identified as *Vibrio parahaemolyticus* on molecular basis in PCR by species specific primer sets (Table 1). Other two strains (*V. alginolyticus, V. fluvialis*) were identified based on colonial morphology. Beside this, another gram-negative bacterium, Escherichia coliwas also purchased from the company to compare the efficacy.

Molecular identification

Bacteria were taken from overnight cultures of a single colony grown on Nutrient agar media. Genomic DNA was extracted using the Monarch® Genomic DNA Extraction Kit, BioLabs, Inc., New Englandfollowing manufacturer's instructions. The extracted DNA was amplified using the primers stated in Table 1. PCR amplifications were carried out in Thermal Cycler (Bio-Rad ®, Hercules, California, USA). The PCR mixture were then exposed to thermal cycling (15 min at 93 ºC for the pre-denaturation; 35 cycles of denaturation at 92 ºC for 40 s; annealing-extension step at 57 ºC for 60 s, and extension at 72 ºC for 1.5 min, with a final extension at 72 ºC for 7 min). A total of 6 µl of the DNA from the amplified

products were analyzed by electrophoresis in a 1% agarose gel (Lonza, USA) with 50X TAE buffer (Trizma base, Acetic acid and 0.5 M EDTA- pH 8.0) stained in SYBR safe DNA gel stain and gel images were viewed under ultraviolet light transilluminator (Bio-Rad).

Table 1. Specific primer for the detection of *V. parahaemolyticus* bacteria.

Target species	Sequence	Amplicon size (bp)	Targeting gene	Reference
V. parahaemolyticus	F- GCAGCTGATCAAAACGTT GAGT R- ATTATCGATCGTGCCACTCAC F- GATTTGGCGAACGAGAAC R- CGTCTCGAACAAGGCG	897 695	flaE	Tarr et al., (2007)

Standardization of LPS extraction method

Under this experiment, Lipopolysaccharide (LPS) was extracted and optimized from the selected available bacterial lyophilized cells following standard method (Westphal and Jann, 1965 and Rezania et al., 2011) with slight modification. The following bacterial LPS mentioned in Table 1 were initially considered for the experiments.

LPS extraction procedure

For the extraction of LPS, 10 gm of bacterial pellet was taken in a 500 ml glass beaker. Then, 2 M 150 ml Calcium Chloride was added drop by drop and homogenized. After that, the mixture was transferred into a centrifuge tube and centrifuged at 7000 rpm for 30 min at 4°C. Then, the supernatant was taken and the pellet was discarded. Then 25% Ethyl Alcohol was added to the supernatant (Original volume=0, Ethyl Alcohol needed= x, 0.25 (0+.95x) = x). The liquid was mixed for 30 min using magnetic stirrer in iced condition. Then, it was centrifuged at 7000 rpm for 30 min at 4°C. After that, the supernatant was taken and the pellet was discarded again. This time, 80% Ethyl Alcohol was added to the supernatant (Original volume=0, Ethyl Alcohol needed= x, 0.8 (0+.95x) = x). Then, it was stored at -20°Cfor 1 hour. After that, the mixture was centrifuged at 7000 rpm for 30 min at 4°C. Finally, the supernatant was discarded and LPS pellet was stored at -20°C for experimental use.

Efficacy assessment of bacterial candidate/selection of best candidate

The best candidate bacterial LPS was selected based on immunogenicity test from the 4 selected species. Prior to do this, pond reared 6.0 g sized tiger shrimp were collected, transferred into wet lab of SRS and acclimatized for two days in 40 L glass aquarium at a density of 20 individuals/tank. LPS was administrated by intramuscular injection (IM) at 75 ng/g body weight. Aliquot was prepared by mixing required amount of LPS with PBS. The LPS containing aliquot was gently incorporated in the ventral muscle with syringe. THC, SOD and immune gene expression was checked at 24, 48, 72 and 96 h post injection.

Experiment II. Development of effective administration methods of LPS to boost immunity

For this study main challenge was to keep experimental animals (average weight 2 g approx.) alive to certain days in glass aquarium. After taking different strategies, pond reared 2 g sized shrimp transferred into wet lab of SRS and acclimatized for two days in 40 L glass aquarium at a density of 20 individuals/tank (Figure 2). LPS was administrated in two different ways with replication, i) Intramuscular Dose, IM (75 ng/ml) and ii) added with commercial feed (5 µg/g). IM administration was done only once while feed was continued throughout the observation period of 72 hrs (Figure 1). Control tank maintained with the same commercial feed having no LPS. Haemocyte cells were counted after 24, 48 and 72hrs of post administration.

Figure 1. Acclimatization and IM administration of LPS.

Figure 2. Lab visit by honorable Minister S M Rezaul Karim, MP

Procedures of challenge experiment

Before executing the challenge experiment, LPS dose was optimized then shrimp survival was assessed under WSSV challenge. The overview of challenge experiment is shown in Figure 2.

a. Test diet formulation and LPS dose optimization

The black tiger shrimp juveniles (*P. monodon*) with an average weight of 6 g were obtained from the grow-out pond of SRS, Bagerhat. Shrimp were randomly divided into four groups (1, 2, 3 and control), each with three replications holding 20 individuals. The experimental juveniles were installed in 40 L glass aquarium and kept in acclimatization for 3 days before feeding. Shrimp feed were prepared by spraying phosphate buffer saline (PBS) solution containing LPS with three different doses e.g., 3, 5 and 7 µgLPS/g diet, then dried at 20 °C for 1 h. Diets were stored in plastic bags at 4 °C before get used. Shrimp were fed twice daily at 08.00 and 18.00 h and the feeding rate was 5-6% of total body weight. After completion of 5 days feeding haemolymph and tissue samples (n=5) from each individual groups were collected and laboratory analysis were done for the following immune parameter THC, SOD and expression of immune related gene. Treatment group that showed the best immunoresponse at the given LPS dose was considered further for challenge experiment.

b. Challenge trial with WSSV infection

Tooutset challenge experiment, one treatment and one control group of juvenile tiger shrimp ($\approx$6.0 g) each having 3 replications with 20 individuals were transferred in to the 40 L glass aquarium containing UV treated water. After 3 days acclimatization, the treatment group were fed with 7 µgLPS/kg LPS coated dietat 5% BW twice daily for 7 days while control group were provided LPS free shrimp diet. After 7 days of feeding trial, experimental animals from the both groups were subjected to WSSV challenge by immersion method (Chotigeat et al., 2004) with moderate aeration. The viral stock solution was prepared following the method of Chang et al. (2008a). Briefly, 0.5 g of the frozen infected specimens of *P. monodon* were minced and then homogenized in 4.5 ml of sterile phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH2PO4). After centrifugation at 400 ×g for 10 min at 4 °C, the supernatant was filtered through 0.45 µm membrane. This viral concentration caused 50% mortality in challenged shrimp in 5 days in a preliminary study. The filtered supernatant was used as the viral stock solution. Equal volume (5ml) of viral stock solution were inoculated per 40-L of rearing water. All parameters weremaintained same in both group during the entire challenge period. Removal of wastes and 30% water change were done daily. Test animals were monitored for 96 hours post infection (h.p.i) and checked daily for mortalities. Dead shrimp were removed, counted for survival and subjected to polymerase chain reaction (PCR) analysis to confirm presence of WSSV. Specific primer set for WSSV were used, electrophoresis of PCR products revealed heavy bands in shrimp infected with WSSV.
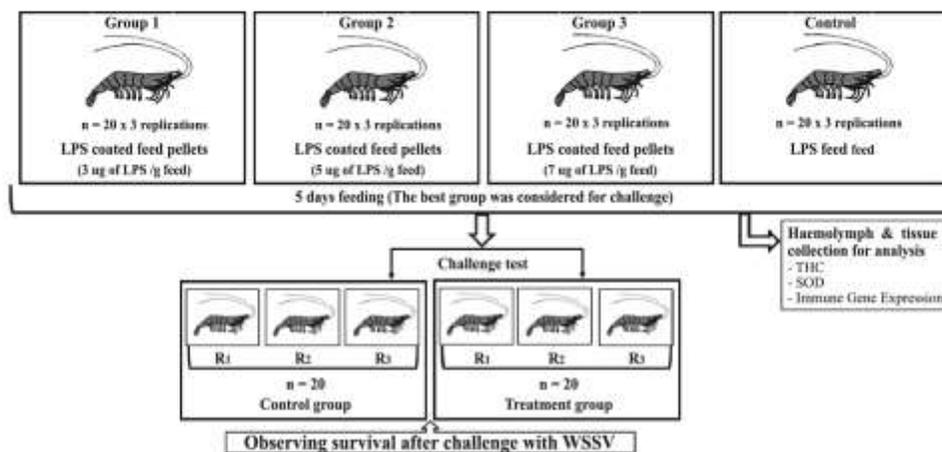
Figure 2. Overview of the challenge experiment.

Total haemocyte count (THC)

Haemolymph was withdrawn from the ventral sinus of each shrimp with a 26-gauge needle. A 100-µl aliquot of haemolymph were immediately diluted with 900 µl of an anticoagulant solution (0.03 M tri sodium citrate, 0.45 M sodium chloride, 0.01 M EDTA, 0.1 M glucose, and 0.026 M citric acid; pH 7.5). A drop of the anticoagulant-haemolymph mixture was placed in a haemacytometer to measure the THC using an inverted phase-contrast microscope (TS100F, Nikon, Japan). The remainder of the mixture were used for subsequent analyses.

Superoxide Dismutase Activity

SOD activity was determined according to the protocol of Creative Enzymes.

(https://www.creativeenzymes.com/pdf/Protocol%20CE_Superoxide%20Dismutase%20EC%201.15.1.1.pdf).

Expression of immune related genes

Immune related gene expression was performed by taking shrimp samples preserved at -80°C from different experimental steps such as selection of the best candidate, LPS dose optimization and WSSV challenge experiment using qRT-PCR (List of the specific primers given in the Table 2). RNA was extracted from the shrimp tissues using RNA Mini Kit (Invitrogen, USA) according to the manufacturer's protocol. The concentration and purity of the total RNA were quantified using A260/280 nm ratio using Spectrophotometer (UV-1800PC, Abbota Corporation). Then RNA were reverse transcribed into cDNA with Verso cDNA Synthesis Kit (Thermo Fisher Scientific Baltics UAB, Lithuania). RT-qPCR was performed on a HYRIS bCUBE QPCR System (London, UK). The reaction mixture consisted a total volume of 20 µl containing 1x (10 µL) of Luna Universal qPCR mix (BioLabs, New England), 10 µM forward (0.2µL) and reverse primers (0.2 µL),

2 µl of cDNA template, and 7.6 µl of sterile ultrapure DNase/RNase-free water (HyClone, Australia). qPCR was performed at 95 °C for 2 min, 40 cycles of 95 °C for 5 s, 60 °C for 11 s and 72 °C for 19 s.

Table 2. Summary details of immune gene primer sequences used for the study

Gene	Primer sequence (5'-3')	Annealing temperature (°C)	Reference
Toll Like Receptor (TLR)	F. CTTAGCCTTGGAGACAAC R. GATGCTTAACAGCTCCTC	53	Deris et al. (2020)
Prophenoloxidase (proPO)	F. CTCCCTAGTCTTCAAGGT R. CATTTCCTGCGAGATACC	54	
Photorhabdus-like Insect A (PIR A)	F. TTGGACTGTCGAACCAAACG R. GCACCCCATTGGTATTGAATG	60	Han et al. (2015)

Experiment III. Efficacy of microalgae on *M. rosenbergii* larval development in the Larvae Rearing Tank (LRT)

To enhance final metamorphosis into PL, black algae powder and spirulina powder was administrated at different frequencies to assess the efficacy on final molting to PL. Flowthrough system with no algal supplement were continued as the control.

Table 3. Efficacy of microalgae on *M. rosenbergii* larval development in Larvae Rearing Tank (LRT)

	Frequency of microalgae administration (per day)	Dose of microalgae (Black algae powder + spirulina powder)	Stocking Density	Replication
T$_1$	4	(2+2) 4 ppm	80 larvae/litter	2
T$_2$	6			
C	-	-		

General hatchery operation protocol for PL production

Before stocking the berried females in the holding tanks, the selected females were disinfected by bathing in 25 ppm formalin solution for about an hour with continuous aeration. The berried females were transferred to the hatching tank with aeration. Salinity of water in the hatching tank were maintained at 12 ppt and the spawner were fed with wholegrain rice and small fish/prawn at the rate of 3-4% body weight, twice daily at morning and evening. The fecal matters and unconsumed feed were siphoned out after every feeding. Water of hatching tank was exchanged daily. Eggs of berried female hatched within three days. After hatching, the spent females weretaken out and the newly hatched larvaewere transferred to the LRTs for rearing up to the post-larvae stage.

After 24 hours of hatching, newly hatched larvae were fed with Artemia nauplli at the density of 4-6 nos/ml of water, twice daily at morning and evening up to 10 days. Thereafter, the larvae were fed 4-6 times/day with prepared feed composing Tilapia/fish muscle, milk powder, hens' egg, wheat flour yeast, tetracycline, vitamin drops at the ratio of 30.25.30.10.4.5.0.5 and 10 drops, respectively. Along with this, in the LRT of T1 and T2, microalgae (Black algae powder + spirulina powder) was provided at a dose of 4.0 ppm

with 4 and 6 times per day, respectively. Fecal material, unused feed, molted shells, etc. were siphoned out prior to feeding. Hygienic condition was maintained in every step and extreme care were taken so that the PL not gets infected. Continuous aeration was provided for sufficient oxygen supply. Water salinity, water temperature, dissolved oxygen, pH and un-ionic ammonia nitrogen were monitored and recorded simultaneously.

Evaluation criteria
Progression rate of metamorphosis in different larval development stage, PL conversion rate and enzymatic activity (amylase, protease, and lipase enzyme activity) were recorded and analyzed for comparison.

Amylase activity
Amylase activity was assayed by starch hydrolysis method of Bernfeld in which the increase in reducing power of buffered starch solutions was measured. The specific activity of amylase was calculated as milligrams of maltose liberated per gram of protein per hour (mg/g/h). The reaction mixture consisted of 0.125 ml of 2% (w/v) starch solution, 0.125 ml of 0.1 M citrate phosphate buffer (pH 7.5) and 0.5 ml enzyme source. The reaction was incubated at 37 °C for 1 hour, and the absorbance was measured at 600 nm against a blank. For the blank, the enzyme source was added just after the incubation period. Maltose solution was used as standard (Bhavanet al., 2010).

Protease activity
The protease activity was estimated by using the casein-hydrolysis method by the method of Furneet al. (2005). Casein was used as substrate. The reaction mixture contained casein at 1% (w/v) (0.25 ml), 0.25ml of 0.1M glycine –NaOH buffer (pH 10) and 0.1ml supernatant (enzyme source). The mixture was incubated for 1 h at 37°C. The reaction was stopped by addition of 0.6 ml 8% (w/v) trichloro-acetic acid solution; kept for 1 h at 2°C; centrifuged at 1800 g for 10 min and the absorbance of supernatant was measured at 280 nm against blank.

Lipase activity
The lipase activity was determined by the evaluation of the degradation of triacylglycerol's, diacylglycerols, and monoacylglycerols to free fatty acids, following the method of Bier (1955). One litre of Polyvinyl alcohol was prepared. A solution of 1% polyvinyl alcohol and 5ml of 0.1 N HCl was heated to 75°C- 85°C. Then they were cooled and filtered. The solution was adjusted to 8.0 with 0.1 N NaOH. Virgin olive oil was added to an aliquot of this solution for obtaining 0.1 M substrate concentration. This mixture was emulsified for 5 min. A mixture of 1 mL of emulsified solution, 0.5 mL of enzyme source and 0.5 mL of phosphate-citrate buffer was incubated for 4 hours at 37°C. To stop the reaction and break the emulsion, 3 mL of 1.1 ethanol-acetone was added. To the reaction mixture, phenolphthalein in ethanol 1% (w/v) was added titrated against 0.01 N NaOH. For the blanks, the same procedure was followed and boiled enzyme was used. Porcine type 2 Lipase was used as standard.

Statistical analysis
After collection of data, statistical analysis was done using Microsoft Excel and GraphPad Prism version 6.0.

Results

Four bacterial isolates were selected for identifying suitable candidate as a better immunomodulators. The selected isolates were *Vibrio parahaemolyticus*, *Escherichia coli*, *Vibrio alginolyticus* and *Vibrio fluvialis*. Among them *Vibrio alginolyticus*, *Vibrio fluvialis* isolates were identified provisionally based on colonial morphology whereas *Vibrio parahaemolyticus* was confirmed by conventional PCR. Figure 3 (a-c) summarizes the specific PCR based and morphological character based detection of the isolates. In species specific PCR assay strain *V. parahaemolyticus* strain was amplified (Figure 3, a) and detected with 897-bp specific fragment, whereas based on distinguished and referendum colony characteristics (Table 4; Figure 3 b, c) other two were identified as *V. alginolyticus and V. fluvialis*.

Table 4. Morphological characteristics of two *Vibrio* spp. strains.

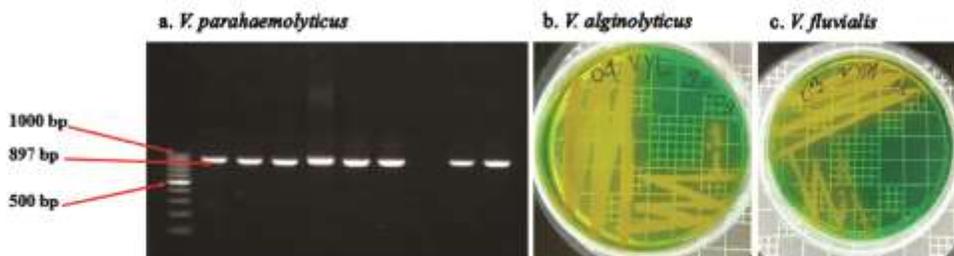| Name of strain	Colony characteristics
1. *Vibrio alginolyticus*	Yellow, large, transparent and round in shape
2. *Vibrio fluvialis*	Yellow, medium, Opaque and round shape

Figure 3. (a). Confirmation of *V. parahaemolyticus* in PCR; (b and c). Colony morphology of *V. alginolyticus* and *V. fluvialis* on TCBS agar plate.

A very simple and high yielding LPS extraction and purification method was developed and standardized with slight modification of previously reported protocol. LPS was successfully extracted from all selected bacteria with optimized protocol (Figure 4).
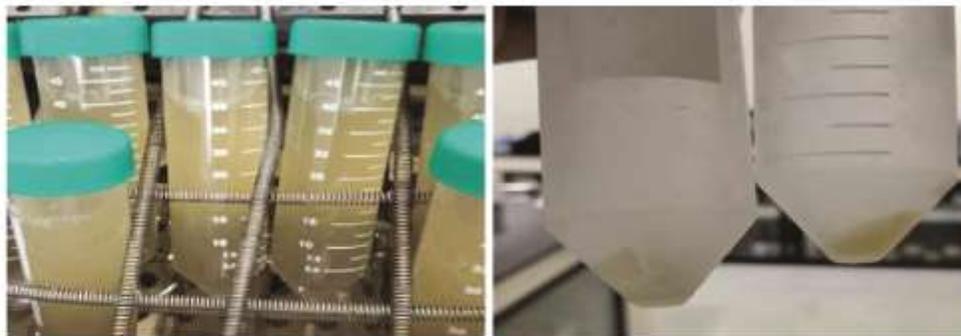

Figure 4. Extracted LPS from the isolated strains.

Prior to know the effect of LPS in shrimp immunity, it was administered to shrimp separately either by IM injection or feeding with LPS coated diet at 75 ng/g BW and 5 µg/g feed. IM showed almost three times more increase in hemocytes followed by LPS provided with feed (Figure 5; a and b).
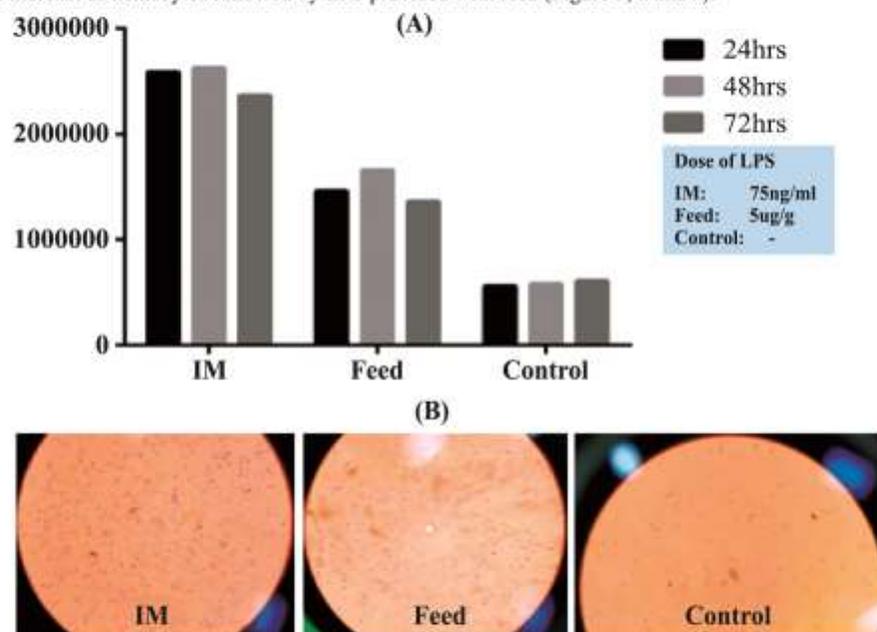

Figure 5. (a) Number of hemocytes/ ml at different methods of LPS administration; (b) Images of hemocytes cell density at different methods of LPS administration.

Figure 6 shows the comparative THC count in four shrimp groups at 24, 48 and 96 hours post inoculation (h.p.i) of LPS extracted from four isolates. THC in all strain gradually increased with specified monitoring period (24 to 96 hours post injection). At 24 h.p.i, THC were more or less equally uplifting for all isolates and at 48 h.p.i THC augmented by *V. parahaemolyticus* and *E. coli*. However, THC maximum at 96 h.p.i for all strains but highest was recorded for LPS of *V. alginolyticus* and the second highest was counted for *E. coli* as well.

Figure 7 illustrating the comparative immunogenicity of shrimp fed LPS coated diet with variable doses (3, 5 and 7 µg/g feed). Treatment 3 (7 µg/g diet) had maximal THC and SOD which significantly different than other treatment and control. Again, for both THC and SOD, no significant differences existed between T1 and T2 but T3 was significantly dominant over all treatment and control. Therefore, LPS dose of T3 (7 µg/g diet) was further considered for challenge experiment. The relative gene expression in different treatment and control groups are depicted in Figure 8. A dose dependent gene expression was expected from the gene expression analysis. However, in respect of TLR, gene expression level concomitantly uplifted with LPS dose expansion respectively. But proPO and PIR A gene expressed heavily in T2 (5 µg/g diet) rather than higher LPS coated diet (T3).
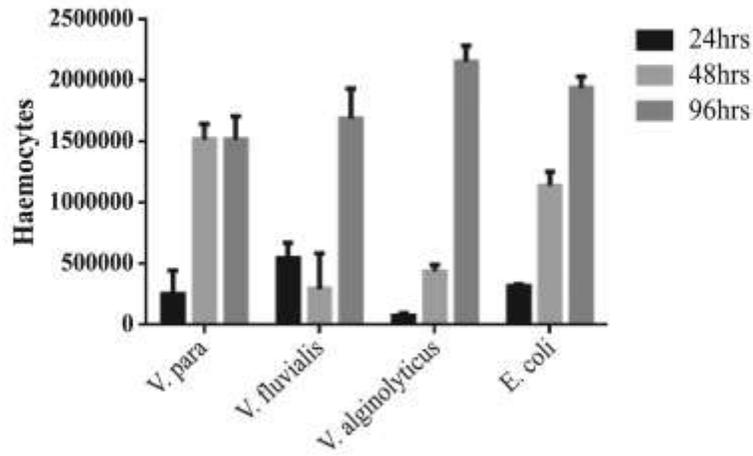
Figure 6. Comparative analysis of Total Haemocyte Count (THC) in shrimps after IM injection of LPS, extracted from four selected isolates.
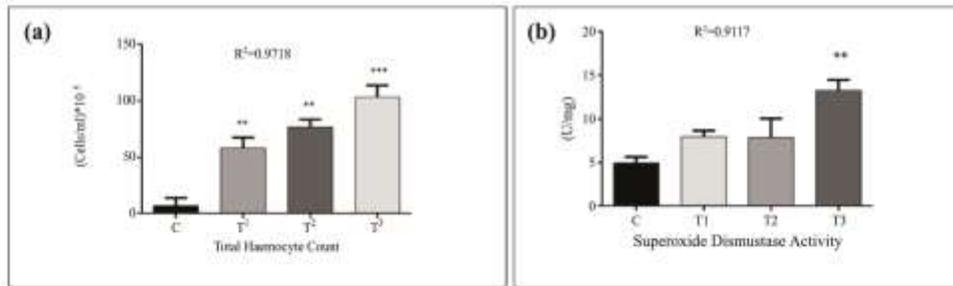

Figure 7. (a) Comparative analysis of Total Haemocyte Count (THC) and (b) Superoxide Dismutase Enzyme (SOD) activity in shrimpsof four different treatments after administration of LPS from *V. alginolyticus.*
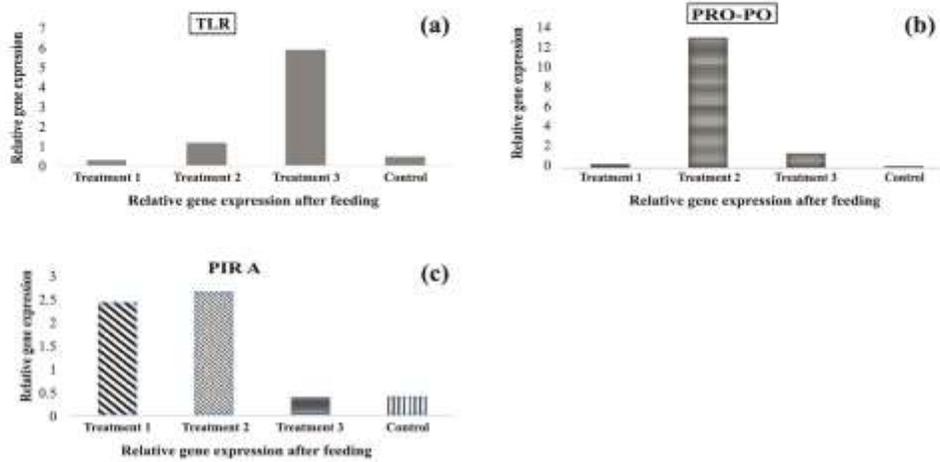
Figure 8. Relative gene expression in different treatment groups after feeding.

The results of challenge experiment are displayed in Figure 9. WSSV challenge results showed that mass mortality commenced on the 3rd day of post-challenge test in the control group (Figure 9) and 50% mortality occurred around 100 h.p.i. Mortalities stopped at 7 d.p.i both in treatment and control group. 85% survival was recorded in the treatment group fed with LPS at the end of the experiment whereas only 15% shrimp survived in control group. Overall, the results manifesting that, LPS can extremely shows disease resistance against WSSV infection of shrimp *P. monodon* farming. The gene expression level for proPO, TLR and PIR A in treatment group significantly increased than in the control group (Figure 10) which reflecting the higher survival rate in treatment group also.
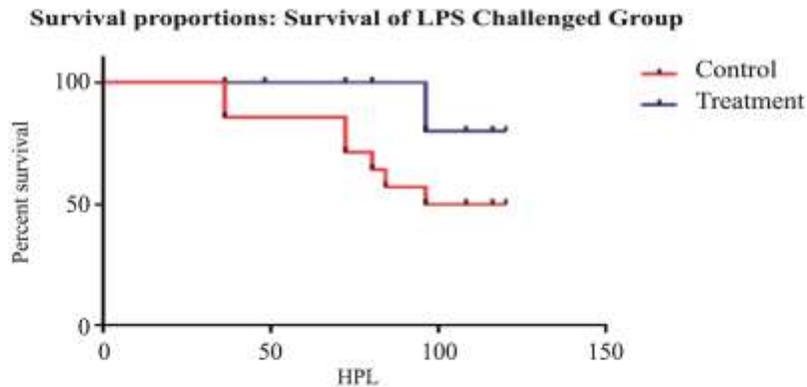

Figure 9. Survival Rate (%) of LPS fed (T3) shrimps and control group after challenged with WSSV infection.
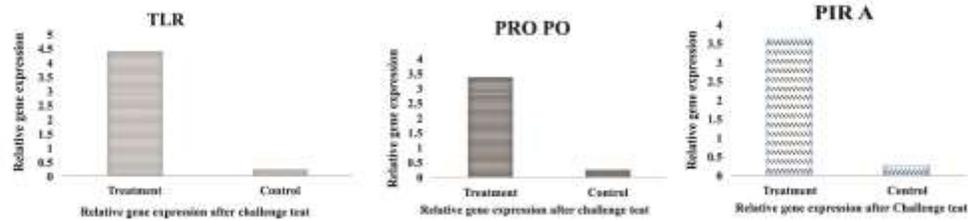
Figure 10. Relative gene expression in treatment and control group after challenge test.

As per the 3rd objectives of the project, Figure 11 is depicting the trends of *M. rosenbergii* stage dispersion through the process of metamorphosis in control (without microalgae) and treatment (with addition of microalgae) LRT. The switching rate of larval stage development as well as conversion of early larval stage to higher stage were comparatively higher and faster in both treatment LRT than in control. According to the statement of Figure 12, PL conversion rate were significantly higher in T2 (95.41 ± 0.28 %) and T1 (86.21 ± 0.51 %) than in control (63.22 ± 0.35 %) LRT. PL production was almost 1.51 times higher in treatment 2 provided with algal supplement for 6 times frequencies in comparison to control LRT without algal supplementation. Another interesting result is that, the different digestive enzymatic activity (protease, lipase and amylase) also found significantly robust in Treatment 2 than Treatment 1 and control (Figure 13, 14 and 15) which is strong influence of microalgae supplements. By and large, the results have shown that the addition of microalgae at appropriate levelssubstantially improved performance of newly hatched larval culture of the *M. rosenbergii*, suggestingthat the black algae along with spirulina supplements in LRT has a promising potential for giant river prawn hatchery development.
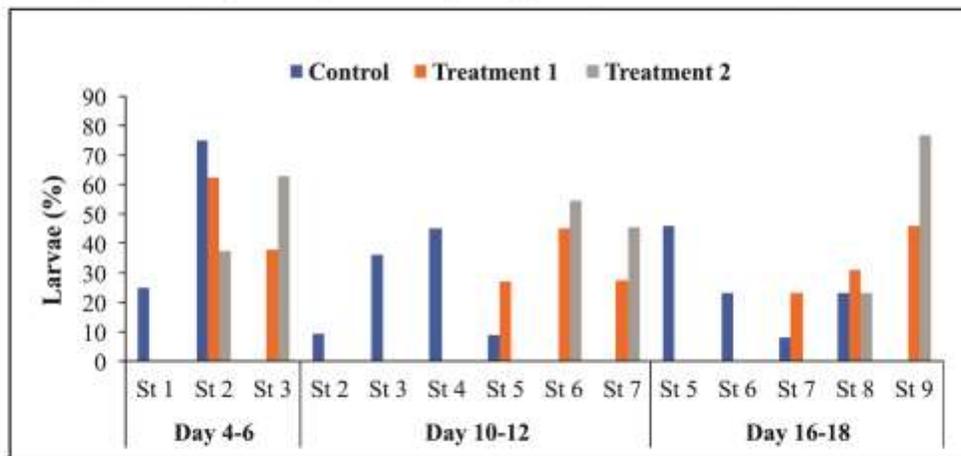
Figure 11. Stage dispersion of *M. rosenbergii* larvae in different Treatments.
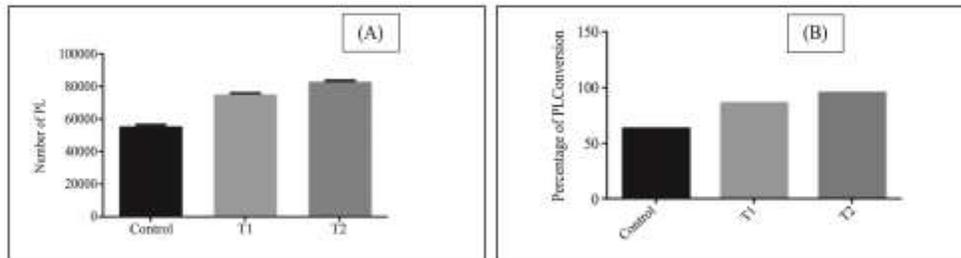
Figure 12. (a) PL production trend (Different letters illustrates significant differences between treatment (p<0.05) where a>b>c and (b) PL conversion rate in different Treatments.
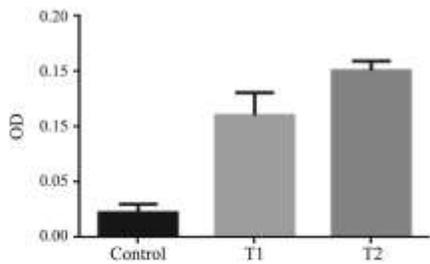
Figure 13. Activity of Amylase enzyme in larvae of different Treatments.
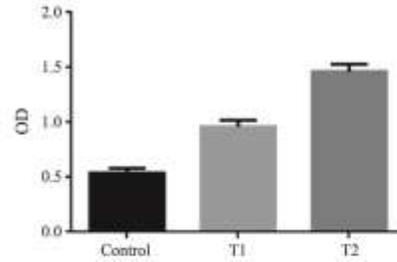
Figure 14. Activity of Protease enzyme in larvae of different treatments.

Figure 15. Activity of Lipase enzyme in larvae of different Treatments.

Discussion

LPS is the main component of cell membrane of almost all gram negative bacteria. It is responsible for the pathological consequences of gram negative bacterial infections. LPS is potent activator of immune system capable of triggering cytokine release from cells of different origin. It is widely used as an inducer of TLR-4 signaling pathway (Kawai and Akira, 2010) and as a mediator of dendritic cell maturation (De Smedt et al., 1996). In this context, several attempts have been made so far to introduce a dozen of 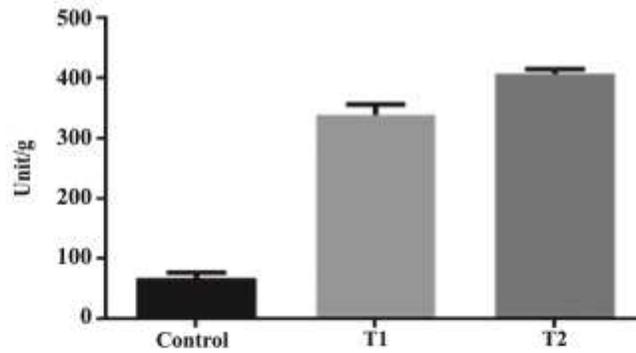techniques for extraction and purification of LPS from different strains of gram negative bacteria (Morrison and Leive, 1975; Ingram and Alexander, 1980; Baba et al., 1988; Al-Harbi and Austin, 1992 and Perdomo and Montero, 2006).

Among the gram negative bacteria, *Vibrio* sp. is a rich source of LPS that can be found at the bacterial cell surface. Newman (2000) administered *E. coli* LPS for juvenile penaeid shrimp, later challenged with WSSV and got better survival rate. Considering these, four candidates of gram negative bacteria and specially three Vibrio sp. were selected for LPS experiment.

Hemocytes are one of cellular immune responses in *P. monodon* (Jiravanichpaisal et al., 2006), hence total haemocyte count is often used as an immunological indicator for shrimp immune level and health status. Total haemocyte counts have been reported to decrease in infected shrimp in comparison to the healthy population (Chang et al., 2008b). Shrimp immune parameters (i.e., total hemocytes count (THC), superoxide dismutase (SOD) activity etc. were significantly affected by exposure or ingestion of ZnO nano particles with immunostimulant property (Ishwarya et al., 2018). Superoxide dismutase activity of shrimp fed diets having 0.5, 1.0and 2.0 g/kg of immunostimulant was considerablysuperior to control shrimp (Ishwarya et al., 2018). Our present study reflected higher activity of different immune properties in *V. alginolyticus* among the four candidates after LPS administration. So, Considering the previous knowledge and literatures, it specifies that *V. alginolyticus*is the best candidate of LPS as an immunomodulators in shrimp.

Among the two administering methods (Oral with feed, Intramuscular injection), better results in immune properties were observed in case of IM administration. But this method might not be economically feasible in large scale application. The oral administration of LPS might be advantageous in minimizing damages to tissues and hemocytesin comparison to the direct LPS injection approach. The LPS-supplemented diet might contribute to enhancement ofintestinal immunity without damaging intestinal epithelia, thus THC and GC counts were not significantly affected. In contrast, when LPS was given to shrimp via injection, the total haemocyte counts decreased, suggesting for cytotoxicity effects to the animals (Lorenzon et al., 1999). The intravenous administration of LPS alsocauses toxicity in mammals, but the oral administration of highdosage of LPS does not inducecell toxicity in mammals, birds orfish (Inagawa et al., 2011). So, the oral administration method of LPS as feed coating or ingredient might be a wise approach to suggest for tendering in large scale.

On the observation of comparative immunogenicity in shrimp after the administration of LPS extracted from the best selected candidate (*V. alginolyticus*) at different doses (3, 5, 7 µg/g feed), treatment 3 (7 µg/g feed) showed best results. All the measured immune properties were found higher in that treatment. The treatment group also showed 85% survival against WSSV infection whereas, the control group showed

50% at a constant time period, which indicates the optimum dose of LPS of V. alginolyticus to be administered orally for attaining best possible results. When, LPS was administered at ahigher dose, lower survival was obtained. Genio et al. (2014) suggested that higher concentration of LPS on feed beyond the assumed optimum level would not have any significance. In their study, shrimp fed with 50 mg dietary LPS elicited the highest protection against WSSV infection with 72% survival. At a higher dose of 100 mg, survival fell to 60%. An improved survival (75% higher than the control) against penaeid acute viremia virus was evident in adult *Marsupenaeus japonicus* fed diet containing Pantoeaagglomerans LPS at a dose of 20 μg/kgbody weight (Takahashi et al., 2000). A similar study by Felix (2005) reported that at optimum of dose of 30 mg *E. coli* LPS/kg diet was enough to protect juvenile *P. monodon* against *Vibrio parahaemolyticus* infection with a reported survival of 75%.

To further assess molecular effects of LPS supplement on host immune system, transcript levels of genes encoding the Toll Like Receptor (TLR), Prophenoloxidase (proPO), and Photorhabdus-like insect A (PIR A) were determined in the black tiger shrimp. These genes of interest were previously shown to be induced immunity in black tiger shrimp and in different post larvae stages of giant tiger prawns which is the part of host defense mechanisms against *V. harveyi* (Charoensapsri et al., 2009) and *V. parahaemolyticus* (Deris et al., 2020). Therefore, relative expression levels of these aforementioned transcripts in the black tiger shrimp on day 05 after fed with the LPS supplement diets (Groups 1, 2 and 3) to those fed with the non-LPS as a control diet were determined. For the TLR gene expression level increased in an equal rhythm with LPS dose intensity. The TLR expression level in T_3 (7μg/g feed) fed shrimp was significantly induced by 19, 6 and 12.5-folds in comparison to T_1, T_2 and control group respectively. In contrast LPS did not seem to affect the transcript level with the same mannerism for PIR A and proPO genes. This suggested that the up-regulation of different immune gene was not specific to LPS treatment. It is important to emphasize that, the TLR gene as well as the Toll-pathway plays key roles in regulating the innate immune response in tiger shrimp (*P. monodon*) against WSSV and *V. harveyi* infection (Deepika et al., 2014 and Dechamma et al., 2015) which was gradually induced as per LPS dose upliftment in this study. Li et al. (2018) clarified the defense mechanism of TLR gene in shrimp against WSSV. They stated that, a shrimp Toll4 from a total of nine Tolls in *L. vannamei* confers resistance to WSSV thought inducing the NF-κB transcription factor Dorsal to inspire the production of some antimicrobial peptides (AMPs) with antiviral activity. The anti-LPS-factor (ALF) and lysozyme (LYZ) family are identified as the Toll4-Dorsal pathway targeted genes with the ability to interact with viral structural proteins in response to WSSV infection. These emchanism suggest that the Toll receptor induces the expression of AMPs with antiviral activity could be a general antiviral mechanism in invertebrates and Toll pathway established antiviral defense could be conserved during evolution. More interestingly all three immune genes were significantly upregulated their expression level in challenge bioassay, wherein TLR gene induced more than 20 folds in shrimp of treatment group compare to control group. With the promising results of LPS we are providing the evidence of pathogen resistance in the black tiger shrimp when feeding with LPS-supplemented diet. While the LPS containing diet resulted in significantly higher survival rates when exposed to WSSV than the normal diet without LPS. Moreover, the LPS supplement showed induction of some crucial immune-related transcripts such as TLR, proPO and PIR A in shrimp. These findings suggest that LPS as an immunostimulant was able to activate the immune system at a molecular level in tiger shrimp and enhanced disease resistance. LPS supplement is therefore a promising candidate to increase disease resistance in black tiger shrimp

farming. The current study clearly demonstrated that the addition of Microalgae (Black algae powder + Spirulina powder) in Larval Rearing Tank (LRT) of *Macrobrachium rosenbergii* has a significant impact on survival rate and metamorphosis of larvae. The treatment group with 6 times application frequency exhibited the best survival rate (95%) and rapid metamorphosis. Digestive enzymes activity (Amylase, Lipase, Protease) of the larvae was also observed higher in that group; which confirmed better utilization of feed as well as assimilation of energy and nutrition in larvae of that group. Application of microalgae in larval rearing tank not only improves water quality but also removes nitrogenous substances in the system (Mallasen and Valentini, 2006). It has been reported that microalgae are able to produce several bioactive compounds that can obstruct the entry of various harmful pathogens (Lober and Zeng, 2009). Occurrence of microalgae or its constituents, even at small quantity in the gut can activate the production of digestive enzymes and enhance growth performances (Brito et al., 2004). Brown et al. (1997) stated the presence of several growth promoting pigments such as chlorophyll, carotenoids and phycobiliproteins in microalgae. The results have shown that the addition of Microalgae at appropriate levels can substantially improve the performance of larval culture of *M. rosenbergii* having a promising potential of eradicating the existing problems of prawn hatchery and increasing PL production.

Development of Mariculture Practice of Seabass (*Lates calcarifer*) in the Southwest Coast of Bangladesh

Researchers
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Objectives
- To develop cage culture technique of Seabass in coastal water of Bangladesh
- To develop brood of Seabass in coastal environment
- To study growth and survival of Seabass in net cage

Methodology

Experiment-1. Replacement of live feed with commercial/ Trash Fish at same stocking density on Seabass cage culture

Cage culture management and techniques
Same size (5-8 cm) wild seabass fry were stocked. Before installing fry in cages, fries were kept in LRT for two weeks and enforced to take commercial feed. Prior to stocking seabass juvenile in cages, fish were acclimatized to the ambient temperature and salinity prevailing in the cages. Stocking density was based on previous result.

Feeds and feeding

Feed is the major constraint confronting the sea bass culture industry. Initially commercial and trash fish were supplied at the rate of 10% of total biomass in the first two months of culture. After 60 days of culture feeding rate was 5% of total biomass. Feed was provided only when the fish swim near the surface to eat.

After stocking altered the commercial feed with trash fish according the table mentioned in bellow.

Table 01. Experimental design for year 2021-22.

Treatment	Alter Commercial feed with Trash fish	Stocking Density
T_1	25% (25% Commercial feed +75% Trash fish)	6 individuals/m³ (chosen from previous year experiment)
T_2	50% (50% Commercial feed +50% Trash fish)	
T_3	75% (75% Commercial feed +25% Trash fish)	

*All treatments had 3 replications

Fish cage management

Damaged wood was changed with new wood. Damaged plastic drums were changed and some new plastic drums were bought.

Regular observation of cages had been done. Since fish cages were immersed under water all the time, they were vulnerable to destruction by aquatic animals such as crabs, otter, etc. Damaged cages were repaired immediately or replaced with a new one.

In addition to biofouling, the net walls of cages were subjected to siltation and clogging. Biofouling was unavoidable since the net walls usually represent a convenient surface for attachment by organisms such as amphipod, polycheate, barnacles, molluscan spats, etc.

These could lead to clogging and reduce exchange of water and may result in unnecessary stress to the cultured fish due to low oxygen and accumulation of wastes. Feeding and growth would likewise be affected. Mechanical cleaning of fouled nets was done frequently.

Figure 3. Repairing cages.

Water quality and growth parameters monitoring

Water quality parameters *viz.*, temperature, pH, dissolved oxygen, salinity, ammonia, iron, alkalinity etc. of different cage had been monitored weekly. On the other hand, growth of seabass had been monitored fortnightly.

Figure 4. Sampling activity.

Data analysis

All experimental data was analyzed using Microsoft excel and SPSS statistical softwares.

Results and Discussion

Growth performance of *Lates calcarifer* at 105 days

The initial weight was 68.97±1.10 g. The treatment T_1 showed better growth compared to the other Treatment. The weight gain of Seabass has been shown in Figure 07. Average weight of fish on 15 days intervals has been shown in Table 6. At final harvesting maximum average weight was found 84.35±6.255g at T_1 and average length 19.40 cm at T_2. The growth rate was slow because facing problems to adapt with chopped trash fish. Different Water quality parameters of Bhairab river during culture period has been shown in Table 08. After 3.5 months culture period in cages overall 80% survival rate was found.

Table 2. Weight of Seabass during different sampling time (2021-22).

Treatment	Initial Weight (g)	Average Weight (g)						
		15 Days	30 Days	45 Days	60 Days	75 Days	90 Days	105 Days
T_1	68.97± 1.10	69.01±1.04	70.03±1.22	70.67±2.30	76.05±3.33	83.75±5.52	84.01±6.23	84.35±6.25
T_2		68.99±1.01	69.05±1.12	69.06±3.25	72.12±2.23	80.40±3.21	81.40±4.15	82.02±4.50
T_3		68.92±1.09	69.01±1.65	69.12±2.19	70.20±3.05	74.83±3.88	75.02±3.92	76.15±4.22
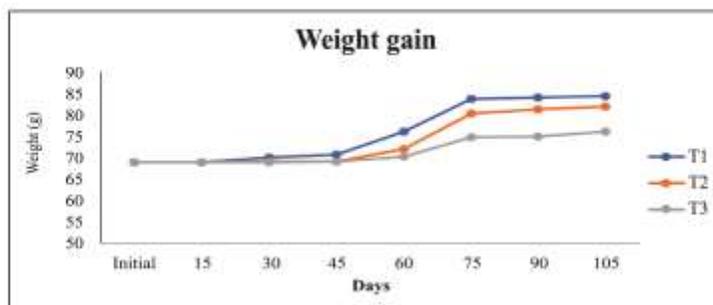
Figure 5. Weight gain of Seabass during the year 2021-22.

Initial length of fish was 18.96 cm. Final average weight was higher in T_2 compared to the other Treatment (Table 07). In Figure 08 ,length gain of Seabass has been presented.

Table 3. Length (cm) of Seabass during different sampling time (2021-22).

Treatment	Initial Length (cm)	Avg. Length (cm)						
		15 Days	30 Days	45 Days	60 Days	75 Days	90 Days	105 Days
T_1		18.96	19.00	19.01	19.08	19.18	19.22	19.29
T_2	18.96	19.00	19.03	19.06	19.16	19.25	19.32	19.40
T_3		19.02	19.03	19.07	19.15	19.20	19.26	19.31
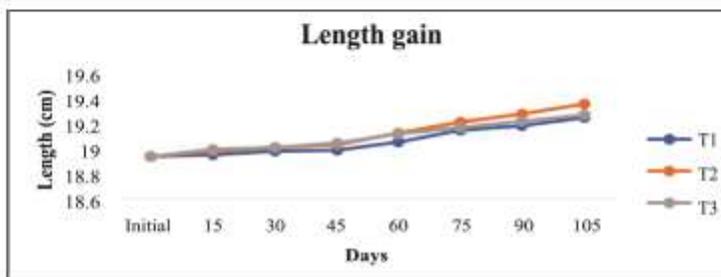
Figure 6. Length gain of Seabass during the year 2021-22

Table 4. Water quality parameter of Bhairab river during culture period (2021-22).

Month	Salinity (ppt)	pH	Turbidity (cm)	Oxygen (mg/l)	Temperature (°C)
March	5.33±0.39	8.20±0.14	37.25±1.48	8.20±0.21	29.55±0.78
April	7.31±0.46	8.27±0.25	38.40±1.11	6.74±0.12	31.73±0.71
May	9.50±0.28	8.05±0.21	34.35±1.20	6.40±0.28	33.60±0.57
June	7.52±0.59	8.03±0.11	30.93±0.13	6.56±0.06	31.45±0.46
July	5.33±0.44	6.91±0.30	31.25±1.39	5.92±0.45	30.81±0.98

Assessment of Stock and Standardization of the Spawning Potential Ratio (SPR) of commercially important marine fish groups of Bangladesh

Researchers

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Objectives

- To estimate the life-history characteristics and stocks of commercially important marine fish species (Tuna and Mackerels) of Bangladesh

- To estimate the biological reference points (BRP) of Tuna and Mackerels

- To standardize Spawning Potential Ratio (SPR) of Tuna and Mackerels

Experiment 1. Estimating the life-history characteristics and stocks of commercially important marine fish species (Tuna and Mackerels) of Bangladesh

Land based data of commercially important marine fish, relationship between total length and weight, length at first sexual maturity of 12 species i.e. *Auxis thazard* (Frigate tuna), *Euthynnus affinis* (Little tuna), *Katsuwonu spelamis* (Skipjack tuna), *Auxis rochei* (Bullet Tuna) *Thunnus albacores* (Yellowfin tuna), *Scomberomorus guttatus* (Indo-pacific king mackerel), *Scomberoides commersonnianus* (Talang queen fish), *Scomberoides tol* (Needlescaled queenfish), *Scomberoides lysan* (Doublespotted queenfish), *Rastrelliger kanagurta* (Indian mackerel), *Rastrelliger faughni* (Indian mackerel) and *S. commerson* (Narrow-barred Spanish mackerel) have been observed. The findings are presented in Table 1.

Table 1. Length at first sexual maturity of 12 marine species.

Species name	Lm
Auxis rochei (Bullet tuna)	41.57 (31.08 - 54.88)
Auxis thazard (Frigate tuna)	24.83 (18.99 - 32.21)
Euthynnus affinis (Little tuna)	37.79 (28.37 - 49.73)
Thunnus tonggol (Long tail tuna)	34.92 (26.31 – 45.84)
Katsuwonus pelamis (Skipjack tuna)	33.30 (25.14 - 43.63)
Scomberoides commersonnianus (Talang queenfish)	60.95 (44.82 - 81.52)
Scomberomorus guttatus (Indo -pacific king mackerel)	56.29 (41.53 - 75.07)
S. commerson (Narrow-barred Spanish mackerel)	60.41 (55.42 – 81.21)
Scomberoides lysan (Double spotted queenfish)	27.84 (21.18 -36.26)
Rastrelliger faughni (Indian Mackerel)	16.08 (12.53 -20.56)
Rastrelliger kanagurta (Indian Mackerel)	12.89 (10.15 -16.36)
Scomberoides tol (Needlescaled queenfish)	23.31 (17.87 -30.18)

About total 870 (male=551 and female=319) individuals of *Auxis thazard* were examined. Estimated size at sexual maturity was at the length of 24.83cm for both sexes. Total 986 (male= 450 and female=536) individuals of *Euthynnus affinis* were examined with sexual maturity was at the length of 37.79 cm. Total 134 (male=74 and female=60) individuals of *Auxis rochei*, 154 (male=90 and female=64) individuals of *Katsuwonus pelamis*, Sum of 167 (male=105 and female=62) individuals of *Thunnus tonggol*, Sum of 198 (male=120 and female=78) individuals of *Scomberomorus guttatus*, about 772 (male=540 and female=232) individuals of *Scomberoides commersonnianus*, about 156 (male=82 and female=74) individuals of *Rastrelliger kanagurta*, Total 134 (male = 86 and female = 48) individuals of *Rastrelliger faughni*, Total 145 (male 85 and female 60) individuals of *Scomberoides tol*, Sum of 34 (male =75 and female = 59) individuals of *Scomberoides lysan* were observed, where the size at sexual maturity were 41.57 cm, 33.30 cm, 34.92 cm, 56.29 cm, 60.95 cm, 12.89 cm, 16.08 cm, 23.31 cm and 27.84 cm respectively.

Table 2. Descriptive statistics and assessed parameters of length–weight relationships (BW = a × TLb) for 12 commercially important marine fish in Bay of Bengal, Bangladesh during July 2021 to June 2022.

Species	N	Total length			Body weight			Regression parameter		95% CL of a	95% CL of b	r²
		Min	Max	Mean	Min	Max	Mean	a	b			
Auxis thazard (Frigate tuna)	870	14	45	32.34±5.6	276	1206	502.6±155.7	0.0358	2.704	0.0163-0.782	2.48-2.926	0.89
Auxis rochei (Bullet tuna)	134	20.5	79	40.25±12.3	78	2052	671.75±213	0.121	2.548	0.08-0.19	2.23-2.65	0.99
Euthynnus affinis (Little tuna)	986	28.5	71.2	46.09±11.08	320	4214	1369.7±150.3	0.0221	2.8364	0.016-0.031	2.74-2.93	0.965
Katsuwonus pelamis (Skipjack tuna)	154	33	62	45.16±8.03	310	2630	1096±477.3	0.0021	3.12	0.002-0.0022	2.79-3.31	0.79
Thunnus tonggol (Long tail tuna)	167	35.56	65.32	50.76±7.6	1102	3086	1925.54±342	0.0151	2.9345	0.0054-0.43	2.63-3.12	0.96
Scomberomorus guttatus (Indo-pacific king mackerel)	198	36	110	53.57±13.54	334	6700	1068.45±465.23	0.0089	2.896	0.0062-0.013	2.80-2.98	0.98
S. commerson (Narrow-barred Spanish mackerel)	149	42.4	124	61.43±27.74	450	10000	2233.74±876.87	0.0072	2.93	0.0061-0.0084	2.76-3.04	0.99
Rastrelliger kanagurta (Indian Macker)	156	19	22	20.71±1.2	64	137	99.6±27	0.00428	3.18	0.0031-0.0047	2.89-3.21	0.98
Rastrelliger faughni (Indian Mackere)	134	20	28	24.21±2.1	114	242	178.8±4.1	0.0797	2.86	0.0022-0.123	2.67-2.93	0.79
Scomberoides tol Needlescaled queenfish	145	36	42	36.5±4.3	128	460	291.3±56.21	0.0056	3.21	0.0089-0.006	3.11-3.25	0.99
S. commersonnianus (T. queenfish)	772	29.5	120	50.09±19.32	170	6500	974±212	0.01	2.9	0.007-0.012	2.8-2.97	0.97
Scomberoides lysan D. spotted queenfish	134	40	51	43.96±4.19	375	750	480.8±91.7	0.006	2.97	0.0003-0.131	2.86-3.16	0.97

n, sample size; SD, standard deviation; Min, minimum; Max, Maximum; a, intercept; b, slope; CL, confidence limits; r2, coefficient of determination; parentheses indicate the range of a mean value.

Figure 1. Picture of 12 commercially important identified marine fish of Bay of Bengal, Bangladesh during July 2021 to June 2022.

Scomberomorus guttatus Indo-Pacific king mackerel

Rastrelliger faughni Indian mackerel

Scomberoides commeronnianus Talangqueenfish

Euthynnus affinis Little tuna

Auxis thazard Frigate tuna

Thunnus tonggol (Long tail tuna)

Auxis rochei Bullet tuna

Scomberoides tol Needlescaled queenfish

Scomberoides lysan Double spotted queenfish

Scomberomorus commerson Narrow-barred Spanish mackerel

R. kanagurta Indian mackerel

Auxis rochei Bullet tuna

Experiment 2. Estimating the biological reference points (BRP) of Tuna and Mackerels

The LBB models were constructed for three fish stocks from the Bay of Bengal. LF datasets of each stock from 2021 to 2022 were combined to increase sample size and representativeness. All LF data exhibited good patterns to reflect resource status and met the requirements of LBB. Figure 3,4,5 shows the accumulated LF data used to estimate priors. The black curve shows the fit of the LBB master equation for each stock, providing estimates for fishery reference points along with their 95% confidence intervals.
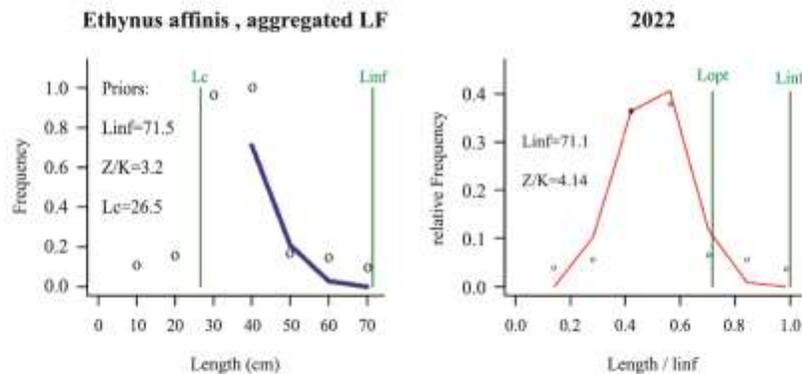

Figure 2. Graphical representation of LBB method for Ethynus affinis.

Here, Lc (length at first capture) was 26.5cm. Linf is the limit body length of this species was 71.5 cm, and Lopt denotes the length at which the maximum sustainable catch is obtained.
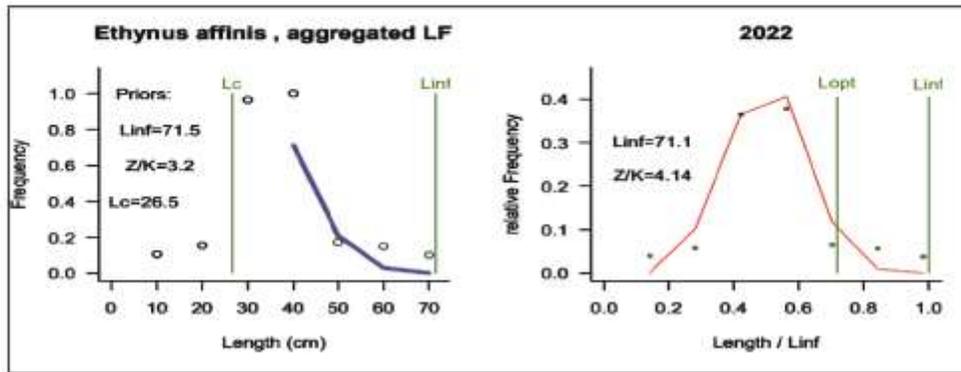
Figure 3. Graphical findings of LBB method for *Scomberoides commersonnianus*.
Here, Lc is the length at first capture was 55 cm, Linf is the limit body length of this species was 156 cm.
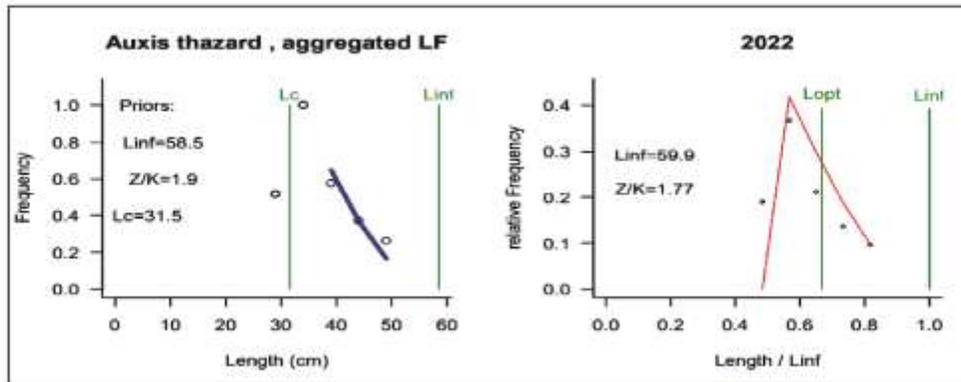
Figure 3. Graphical findings of LBB method for *Auxis thazard*.
Here, Lc is the length at first capture was 31.5 cm, Linf is the limit body length of this species was 58.5 cm.

Table 3. Life-history characteristics of rest 09 Marine Fish using FiSAT II.

Species	n	Linf	K	M
Auxis rochei (Bullet tuna)	134	46.3	0.40	0.79
Katsuwonus pelamis (Skipjack tuna)	154	62.6	1.06	1.45
Thunnus tonggol (Long tail tuna)	167	66	2.67	2.87
Scomberomorus guttatus (Indo-pacific king mackerel)	198	109	0.31	0.53
S. commerson (Narrow -barred Spanish mackerel)	149	119.4	0.8	0.87
Rastrelliger kanagurta (Indian Macker)	156	24.5	1.6	2.5
Rastrelliger faughni (Indian Mackere)	134	23.5	1.9	2.6
Scomberoides tol (Needlescaled queenfish)	145	42.5	1.53	1.81
Scomberoides lysan (D. spotted queenfish)	134	56	0.67	1.13

Experiment 3. Standardizing the Spawning Potential Ratio (SPR) of Tuna and Mackerels.

The Length Based-Spawning potential ratio (LB-SPR) analysis of three fish stocks from the Bay of Bengal were ranged from 1-20% (average 10%) for the total population. Furthermore, it also has shown a low proportion of mature stock to be a new stock (recruitment) in population. The decrease in the SPR level occurs while the size of spawning fish is decreasing in number, both due to the effect of a decrease of selectivity and adult stock. The threshold value of SPR is 40% than can be accepted as a proxy for the Maximum Sustainable Yield (MSY) for recruitment overfishing in a less resilient fish population.
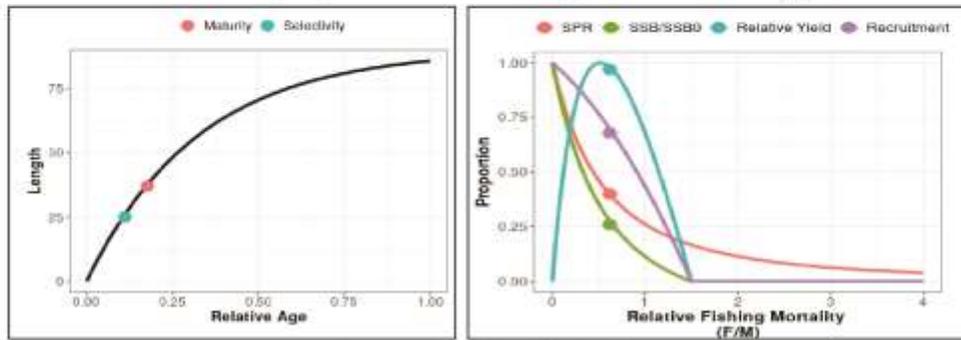
Figure 5. Estimation SPR for *E. affinis.*

a) Growth curve with relative age, and b) SPR and relative yield curves as a function of relative fishing mortality. In case of Figure a, selectivity point is in front of mortality point i.e fish are captured before mature stage.
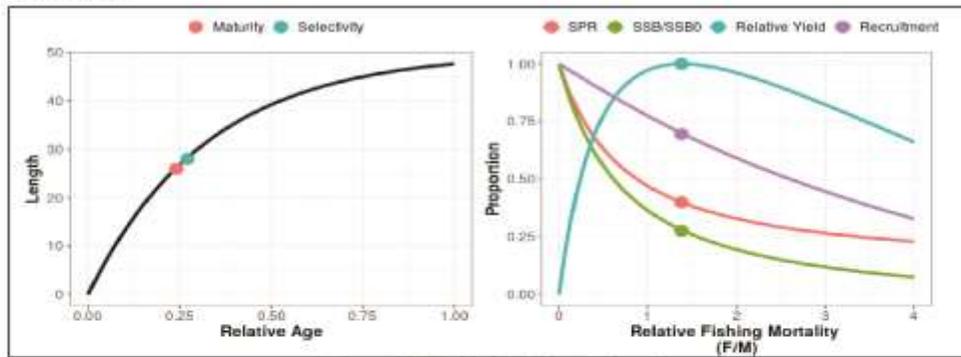
Figure 6. Estimation SPR for *A. thazard*

a) Growth curve with relative age b) SPR and relative yield curves as a function of relative fishing mortality. In case of figure a, mortality point is in front of selectivity point i.e fish are captured after mature stage.
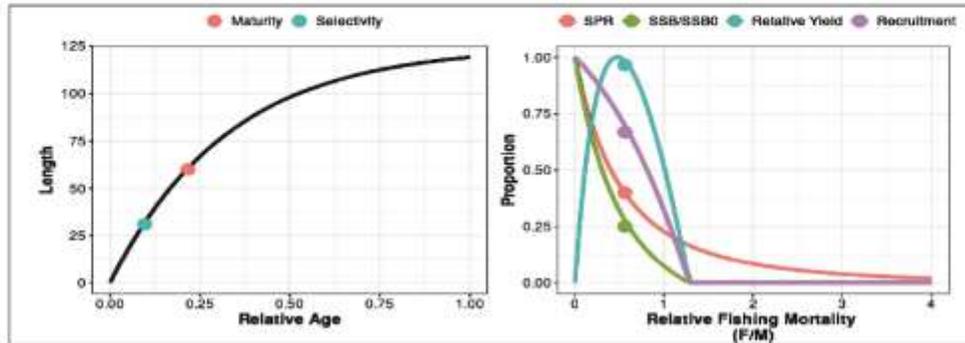
Figure 7. Estimation SPR for *A. thazard*

a) Growth curve with relative age b) SPR and relative yield curves as a function of relative fishing mortality. In case of figure a, mortality point is in front of selectivity point i.e fish are captured after mature stage.
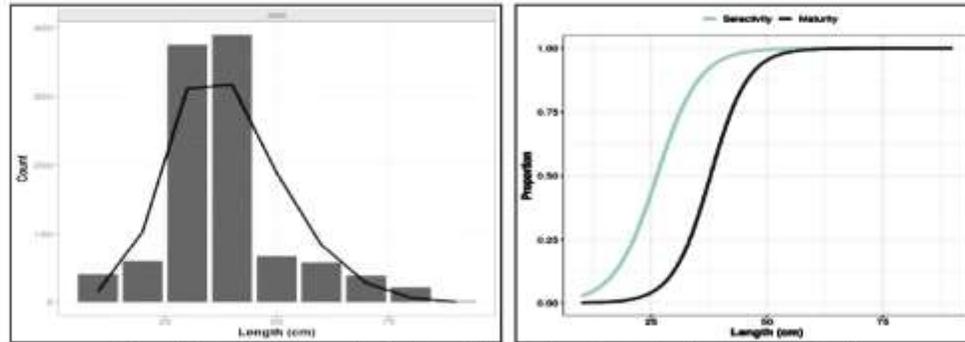
Figure 8. Length frequency distribution and Selectivity and maturity size of *Ethynus affinis*.
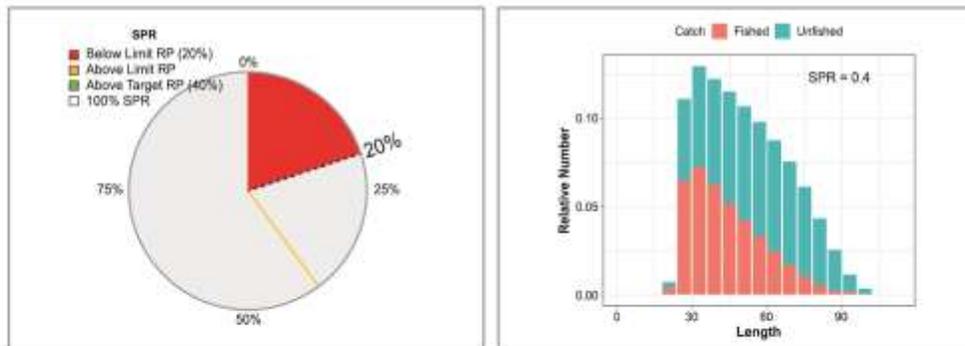
Figure 9. a) Estimation of SPR and total stock of *Ethynus affinis* b) expected (equilibrium) size structure of the catch and the expected unfished size structure of the vulnerable population.
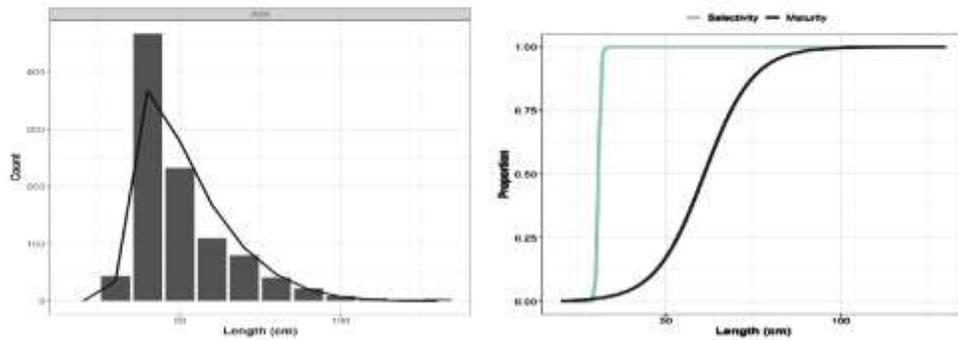
Figure 10. Length frequency distribution and Selectivity and maturity size of Scomberoides commersonnianus.
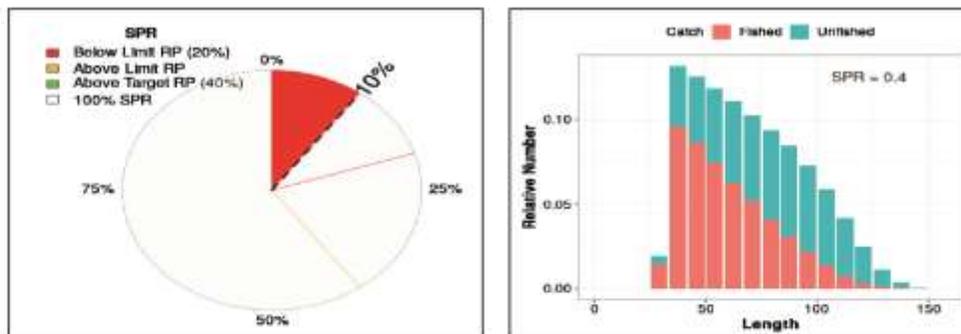
Figure 11. a) Estimation of SPR and total stock of *S. commersonnianus* b) expected (equilibrium) size structure of the catch and the expected unfished size structure of the vulnerable population.
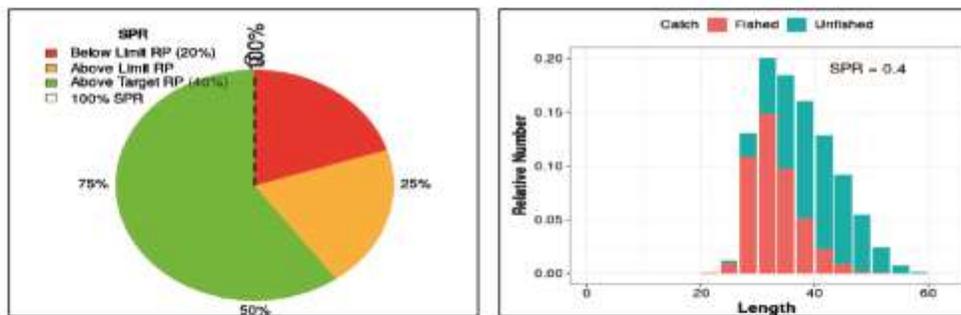
Figure 12. a) Estimation of SPR and total stock of *A. thazard* b) expected (equilibrium) size structure of the catch and the expected unfished size structure of the vulnerable population.
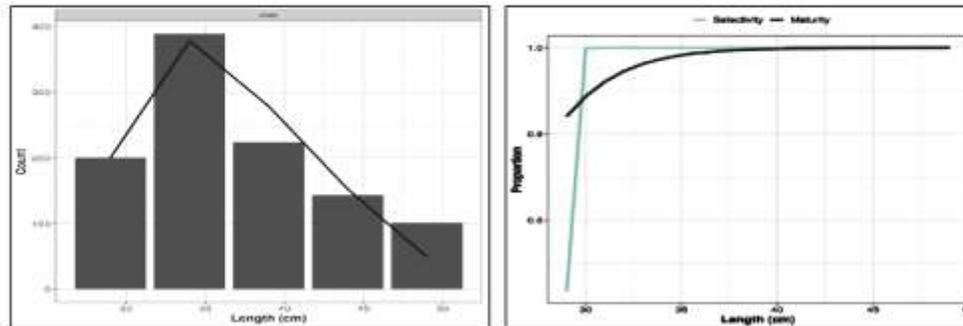
Figure 13. Length frequency distribution and Selectivity and maturity size of *Auxis thazard*.

Domestication and Breeding of Blue swimming crab (*Portunus pelagicus*) and Horseshoe Crab (*Tachypleus* sp.) of the Bay of Bengal, Bangladesh

Researchers

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Objectives

- To scrutinize the abundance of Horseshoe Crab (*Tachypleus* sp.) and Blue Swimming crab (*Portunus pelagicus*) in the coast of Bangladesh

- To study the food and feeding habit and reproductive biology *viz*; fecundity, gonadosomatic index (GSI), egg diameter, sex ratio of the Horseshoe Crab and Blue Swimming crab

- To domesticate the Blue Swimming crab (*P. pelagicus*) and Horseshoe Crab (*Tachypleus* sp.) under captive/ hatchery conditions

- To develop breeding technology of Blue Swimming crab (*P. pelagicus*) in captive/ hatchery conditions

- To develop larval and nursery management technique of Blue Swimming crab

Experiment 1. Scrutinizing the abundance of Horseshoe Crab (Tachypleus sp.) and Blue Swimming crab (Portunus pelagicus) in the coast of Bangladesh

Till date, two species of Horseshoe Crab (*Carcinoscorpius rotundicauda, Tachypleus gigas*) were collected from Chowfaldandi, Sonadia of Cox's Bazar. It has been reported that these two species inhabit in indo-pacific region (Tanu and Noguchi, 1999, Chowdhury and Hafzuddin, 1980). Besides these, six species of swimming crab (*Portunus pelagicus, Portunus sanguinolentus, Charybdis feriata, Charybdis natator, Charybdis lucifera* and *Necora puber*) are found in Cox's Bazar and Saint Martin's Island.

Experiment 2. Studying the food and feeding habit and reproductive biology viz; fecundity, gonadosomatic index (GSI), egg diameter, sex ratio of the Horseshoe Crab and Blue Swimming crab

We examined the batch fecundity of female Blue Swimming crab (*Portunus pelagicus*) that were collected monthly at landing sites from July 2021 to June 2022, calculated the relationships with body size, egg mass and month of the year and determined the size at which females became potentially reproductive in the population inhabiting cox's Bazar (Bay of Bengal). Fecundity values ranged from 309,468 to 2,836,355 nos (mean = 1426,638±56,975 [±SE]). To determine the sex ratio of Blue swimming crab, sampling was done in monthly basis and resulted in higher female ratio in the sample with 71%. To study the GSI of Blue swimming crab, that was purchased from BFDC fish landing center, Cox's bazar and then calculated. The average GSI was 7.27. Egg diameter of Blue swimming crab was also been calculated which revealed the average egg diameter 0.194 mm.

Generally, Horseshoe crab breeds in April-May in nature. Collected samples were not mature enough to study the fecundity, gonadosomatic index (GSI) and egg diameter. However, mature Horseshoe crab could not be collected for further study.
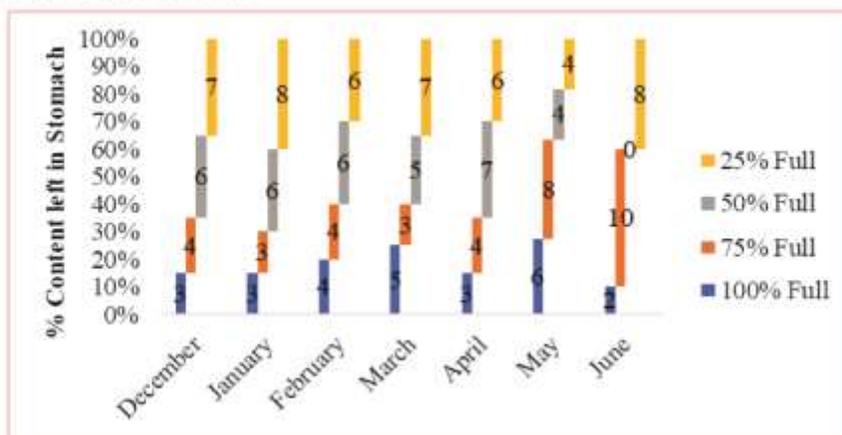
Table 1. Monthly gut content remained in Crabs.

Month	Major Food group	2nd most remaining
December	Molluscan remains	Fish remainings
January	Molluscan remains	Fish remainings
February	Molluscan remains	Fish remainings
March	Fish remaining	Molluscan remaings
April	Fish remaining	Miscellaneous
May	Miscellaneous	Fish remaining
June	Miscellaneous	Molluscan remaining
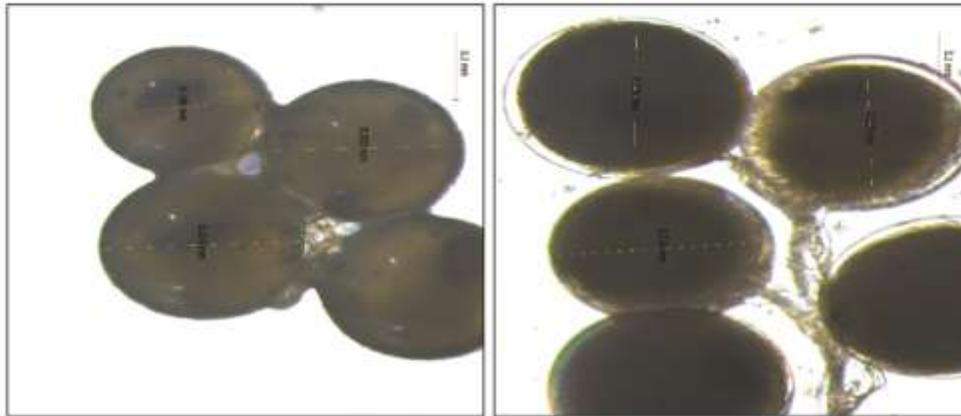
Figure 3. Measuring egg diameter.

Experiment 3. Domestication of Blue Swimming crab (P. pelagicus) and Horseshoe Crab (Tachypleus sp) under captive/hatchery conditions

Domestication of Horseshoe crab is being under processed, about 3 types of habitats had been modified as treatment where first treatment was sandy bottom habitat, second one was muddy bottom and the final treatment was combination of sandy and muddy bottom habitat. Horseshoe crabs were being regularly fed with plankton and rotifer (20 indi./ml) twice a day. About 40 Horseshoe crabs were collected and domesticated in MFTS Hatchery.

Total 60 Blue swimming crabs were collected and Habituated in MFTS Hatchery. Water temperature was maintained at 25-30 °C, water salinity was 30 ppt with continuous aeration and daily feeding ratio was at 5% of body weight with fresh marine squid, bivalve (Oyster) or fish meat (Tilapia, leaf fish) alternately. Total 16 berried female crabs were identified when water level was reduced. Berried females were then transferred individually to 500-liter tank with aerated sea water at 30 ppt salinity. Size of berried crab was average 7.8 cm carapace length and 165 g total weight.

$$y = 0.0059x^{3.9151}$$
$$R^2 = 0.8922$$
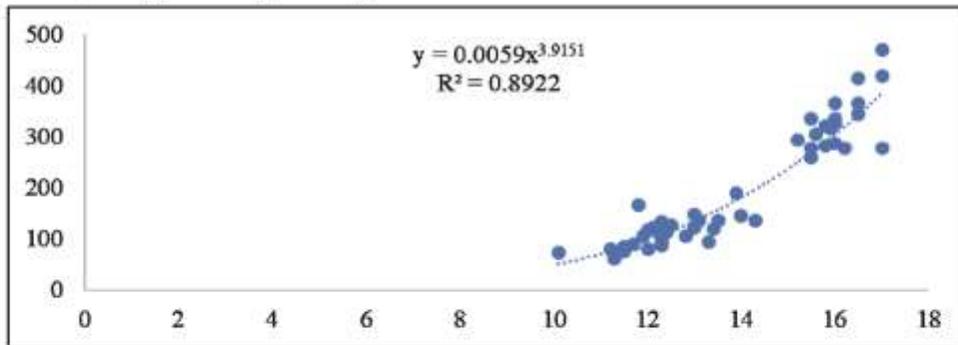
Figure 4. Length weight relationship of hatchery reared Blue swimming crab.

Experiment 4. Breeding technology development of Blue Swimming crab (P. pelagicus) in captivity

Total 50 gravid brood crabs were sorted. The crabs were examined for ovarian maturity by looking through the transparent membrane between the junction of the first abdominal segment and carapace. Mature ovaries were dark orange. De-chlorinated sea water was used for this experiment that was brought from Modina hatchery. During the experiment, collected crabs were subjected to a bath treatment of 100μl/l of a 40% formalin solution for disinfection. After the formalin bath, every 500L fiber glass tank with sand was used for rearing brood; substrate and PVC pipes (20 cm diameter x 30 cm length) were also kept as shelters. In this system each tank contains two crabs. Water temperature was maintained to 25-27ºC, water salinity was 30ppt, and daily feeding ratio was at 5% of body weight of fresh marine squid, bivalve or fish meat (Tilapia, leaf fish) alternately. Water quality parameters were monitored daily following standard methods and a daily management schedule were maintain for siphoning out waste material from the 500 L fiber glass tank. Eyestalk ablation was applied for selected 35 broods to spawning by a blunt scissor. After eyestalk ablation, gravid crabs were again treated in 100μl/l formalin bath and then transfer to a 500 L fiber tank. About 16 crabs were used for it. In this time individual tank were used for two crabs. Another technique was applied parallelly i.e., each 3000 rectangular tanks filled with seawater contained 19 gravid crabs for early brood development. Water exchange was done weekly basis. Water temperature was maintained to 25-30ºC, water salinity was 30 ppt with continuous aeration. Eggs released by the female become attached to the pleopod hairs of the abdominal flap. Sampling for egg carrying or berried females was done. Total 16 berried crabs are found when water levels were reduced during the water change. Berried females were then transferred individually to 100-liter tank with aerated sea water at 32 ppt. Hatching occurs 7-14 days after berried maintain temperatures of 26.5-31°C. Each brood crab of Portunus pelagicus was given 0.7-1.7 million zoeae.

Experiment 5. Development of larval and nursery management technique of Blue Swimming crab.

Larvae management

Zoeae were stocked at a density of 60 individuals per liter in 6 circular concrete tanks respectively contain 500 L seawater and fed with the rotifer *Brachionus rotundiformis* at a density of 10-15 rotifers/ml. The microalgae Nannochloropsis sp. was maintained in the rearing tanks at 50,000 cells/ml as food for the B. rotundiformis. Brine shrimp Artemia nauplii were also given at 0.5-3/ml to zoea 3 stage and larger larvae. In this experiment feed was variables. The zoeae were reared at a salinity of 28-30 ppt and water temperature of 26-30.5ºC and a natural photoperiod of 11-13 hours light and 11-13 hours dark. The rearing water was replaced at a daily rate of 30% starting on day 3 and increasing up to 80% as larvae grow bigger or when disease-causing luminescent bacteria are detected in the water and larvae.

Nursery management

Megalopa were nursed in circular concrete tanks. The stocking density of 3-5 days old megalopa in nursery tanks were reduced to 1500-2000 inds./3000litre of water. Black nets were placed at the bottom as substrates and some were suspended in the water column.

New experiment was set up with three treatments (T_1= Artemia umbrella from Z1-Z4 single time a day, T_2= Rotifer from Z1- Z2 and T_3= Artemia only from Z1-Z4). Feeding was done six times a day where artemia/ rotifer was being used with a rotation of mix feed (6 am= Rotifer, 9 am= Mix feed, 12 pm= Rotifer, 3 pm= Mix feed, 6 pm= Rotifer, 12am= Rotifer). At 9 pm enzyme was being used for better digestion. This study showed better survival though out the experiment however at 14th day of the experiment temperature dropped for sudden and prolonged rain in Cox's Bazar and metamorphosis was being interrupted. Metabolism was decreased that resulted in less feed consumption and finally fungal attack happened. This study showed

better survival rate in T_2 as low consumption of artemia umbrella resulted in artemia biomass in the tank and water quality was deteriorated for higher biomass in the tank. Food consists of newly hatched and adult Artemia. As soon as the megalopa molt to crablet stage, they were fed with minced trash fish, mussel, oyster or small shrimp Acetes twice daily ad libitum. About 30-50% of the volume of the rearing water (26-30 ppt) was replaced daily during the first 5 days and every two days thereafter.

The survival rate from Zoea 1 to 4 days old to Megalopa was 6.8% in treatment 2. The survival from Megalopa to crablet (1-3 g bodyweight) after 20 days in hatchery tanks was <5% in Treatment 2.

Table 2. Rational survival rate of Blue Swimming crab (zoea to crablets).

	Larval Stage			No of individuals			Survival Rate		
Day	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
0	Z1	Z1	Z1	30000	30000	30000	100	100	100
4	Z1	Z1	Z1	27000	22000	22000	90	73.33	85
5	Z2	Z2	Z2	24000	18000	20000	80	60	72
7	Z3	Z3	Z3	15000	16000	18000	50	53.33	54
10	Z4	Z4	Z4	10000	15000	10000	33.33	50	33.33
12	M	M	M	5000	13000	5000	16.67	43.33	16.67
13	M	M	M	3000	11000	4000	10	36.67	12
14	M	M	M	700	1500	800	2.33	6.82	2.80
16	M	M	M	600	1400	560	2.0	4.66	1.9
18	C	C	C	550	1300	420	1.83	4.33	1.4
20	C	C	C	210	1236	380	0.70	4.12	1.27
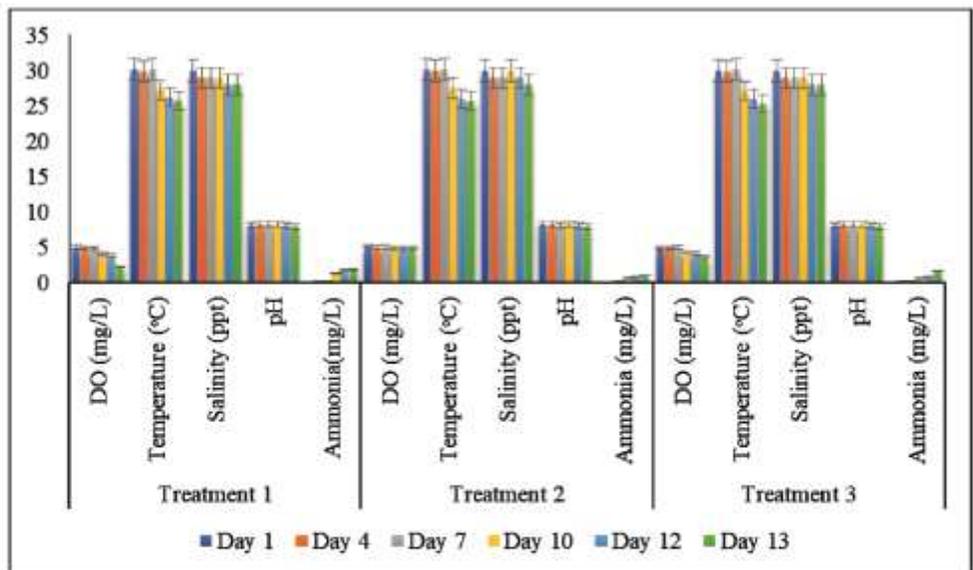
Figure 6. Water quality analysis during rearing of zoeae and Megalopa in Circular tank.
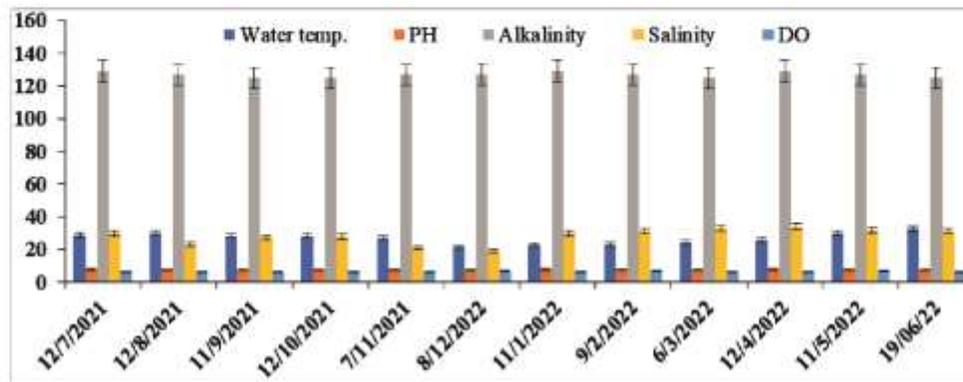
Figure 7. Water quality analysis of Brood Crabs during Domestication in cistern tank.

Development of Mariculture Practice of Some Important Fin Fish (Seabass, Mullet) in The South-East Coast of Bangladesh

Researchers

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Objectives

- To develop a proper nursery and grow-out management protocols for cage culture of suitable species (Mullets, Seabass)

- To optimize stocking density and evaluate production performance in cage culture

- To develop suitable species (Mullets, Seabass) cage culture co-management practice through pilot Project

Achievement

Study-1. Development of a proper nursery and grow-out management protocols for cage culture of suitable species (Mullets, Seabass)

Experiment on Seabass/Mullet pond culture

Nursery practices of Seabass and Mullet in on-station ponds

Methodology

Four (04) on-station ponds were prepared for stocking of nature-borne Seabass and Mullet fries. Mullet fry was available from early March. The ponds were dried and then fertilization and liming was done.

After 07 days of fertilization and liming, we watered those ponds. As the ponds were ready, the desired Mullet fry were stock whenever they are available in the wild. Fry of Seabass were stocked in 02 ponds as they were available on August. The culture practice of Seabass in the ponds is given in the following Table.

Seabass were stocked on August 15, 2021

Stocking and Sampling In pond	Pond 1 (Seabass)	Pond 2 (Seabass)	Pond 3 (Mullet)	Pond 4 (Mullet)
Stocking density	50/Dec	70/Dec	30/Dec	45/Dec
Initial avg. wt. of fish (g)	35±11.37	35±11.86	25±5.50	25±5.25
Feeding	Live Tilapia fry and Formulated feed	Live Tilapia fry and Formulated feed	Died (Within 2 days)	Died (Within 2 days)
Avg. wt. of the last sampling	769±112.25	788±111.35	-	-
Net Av. weight gain (g)	734	753	-	-
Total culture period (Ongoing)	240 days	240 days	-	-

Table 1. Seabass and mullet Pond culture.

Result

Average Daily Growth Rate and Specific Growth Rate of Seabass during pond culture.

Figure 1. ADGR and % SGR of Seabass in Ponds.

Average daily growth rate showed lower growth than the usual. It may happen due to the lower interest in food thriving as they were in a confined condition. Other reason may be, some individual shows dominating food thriving those results in lower average growth. And additionally in case of pond, salinity falls often, result in lower appetite and lower growth rate (Figure 1).

Study-2. Optimization of stocking density and evaluation of production performance in cage culture.

Cage preparation

A total of 12 circular cages have been prepared with double layered, knotless net, 12 plastic drums per cage for better bouncy. Mooring (9ft long, 3.5-inch diameter) were done to resist the stronger tidal and wave action. Cover net was established over the cages where Mullet were stocked. For a larger access to the cages and the cage culture research, a mechanized boat was arranged by the MFTS, Cox's Bazar.

The details of the fixed net cages were as follows-

- Shape. Round
- Inner Diameter. 6 meters
- Outer Diameter. 7 meters
- Depth. 2 meters
- Frame. 110 mm HDPE Pipe were used
- Body Net. Nylon net 40 mm Mesh size were used
- Cover Net. Nylon net 20mm Mesh Size were used
- Float. Concealed Plastic drum-Length 990 mm were used
- Rope. Green fabricated Nylon 1.5inch diameter thick rope used for mooring
- Mooring. 4 nos. of 50 kg weighted mooring were used for fixing the cage

Methodology

After the preparation of the net cages, we stocked Seabass and Mullet in different densities in the cages. We stocked Seabass in 03 cages and Mullet in Two cages. Stocking densities, initial weight, final weight, feed type and culture period of both Seabass and Mullet are given in the following Tables.

Stocking date. 16 November 2021, **Cage size . 32 m^3**

Table 2. Seabass cage culture.

Parameters	48 Seabass / Cage (C1)	72 Seabass / Cage (C2)	96 Seabass / Cage (C3)
Initial weight (g)	100.12±10.05	100.43±15.09	100.41±12.01
Final weight (g)	697.82±9.47	674.97 ±10.55	656.43±10.88
Net weight gain (g)	597.7±8.32	574.54±10.01	556.02±9.93
Average daily growth rate	3.30±0.11	3.17±0.14	3.07±0.21
% Specific growth rate	1.07±0.09	1.05±0.10	1.04±0.07
FCR	2.03±0.08	2.10±0.06	2.48±0.10

Result

Average Daily Growth Rate and Specific Growth Rate of Seabass during net cage culture
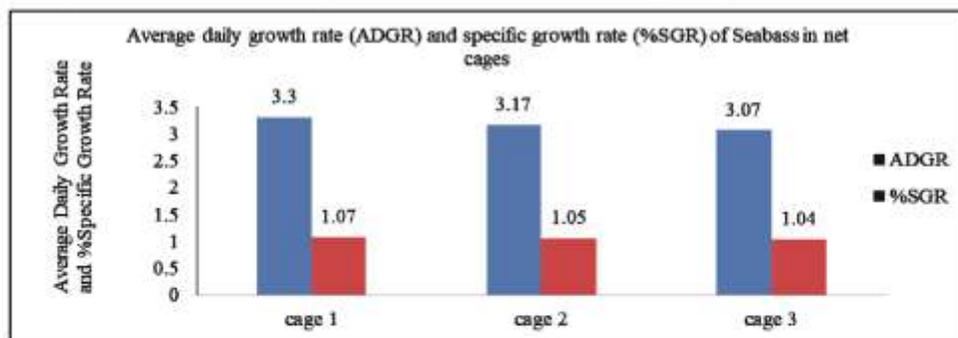
Figure 2. ADGR and %SGR of Seabass in Net Cages.

Average daily growth rate shows lower growth than the usual. It may happen due to the lower interest in food thriving as they are in a confined condition. Other reason is sometime some individual shows dominating food thriving those results in lower average growth. (Fig. 02)
Stocking date. 16 November 2021 **Cage size.** 20 m^3

Table 3. Mullets cage culture.

Parameter	15 Mullet / Cage (C6)	20 Mullet / Cage (C7)
Initial weight (g)	350.14±5.70	350.63±7.52
Final weight (g)	822.75±10.26	813.32±11.39
Net weight gain(g)	472.61	462.69
Feeding	Natural Feed	Natural Feed
Culture period (Days)	181	181

Result

Average Daily Growth Rate and Specific Growth Rate of Mullet during net cage culture
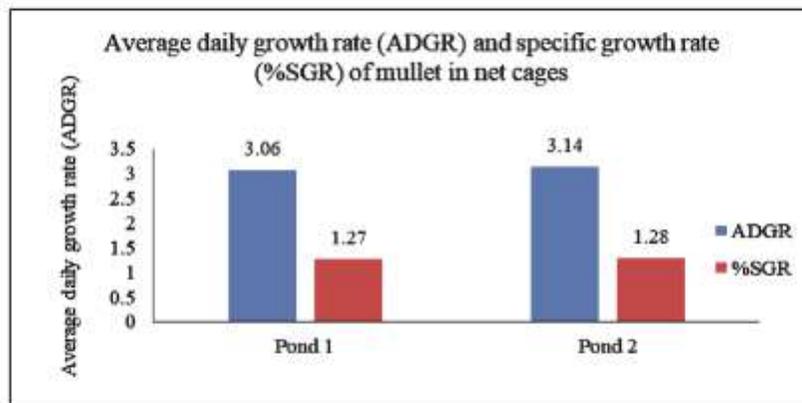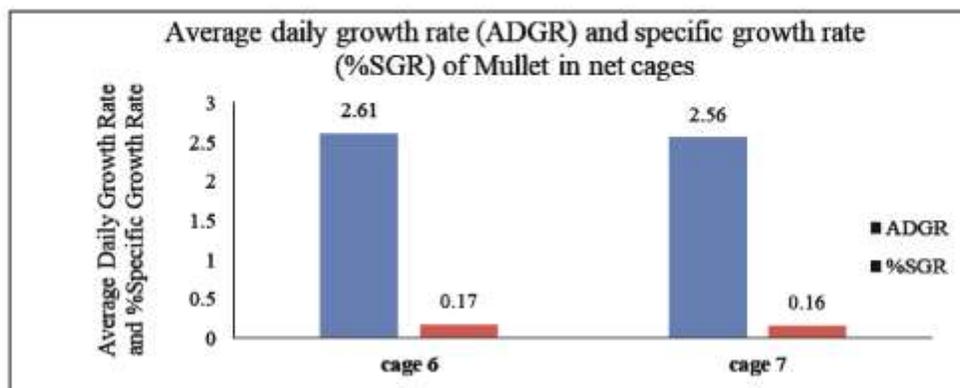
Figure 03. ADGR and %SGR of Mullet in Net Cages

Average daily growth rate showed lower growth than the usual. It may happen due to the lower interest in food thriving as they were in a confined condition. Other reason is some individual shows dominating food thriving those results in lower average growth (Figure 3).

Table 4. Water quality data of cage culture site (Moheshkhali Channel).

	Days	Temperature (°C ±SE)	Salinity (‰ ± SE)	DO (mg/l ± SE)	pH (± SE)	NO_2 (mg/l ± SE)	NH_3 (mg/l ± SE)
Nov	15	22.4 ± 0.1	30.2± 0.2	5.5± 0.32	7.2± 0.1	0.015± 0.002	0.02± 0.002
Dec	30	22.8 ± 0.2	30.8± 0.3	5.8± 0.25	7.2± 0.1	0.020± 0.001	0.02± 0.002
	45	21.4 ± 0.2	31.3± 0.3	6.0± 0.50	7.4± 0.2	0.021± 0.002	0.03± 0.002
Jan	60	21.1 ± 0.1	31.9± 0.3	5.6± 0.45	7.3± 0.2	0.018± 0.001	0.02± 0.003
	75	21.5 ± 0.3	32.0± 0.4	5.9± 0.14	7.1± 0.1	0.025± 0.003	0.01± 0.004
Feb	90	21.9 ± 0.1	31.1± 0.2	6.0± 0.14	7.2± 0.1	0.019± 0.004	0.02± 0.002
	105	23.7 ± 0.0	30.7± 0.3	5.4± 0.23	7.3± 0.1	0.023± 0.002	0.02± 0.004
Mar	120	24.4 ± 0.1	29.3± 0.1	5.7± 0.27	7.2± 0.2	0.022± 0.003	0.03± 0.001
	135	26.3 ± 0.2	28.4± 0.3	5.8± 0.26	7.2± 0.1	0.024± 0.001	0.02± 0.002
Apr	150	26.7 ± 0.3	27.6± 0.2	5.5± 0.23	7.1± 0.3	0.021± 0.001	0.02± 0.002
	180	25.1 ± 0.2	27.4± 0.2	5.6± 0.18	7.1± 0.3	0.019± 0.002	0.03± 0.003

Study 03. Development suitable species (Mullets, Seabass) cage culture co-management practice through pilot project

Research work is in progress. Optimization and production performance study is going on in different stocking densities with regular feeding and monitoring. After coming to a conclusive finding, we will working on co-management in the final year.

Identification and Culture Practice of Commercially Important Seaweeds in Bangladesh Coast

Researchers

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Md. Golam Mostofa, SO

Objectives

- To make a detailed inventory of available seaweed species in Bangladesh coast
- To develop culture technique (indoor to field) of selected seaweed in St. Martin and other suitable areas
- To develop in-vitro tissue culture technique for some selected seaweed species
- To develop value-added products from selected seaweeds

Achievements

Study-1. Inventory of available seaweed

Surveys were conducted in and around Cox's Bazar (St. Martin Island, Teknaf, Inani, Bakkhali, and Sonadia), Bagerhat (Shala river, Pashur river, and Vhula river), Patuakhali (Fatrarchor), and Noakhali (Nijhum Dip) from September 2021 to March 2022. Special emphasis was given to the identification of mangrove seaweed species as they have a special character than other seaweed species and can be found throughout the year. Different species of seaweed i.e. *Ulva compressa, Bostrychia radicans, Caloglossa beccarii, Catenella impudica, Catenella nipae, Chaetomorpha aerea, Ulva reticulate, Colpomenia peregrine, Bangia fuscopurpurea, Grateloupia lanceolate, Ulva intestinalis, Hypnea musciformis, Dictyota dichotoma, Asparagopsis taxiformis, Padina tetrastromatica, Eucheuma cottonii, Codium bursa* were collected randomly by hand-picking from the study area at the time of low-tide. Fresh samples were taken into plastic jars and then kept in the icebox for laboratory work. In the laboratory, samples were gently brushed under running seawater and rinsed with distilled water. Herbarium pressing frames were used to dry the seaweed sample instead of direct sun drying. Wet papers were exchanged one day at a time for a total of three days and finally the dry seaweed samples were preserved in the laboratory. A total of ten (10) seaweed samples were identified as a new species this year. The total number of seaweed species was one hundred and fifty-four (154).

Acetabularia calyculus   *Gracilaria salicornia*   *Codium bursa*

Figure 1. Newly identified seaweed species collected from Saint Martin's Island.

Table 1. Availability and distribution of seaweed.

Area	Type	Species
St. Martin	Phaeophyta, Chlorophyta and Rhodophyta	*Padina tetrastromatica, Padina fraseri, Halimeda minima, Halimeda discoidea, Enteromorpha torta, Ulva compressa* and *Eucheuma cottonii*
Bakkhali	Phaeophyta, Chlorophyta and Rhodophyta	*Enteromorpha intestinalis, Hypnea* sp., *Chaetomorpha aerea, Ulva compressa* and *Ulva reticulate*
Inani	Phaeophyta, Chlorophyta	*Enteromorpha intestinalis, Hypnea* sp., *Ulva compressa* and *Ulva reticulate*
Sonadia	Chlorophyta and Rhodophyta	*Catenella nipae, Catenella impudica, Bostrychia radicans, Caloglossa beccarii, Colpomenia sinuosa* and *Chaetomorpha aerea*
Bagerhat	Chlorophyta and Rhodophyta	*Bostrychia radicans, Caloglossa beccarii, Catenella impudica, Catenella nipae, Colpomenia sinuosa* and *Chaetomorpha aerea*
Patuakhali	Chlorophyta and Rhodophyta	*Bostrychia radicans, Caloglossa beccarii, Catenella impudica, Catenella nipae, Colpomenia sinuosa* and *Chaetomorpha aerea*
Noakhali	Chlorophyta and Rhodophyta	*Caloglossa beccarii, Catenella impudica, Catenella nipae* and *Chaetomorpha aerea*

Catenella nipae   *Catenella caespitosa*   *Caloglossa beccarii*   *Caloglossa stipitate*

Bostrychia kelanensis   *Caloglossa bengalensis*   *Chaetomorpha aerea*

Figure 2. Newly identified mangrove seaweed species.

The Sundarban mangrove forest is one of the renowned mangrove forests and a UNESCO heritage site in the Bay of Bengal. During our inventory, we observed that several seaweed species possess decent association with mangrove species. Mangrove provides a substrate for the attachment of seaweed as they were found to be attached to the roots and barks of the mangrove trees. These epiphytic mangrove seaweeds are also available throughout the year and can survive in adverse conditions (zero salinity).

Figure 3. Seaweed attachment in different mangrove areas.

Study-2. Seaweed culture

Experimental culture sites of seaweeds were set up in sheltered intertidal zones of Bakkhali river estuary at Nuniarchora (N21°28.482, E091°57.867), Kutubdia (N21°47'5.0208", E91°50'21.2892"), Chowfoldondi (N21°30'13.1076", E91°59'38.8392"), and Saint Martin (N20°36.971, E092°19.459). A culture experiment was set up in January in four sites. Coir rope was used as net material for substrate with a horizontal net size of square (4m×4m) coir rope. Four corners of the nets were tied with rocks or bamboo with plastic floats placed 25 cm above the bottom. Micronutrient-enriched seaweed species *S. ilicifolium* were selected for culture experiments in study sites. Seeding was done by inserting the young fragments of seaweed with an average of 4±0.5 kg fw (fresh weight) and 5 cm length in the twists of the coir ropes with the short string length at a density of seaweed seed were 10-15 seed/m^2.

At the Chowfoldondi site, a floating raft method was made of bamboo poles and recycled plastic drums. A 1.50 cm mesh size plastic net was placed in the lower part of the frame to minimize the wave action and crop loss caused by plant rupture from the base, especially during adverse weather. All rafts were rope-tied, placed in the culture site, and anchored to help stabilize the structure. The structure's anchor was placed to raise and fall vertically during the tidal action.

Between January 2022 to March 2022, a total of 13 partial harvests of *S. ilicifolium* were made in four sites (Saint Martin, Nuniarchora, Kutubdia, and Chowfoldondi). Due to natural calamity in the Kutubdia site, only one partial harvest was made as the culture structure was damaged by high wave action. Additionally, because of a government development project (airport extension), the water quality has deteriorated, resulting in no partial harvest at the Nuniarchora site. Partial harvesting (kg) of *S. ilicifolium* in four sites is given in Table 2.

Table 2. Partial harvesting (kg/m2) of *S. ilicifolium* during the culture period in four sites.

Culture sites	Partial harvesting *S. ilicifolium* (Mean±SD) kg					
	15th day	30th day	45th day	60th day	75th day	90th day
St. Martin	16.32± 0.68	17.42± 0.59	19.37± 0.27	17.81± 0.45	15.75± 0.23	14.40± 0.55
Chowfoldondi	13.56± 0.46	15.23± 0.55	17.44± 0.79	16.34± 0.37	13.24± 0.88	11.38± 0.31
Kutubdia	09.45± 0.85	-	-	-	-	-
Nuniarchora	-	-	-	-	-	-

The maximum daily growth rate of *S. ilicifolium* was 5.04±0.09% day-1 on the 15th day in St. Martin, and the minimum daily growth rate was observed at 1.03±0.10% day-1 on the 90th day in Chowfoldondi. The daily growth rate (%/day) of *S. ilicifolium* in four sites is given in Table 3.

Table 3. Daily growth rate %/day of *S. ilicifolium* on culture period in four sites.

Culture sites	*S. ilicifolium* DGR %/day (Mean±SD)					
	15th day	30th day	45th day	60th day	75th day	90th day
St. Martin	5.04±0.09	4.87±0.14	4.78±0.13	3.73±0.14	2.68±0.16	1.14±0.19
Chowfoldondi	4.83±0.11	3.98±0.16	4.14±0.19	2.92±0.07	2.34±0.12	1.03±0.10
Kutubdia	4.25±0.25	-	-	-	-	-
Nuniarchora	-	-	-		-	-

Harvest at the end of the 90-day duration of culture period in four sites results in the absolute maximum biomass yields (16.85±0.46 kg fw/m^2) for *S. ilicifolium* in Saint Martin and the lowest biomass (0 kg fw/m2) in Nuniarchora due to unfavourable condition (Figure 4).
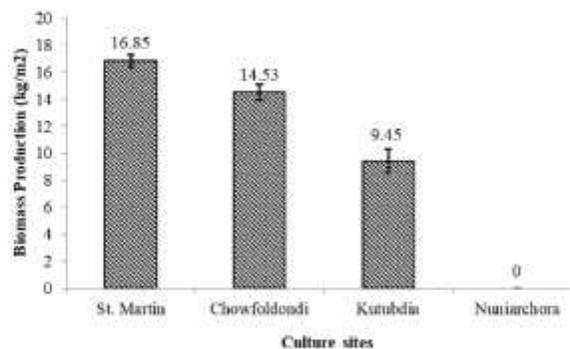
Figure 4. Biomass production (kg/m2) of *S. ilicifolium* on 90 days of culture period in four sites.

The hydrological data are shown in Table 4.

Table 4. Hydrological data of four culture sites.

Experimental Sites	Mean values of hydrological data					
	Temperature (°C)	Salinity (‰)	DO (mg/l)	pH	Alkalinity (ppm)	Transparency (cm)
St. Martin	25.2	33.5	7.6	8.2	120	92.0
Chowfoldondi	24.1	30.8	6.5	7.3	105	68.5
Kutubdia	23.7	30.6	6.1	7.6	118	49.0
Nuniarchora	23.2	31.2	6.4	7.8	113	45.2

Indoor seaweed culture

Seaweed species (*Ulva intestinalis*) was stocked in laboratory condition of MFTS, Cox's Bazar in the long line (4m) and tray (0.45m x 0.30m x 0.08m) method. Raw seawater with continuous aeration and artificial light was provided. Water was partially exchanged every seven days interval. Growth and water quality parameters were measured every twenty days intervals. Weight increase of *U. intestinalis* under different culture methods in laboratory condition are shown in Table 5.

Table 5. Weight (g) increase of *U. intestinalis* on different culture methods.

Culture method	*U. intestinalis* fresh weight (gm) (Mean±SD)				
	Initial (gm)	20th day	40th day	60th day	80th day
Long line (4m)	40.0± 0.25	53.3± 0.58	72.67±3.85	81.62±4.78	95.19±5.09
Tray (0.01m³)	40.0± 0.25	59.5± 0.41	95.93±3.39	118.87±5.61	128.48±4.26

The hydrological data in the laboratory are shown in Table 6.

Table 6. Hydrological data recorded in different culture methods recorded at the laboratory.

Experimental Method	Mean values of hydrological data				
	Temperature (°C)	Salinity (‰)	DO (mg/l)	pH	Alkalinity (ppm)
Indoor	26.4	30.6	7.6	7.8	110

Figure 5. Indoor culture of seaweed.

Study-3. In-vitro tissue culture of seaweed

Seaweed seeds of (*Ulva lactuca, Ulva intestinalis, Gracilaria verrucosa*) have been produced in laboratory conditions through the tissue culture technique at MFTS, BFRI. For seaweed tissue culture, callus induction and thallus/fragment regeneration methods are widely applied. In the callus induction method, semisolid media is used, which consists of agar and a mixture of macronutrients and micronutrients for the given cell type. On the other hand, the thallus regeneration method conducts in liquid media using PES or VS media composition to regenerate thalli or fragments. In our study, laboratory test trials were conducted to assess the effectiveness of the production of seedlings by the fragmented regeneration method. Spores of *U. lactuca, U. intestinalis* and *G. verrucosa* are now available at MFTS, and farmers can collect the spore from the MFTS. Land-based seaweed cultures are now possible throughout the year through this process. Procedures are described below.

Tissue isolation and purification

Fresh samples of *U. lactuca, U. intestinalis* and *G. verrucosa*, free of any other contaminated algae, were gathered from the field and cleaned in clean seawater to eliminate mud and other foreign materials before being transported to the laboratory in a thermocol box under cool conditions. In addition, selected vegetative thallus was cleaned thoroughly in autoclaved seawater using a soft painter brush to remove the epiphytic contaminants, including dirt and biofilms.

Optimize environmental conditions for seaweed tissue culture

The thalli (0.25 mg) were chopped into 2 ± 1 mm in size pieces and cultured in flat-bottomed round aerated flasks with 10 ml/L PES medium under white fluorescence tube lights at 30 µmol photon $m-2$ $s-1$ irradiance with 12.12 light and dark photoperiod at $25\pm1°C$. The media was added every 3 days, and the medium was changed every 10 days. After 55 days, matured thali was harvested.

PES media composition

NaNO$_3$: 350 mg
Glycerine Phosphate.Na : 50 mg
Fe EDTA (2Na)   : 18.8 mg
PII metals   : 25 ml
Vitamin mixtures   : 1 ml
Tris   : 500 mg
pH   : 7.8
Distilled water   : 100 ml

Results

From the fragment regeneration method, after 30 days, from 0.258 g of fragmented *U. lactuca*, about 122 g of matured *U. lactuca*, 2.0 g of fragmented *U. intestinalis*, about 752 g of matured *U. intestinalis* and 1.29gm of fragmented *G. verrucosa* about 15 g of matured *G. verrucosa* was found (Table 7).

Table 7. Growth performance of seaweed species obtained in fragment regeneration method.

No.	Species name	Culture period	Initial fresh weight (g)	Final fresh weight (g)
01	*Ulva lactuca*	30 days	0.258 ± 0.01	122 ± 2.59
02	*Ulva intestinalis*	30 days	2.0 ± 0.05	752 ± 4.28
03	*Gracilaria verrucosa*	30 days	1.29 ± 0.08	15 ± 1.20

Figure 6. Tissue culture of seaweed.

Study-4. Value-added product development

Different seaweed value-added products have been developed from seaweed species *Hypnea* sp. and *Sargassum* sp. with the collaboration of Zahanara Green Agro and Mahi Agro Ltd. These products include seaweed extract, seaweed face pack, seaweed uptan, seaweed momo sauce, seaweed egg barfi, seaweed dates and peanuts, seaweed nutria-dessert, seaweed carrot toffee, seaweed ginger toffee, and seaweed soup. These products are now available in the local market and have growing customer attention.

Figure 7. Seaweed value-added products.

Commercially significant seaweed species

Identification of commercially important seaweed species for Bangladesh aspects was conducted throughout the experimental period. As a result, three seaweed species were identified as commercially, and the economically important and enlisted total number of commercially important seaweed were twenty-six. Among them, one was Chlorophyta (*Ulva reticulata*) and another two were Rhodophyta (*Gracilaria verrucosa, Gelidium pusillum*).

Conclusion

The culture of *S. ilicifolium* at our South Eastern coast shows better biomass yield and daily growth rate in the floating raft method. These species can be successfully cultured at a large scale in our coastal area. However, successful development of seaweed culture requires appropriate natural environmental conditions, available technical support and social acceptance. Additionally, tissue culture activity will ensure the seaweed seeds throughout the year. The land-based year around seaweed production can be possible whether the successful practice of indoor culture and tissue culture of commercially important seaweed species will open a new era of seaweed culture in our country. Furthermore, developing value-added products from seaweeds will ensure diversification of seawed, meet the nutritional demand, and ensure food security for the mass population of the country. So we need more research on seaweed culture and product development.

Figure 8. Seaweed culture net set-up, observation, and harvesting.

Breeding and Culture Potential of Marine Oyster and Green Mussel in the Bay of Bengal Bangladesh Coast

Researchers

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Objectives

- To develop culture techniques of oyster and green mussel in Cox's Bazar and other suitable areas

- To develop breeding techniques of oyster in captivity

- To develop larval rearing and nursery management techniques of oyster

Achievements (2021-2022)

Study -01. Development of culture techniques of oyster and green mussel

Methodology for outdoor culture system

Off-bottom culture methods were followed to develop the culture technique of oyster and green mussel. In this experiment, three culture substrate (Plastic fruit basket, stainless still basket and net bag) were used. Three different densities of oysters and green mussel considered as treatments with three replications. Oysters and green mussel were suspended from floating raft structures. Different culture substrate and stocking densities (Table 1) of oyster and green mussel were laid on trays and allowed to grow until marketable size.

Table 1. Different stocking densities of oyster and green mussel were considering as substrate volume.

Culture substrate	Species	Treatment	Density(No./fit^3)	Replication
Fruit basket	Green mussel	T_1, T_2, T_3	50,70, 90	3
Stains still tray		T_1, T_2, T_3	12,18,25	3
Plastic net bag		T_1, T_2, T_3	12,18,25	3
Fruit basket	Oyster	T_1, T_2, T_3	15,20,30	3
Stains still tray		T_1, T_2, T_3	6,8,12	3
Plastic net bag		T_1, T_2, T_3	12,18,25	3

Results

Growth evaluation in outdoor culture system

Green mussel

Initially 40.12 ± 1.25g green mussels were stocked in different culture substrate such as fruit basket, stainless still and net bag. After the completion of the experiment in fruit basket substrate, higher final weight were found in T_1 (100.16 ± 4.94 g) followed by T_2 (99.49 ± 5.08 g) and T_3 (93.86 ± 2.28 g), respectively (Table 2). Comparatively higher final weight were found in T_2 (88.16 ± 2.59g) than T_1 (87.16 ± 8.14 g) and T_3 (88.52 ± 5.98 g) groups where green muscle cultured in stainless still substrate (Table 3). However, in net bag substrate higher final weight were found in T_1 (103.49 ± 1.52 g) followed by T_2 (102.19 ± 6.05 g) and T_3 (99.19 ± 3.04 g), respectively (Table 4). Among three culture substrates significantly higher yield was observed in net bag substrate.

Table 2. Different stocking densities of green mussel were considering as fruit basket substrate.

Green mussel	Fruit basket substrate			Significance	CV (%)	p-value
	T_1	T_2	T_3			
Initial weight (g)	41.48 ± 1.51^a	40.48 ± 2.09^a	41.85 ± 1.09^a	NS	4.17	0.085
Final weight (g)	100.16 ± 4.94^a	99.49 ± 5.08^b	93.86 ± 2.28^c	*	7.44	0.024
Survival ret (%)	88.88 ± 5.55^a	91.66 ± 4.16^a	88.88 ± 4.81^a	NS	11.58	0.046
Yield ton/ha	53.75 ± 2.74^c	85.54 ± 1.42^b	133.94 ± 9.02^a	*	6.38	0.024

Table 3. Different stocking densities of green mussel were considering as stains still tray substrate.

Green mussel	Stains still tray substrate			Significance	CV (%)	p-value
	T_1	T_2	T_3			
Initial weight (g)	47.82 ± 2.46^a	48.82 ± 0.60^a	48.85 ± 2.02^a	NS	1.12	0.057
Final weight (g)	87.16 ± 8.14^a	88.16 ± 2.59^b	88.52 ± 5.98^c	*	7.44	0.065
Survival ret (%)	88.66 ± 11.54^a	73.33 ± 11.54^c	86.66 ± 6.66^b	*	8.58	0.045
Yield ton/ha	20.49 ± 4.69^c	18.32 ± 6.39^b	74.85 ± 10.73^a	*	2.48	0.019

Table 4. Different stocking densities of green mussel were considering as net bag substrate.

Green mussel	Net bag substrate			Significance	CV (%)	p-value
	T_1	T_2	T_3			
Initial weight (g)	40.15 ± 1.05^a	39.15 ± 1.05^a	39.52 ± 1.53^a	NS	2.17	0.095
Final weight (g)	103.49 ± 1.52^a	102.19 ± 6.05^b	99.19 ± 3.04^c	*	5.34	0.031
Survival ret (%)	88.88 ± 5.55^a	92.59 ± 3.20^a	97.22 ± 2.40^a	NS	11.53	0.041
Yield ton/Ha	35.66 ± 2.07^c	55.10 ± 5.09^b	150.03 ± 5.89^a	*	4.38	0.034

In the beginning of the experiment, 45.72 ± 1.65 g oysters were stocked in fruit basket, stainless still and net bag. The end of the experiment, higher final weight of oysters were found in T_2 (95.49 ± 3.17 g) followed by T_3 (90.52 ± 5.03 g) and T_1 (89.96 ± 0.66 g) in fruit basket substrate systems (Table 5). When considering stainless still substrate, higher growth was found in T_1 (106.12 ± 3.45 g) followed by T_2 (98.23 ± 3.13 g) and T_3 (95.12 ± 4.73 g), respectively (Table 5). However, comparatively bigger oyster was found in T_2 (89.19 ± 6.11 g) than T_1 (87.83 ± 6.65 g) and T_3 (79.86 ± 5.50 g) in net bag substrate culture methods (Table 7). Among three culture substrates significantly higher yield was observed in stainless still substrate.

Table 5. Different stocking densities of oyster were considering as fruit basket substrate.

Oyster	Fruit basket substrate			Significance	CV (%)	p-value
	T_1	T_2	T_3			
Initial weight (g)	47.82±2.46[a]	49.82±1.53[a]	48.18±2.95[a]	NS	5.17	0.075
Final weight (g)	89.96±0.66[c]	95.49±3.17[a]	90.52±5.03[b]	*	2.44	0.024
Survival ret (%)	73.33±11.54[c]	80.00±10.00[b]	84.44±0.00[a]	*	11.47	0.041
Yield ton/ha	17.77±2.91[c]	41.24±6.47[b]	74.22±2.49[a]	*	6.38	0.024

Table 6. Different stocking densities of oyster were considering as stains still tray substrate.

Oyster	Stains still tray substrate			Significance	CV (%)	p-value
	T_1	T_2	T_3			
Initial weight (g)	39.12±1.51[a]	43.22±1.53[a]	40.12±1.51[a]	NS	1.17	0.065
Final weight (g)	106.12±3.45[a]	98.23±3.13[b]	95.12±4.73[c]	*	1.17	0.065
Survival ret (%)	88.88±4.81[a]	90.74±3.20[a]	87.50±4.00[a]	NS	11.58	0.046
Yield ton/ha	57.36±5.06[c]	84.55±2.61[b]	127.14±1.55[a]	*	8.48	0.029

Table 7. Different stocking densities of oyster were considering as net bag substrate.

Oyster	Net bag substrate			Significance	CV (%)	p-value
	T_1	T_2	T_3			
Initial weight (g)	45.82±3.01[a]	47.82±3.51[a]	48.18±0.95[a]	NS	2.15	0.091
Final weight (g)	87.83±6.65[b]	89.19±6.11[a]	79.86±5.50[c]	*	5.29	0.029
Survival ret (%)	86.66±11.54[a]	86.66±5.77[a]	86.66±6.66[a]	NS	11.59	0.047
Yield ton/ha	12.23±1.06[c]	24.89±0.16[b]	67.18±5.14[a]	*	4.32	0.034

Methodology for indoor culture system

In MFTS hatchery complex, three 100L tank were used for the rearing of green mussel and oyster. Average initial weight of oyster and green mussel was 52.10±1.65 g and 42.12±1.65 g, respectively. Three different densities of oysters and green mussel were considered as treatment with two replications (Table 8).

Table 8. Different stocking densities of oyster and green mussel were stocked in fiberglass tank.

Treatment	Species	Initial weight	Density sp/100 l	Replication
T_1	Green mussel	42.13±3.15	30	2
	Oyster	52.12±5.15	30	2
T_2	Green mussel	42.83±4.15	45	2
	Oyster	52.32±4.12	45	2
T_3	Green mussel	42.73±2.15	60	2
	Oyster	52.42±2.25	60	2

Results

Growth evaluation in indoor culture system

Moderate growth of oyster was measured in indoor condition. Among the three Treatments, higher final weight of green mussel was found in T_1 (101.16±7.89g) followed by T_2 (96.16±1.79g) and T_3 (88.52±3.75g), respectively (Table 9). However, higher final weight of oyster were found in T_1 (89.63±9.08g) compared to other T_3 (83.52±2.94g) and T_2 (80.49±1.47g) groups respectively (Table 10).

Table 9. Different stocking densities of green mussel were considering in indoor culture system.

Green mussel	Indoor culture system			Significance	CV (%)	p-value
	T_1	T_2	T_3			
Initial weight (g)	42.48±1.09[a]	42.82±2.04[a]	41.85±1.09[a]	NS	6.17	0.075
Final weight (g)	101.16±7.89[a]	96.16±1.79[b]	88.52±3.75[c]	*	11.34	0.021
Survival ret (%)	75.66±11.54[a]	65.66±5.77[a]	60.66±6.66[a]	NS	10.53	0.066

Table 10. Different stocking densities of oyster were considering in indoor culture system.

Oyster	Indoor culture system			Significance	CV (%)	p-value
	T_1	T_2	T_3			
Initial weight (g)	49.15±3.55[a]	49.15±1.70[a]	45.18±0.95[a]	NS	2.17	0.095
Final weight (g)	89.63±9.08[a]	80.49±1.47[b]	83.52±2.94[c]	*	5.34	0.031
Survival ret (%)	86.86±11.54[a]	80.26±5.77[b]	78.33±6.66[c]	*	1.58	0.026

Study -02. Develop breeding techniques of oyster in captive condition

Breeding Methodology and results

For developing the breeding program of oyster we collected 20 broods from wild source, specifically from Inani beach, Kutubdia and Moheskhali channel. Then sacrificed the oyster brood and collected the gonad part for artificial breeding. After that, gonadal part striped into transparent water jar and mixed all the striped materials for fertilization but we didn't get any successful results. Besides that, we preserved some parts of gonad for histological studies from January to June, 2022. Early developing oysters appeared in January-March and May-June while late developing oysters occurred during Jan-June. Ripe oysters occurred from March-June and absence in January and February. Spawning commenced in April as evidenced by the presence of spawning oysters in the histological preparations. There was a single prolonged spawning season from April to June with spawning peak in April. The absence of spawning oysters from January to March showed that no spawning activity took place during these months. Spent oysters occurred from April to June. Undifferentiated stage as a preparatory step for the next spawning season was reported during January-April. The number of male oysters were very poor than the female oysters (Table 11).

Table 11. Number of oyster (male and female) in different stages of gonadal maturation from January-June.

Month	Total	Male	Female	ED	LD	Ripe	SW	Spent	UN
January	10	2	5	4	3	0	0	0	3
February	10	0	8	3	5	0	0	0	2
March	10	1	7	2	3	3	0	0	2
April	10	1	7	0	2	2	4	1	1
May	10	0	10	1	3	2	2	2	0
June	10	2	8	3	1	2	3	1	0

*ED= Early developing, LD= Late developing, SW= Spawning, UN= Undifferentiated

Study -03 Developments of oyster larval rearing techniques

We didn't get positive result due to unsuccessful artificial breeding.

Outdoor spat monitoring methods and results

For the collection of spat, 4 types of substrates (spat collector) such as tiles, kortal, bolder and oyster shell were used. Spat collector substrates were hanged from the raft (each with 10-12 cm gap) having 1.5 m length following experimental design. After setting the spat collector, fortnightly regular monitoring was done to observe spat settlement. Water quality data were also recorded fortnightly. Different spat substrates of oyster and green mussels were hanged from raft. Among the four Treatments higher number of total spats were found in T_1 (77.00±2.00) followed by T_3 (62.00±2.00), T_4 (53.00±3.00) and T_2 (46.00±3.00) respectively (Table 12).

Table 12. Different spat collector of oyster and green mussel were stoked under floating raft.

Spat collector	Average oyster spat/unit	Average green mussel spat/unit	Total spat/unit
T_1 (Tiles)	12±1.00	65±2.00	77±2.00
T_2 (Bolder)	25±2.00	21±2.00	46±3.00
T_3 (Kortal)	24±3.00	38±3.00	62±2.00
T_4 (Oyster shell)	32±2.00	21±1.00	53±3.00

Development of Culture Technique for Live Feed Isolation from the Bay of Bengal

Researchers

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Objectives

● To identify the commercially important microalgae from the Bay of Bengal

- To isolate and scale up the microalgae in vitro condition

- To investigate the nutritional status of the isolated microalgae

- To investigate the cause of water quality deterioration in the live feed habitat at the coastal region of Cox's Bazar.

Achivements

Experiment 1. Identification of the available marine microalgae species

Methodology

For identification, samples were collected from different points of Bay of Bengal. Plankton net was used to collect the samples and preserved in sample bottle with using 5% formalin. Sample were observed under light microscope (Leica DM1000) and identified based on color, size and distinctive morphological features.

Results

During this experimental period, total 19 species of 15 different genera and 7 classes had been identified. Table 1 shows the list of class, order, family and genus of the identified microalgae.

Table 1: Identified marine microalgae species from Bay of Bengal

Class	Order	Family	Genus
Bacillariophyceae	Bacillariales	Bacillariaceae	*Nitzschia*
	Coscinodiscales	Hemidiscaceae	*Hemidiscus*
	Naviculales	Naviculaceae	*Navicula*
Chlorodendrophyceae	Chlorodendrales	Chlodendracea	*Tetraselmis*
Coscinodiscophyceae	Coscinodiscophyceae	Chaetocerotaceae	*Chaetoceros*
		Coscinodiscales	*Coscinodiscus*
		Thalassiosirales	*Skeletonema*
			Thalassiosira
	Stephanodiscales	Stephanodiscaceae	*Cyclotella*
		Hemiaul aceae	*Eucampia*
Cyanophyceae	Oscillatoriales	Oscillatoriaceae	*Oscillatoria*
Dinophyceae	Gonyaulacales	Ceratiaceae	*Ceratium*
Eustigmatophyceae	Eustigmatales	Monodopsidaceae	*Nannochloropsis*
Fragilariophyceae	Thalassionematales	Thalassionemataceae	*Thalassionema*
	Araphidineae	Fragilariaceae	*Fragilaria*

Discussion

Plankton availability varies depending on the season. Among these 15 genera; *Skeletonema*, *Chaetoceros* and *Nitzschia* were found as the most common species during this sampling tenure.

Experiment 2. Isolation and scale up of microalgae in laboratory condition

Methodology

For isolation, collected samples (using plankton net) were carried out into the laboratory and cultured in laboratory condition. Different culture medium (Conway medium, f/2 medium etc.) were used for culture with maintaining different pH and salinity. Serial dilution method and physical separation (using Pasteur pipette) method were followed to isolate single species.

Results

Two different phytoplankton species (*Skeletonema* sp. and *Chaetoceros* sp.) and one zooplankton (*Cyclops* sp.) species have been successfully isolated during the experimental time period.

Discussion

Native strains show a better adaptability to environmental conditions of their isolation area. In this study, different commercially important native species of Bay of Bengal was isolated and adapted into a laboratory conditions. So that, a pure stock will be ensured for future studies. Moreover, different physiological studies (growth pattern, productivity, cell multiplications rate etc.) had done for each species.

Skeletonema sp.   *Chaetoceros* sp.   *Cyclops* sp.

Figure 1. Isolated plankton species during the experimental period

Experiment 3. Investigation of the nutritional status of the isolated microalgae

Methodology

To analyze the nutritional profile of the isolated microalgae all the isolated species were cultured in laboratory condition. Mass cultures were maintained using 20 liters' tanks. Biomasses were harvested at the stationary phase of a specific species. Harvested biomasses were dried at 60 °C for 12 hours. Finally, the biomasses were processed for proximate, amino acid and fatty acid analyses. Proximate composition (protein, lipid and carbohydrate) was analyzed using biochemical extraction method. Amino acids were analyzed using SYKAM amino acid analyzer and fatty acids were analyzed using gas chromatography mass spectrophotometry (GCMS).

Results

Proximate (Figure 2), amino acid (Table 2) and fatty acid (Table 3) profile of the isolated microalgae are given below. The proximate content (protein, lipid and carbohydrate) showed a significant difference ($p<0.05$) among the species. *Skeletonema* sp. showed highest protein ($41. 89 \pm 4.47\%$) and carbohydrate ($22.63 \pm 2.14\%$) content compared to Chaetoceros sp. ($31.73 \pm 1.07\%$ and $8.26 \pm 1.23\%$) respectively. In contrast, *Chaetoceros* sp. showed highest lipid content ($26.33 \pm 3.05\%$) compared to *Skeletonema* sp. ($18.26 \pm 2.95\%$).
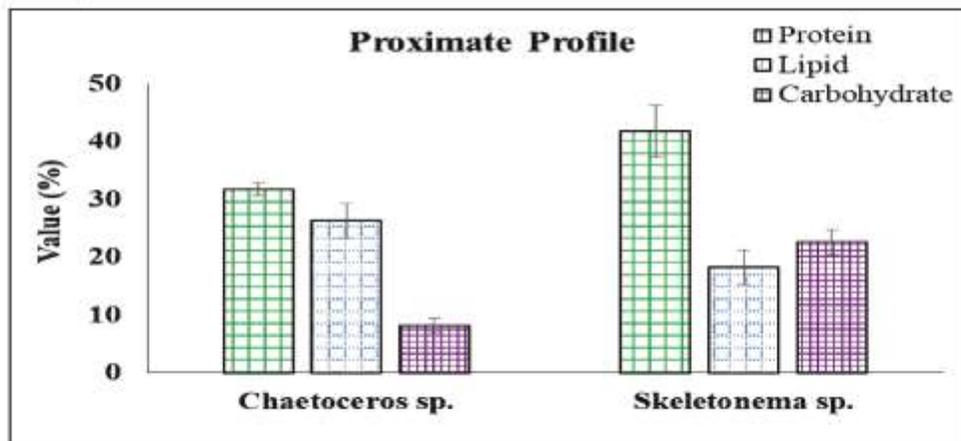

Figure 2. Proximate composition of the isolated microalgae

Table 2. Amino acid content (% amino acid) in the cultured microalgae species

Parameters	Code name	Types	Species	
			Chaetoceros sp.	*Skeletonema* sp.
Histidine	HIS	EAA	3.40 ± 0.04	2.46 ± 0.05
Isoleucine	ILE	EAA	6.97 ± 0.15	4.87 ± 0.02
Leucine	LEU	EAA	10.35 ± 0.34	9.50 ± 0.05
Lysine	LYS	EAA	3.64 ± 0.01	4.46 ± 0.03
Methionine	ME T	EAA	9.68 ± 0.06	2.43 ± 0.06
Phenylalanine	PHE	EAA	5.80 ± 0.22	6.50 ± 0.05
Threonine	THR	EAA	3.76 ± 0.28	4.83 ± 0.01
Tyrosine	TYR	EAA	6.28 ± 0.26	3.41 ± 0.04
Valine	VAL	EAA	8.20 ± 0.35	5.41 ± 0.00
Alanine	ALA	NEAA	6.46 ± 0.17	8.05 ± 0.04
Arginine	AR G	NEAA	3.94 ± 0.21	5.71 ± 0.02
Aspartic acid	ASP	NEAA	9.00 ± 0.10	12.12 ± 0.04
Glutamic acid	GLU	NEAA	8.87 ± 0.01	12.92 ± 0.57
Glycine	GLY	NEAA	5.16 ± 0.29	7.12 ± 0.10
Cysteine	CYS	NEAA	1.94 ± 0.08	ND
Seronine	SER	NEAA	4.03 ± 0.09	5.01 ± 0.07
Proline	PRO	NEAA	2.53 ± 0.04	5.19 ± 0.11

Values are means ± standard error of duplicate measurements. EAA: Essential Amino Acid, NEAA: Non-Essential Amino Acid, ND: Not detected

Table 3. Fatty acid content (% fatty acid) in the cultured microalgae species

Carbo n	Fatty acid	Types	Species	
			Chaetoceros sp.	*Skeletonema* sp.
C8:0	Octanoic acid	SAFA	0.09 ± 0.00	2.12 ± 0.10
C10:0	Decanoic acid	SAFA	0.12 ± 0.00	0.34 ± 0.04
C12:0	Lauric acid	SAFA	0.05 ± 0.00	0.50 ± 0.06
C13:0	Tridecanoic acid	SAFA	0.06 ± 0.00	1.89 ± 0.70
C14:0	Myristic acid	SAFA	2.51 ± 0.09	7.68 ± 0.06
C16:0	Palmitic acid	SAFA	16.27 ± 0.11	3.00 ± 0.06
C18:0	Stearic acid	SAFA	4.01 ± 0.42	1.15 ± 0.27
C20:0	Arachidic acid	SAFA	12.15 ± 1.79	1.12 ± 0.71
C17:0	Heptadecanoic acid	SAFA	0.30 ± 0.00	ND
C21:0	Heneicosanoic acid	SAFA	10.18 ± 0.42	0.89 ± 0.35
C22:0	Behenic acid	SAFA	0.06 ± 0.06	ND
C23:0	Tricosanoic acid	SAFA	ND	ND
C24:0	Lignoceric acid	SAFA	ND	ND
C16:1	Palmitoleic acid	MUFA	21.26 ± 0.60	73.80 ± 1.52
C18:1	Oleic acid	MUFA	0.37 ± 0.01	0.20 ± 0.20
C20:1	cis -11 -Eicosenoic acid	MUFA	1.60 ± 0.09	0.04 ± 0.02
C22:1	Eruic acid	MUFA	17.09 ± 0.21	0.28 ± 0.20
C24:1	Nervonic acid	MUFA	0.02 ± 0.01	0.06 ± 0.01
C18:2n -6	Linoleic acid	n6 -PUFA	0.62 ± 0.04	3.06 ± 0.00
C20:3n -6	Eicosatrienoic acid	n6 -PUFA	1.42 ± 0.18	1.56 ± 0.44
C20:4n -6	Arachidonic acid	n6 -PUFA	1.32 ± 0.35	0.15 ± 0.07
C18 -3n -3	Linolenic acid	n3 -PUFA	9.83 ± 0.16	2.05 ± 1.37
C20:5n -3	Eicosapentanoic acid	n3 -PUFA	0.04 ± 0.02	0.08 ± 0.05
C22:5n -3	Docosapentaenoic acid	n3 -PUFA	$0.01 \pm 0 .00$	0.04 ± 0.01
C22:6n -3	Docosahexaenoic acid	PUFA	0.65 ± 0.02	ND

Values are means ± standard error of duplicate measurements. SAFA: Saturated Fatty Acids, MUFA: Mono Unsaturated Fatty Acids, PUFA: Poly Unsaturated Fatty Acids

Discussion

Skeletonema sp. and *Chaetoceros* sp. both two species were found commercially important considering their total profile. To utilize all these species at food, feed, pharmaceuticals and nutraceuticals industries as raw material, it is important to know the nutritional profiles. The outcomes of this study will help to select a suitable species on basis of purpose of use.

Experiment 4. Investigation of the cause of water quality degradation in live feed habitat (sampling station)

Methodology

Five different locations were set to evaluate the water quality parameters (Table 4). The physico-chemical parameters (humidity, rainfall, salinity, temperature, pH, DO, alkalinity, ammonia, nitrite, nitrate, TDS, and silica) and bacteriological test of the selected microalgae habitat were performed monthly. The parameters (pH, ammonia, alkalinity, nitrite, nitrate, TDS, and silica) were tested using water quality multi-parameter (HANNA HI98194) and HANNA COD and multiparameter bench photometer (HANNA HI83099). For bacteriological analysis various selective agar media had been used to determine the amount (cfu/mL) of bacteria present in live feed habitat. Seawater samples were collected from the different depth of the selected habitat. Diluted sample was used to observe the growth of bacteria in agar plate.

Table 4. Sampling locations

Station	Station name	Latitude	Longitude
1	Khurushkhul	21° 30' 15.79" N	91° 59' 39.31" E
2	Laboni Point	21° 25' 34.726" N	91° 58' 13.4" E
3	Dariyanagar	21° 23' 36.888" N	91° 59' 50.1" E
4	Pechar dip	21° 20' 19.608" N	92° 1' 37.05" E
5	Inani	21° 14' 5.244" N	92° 2' 42.03" E

The stations were selected considering some criteria which are commonly being observed in this southern coast region. Table 5 summarizes the basis common characteristics of the sampling locations.

Table 5. Sampling locations Characteristics

Characteristics	S1	S2	S3	S4	S5
Surface runoff	Yes	Yes	Yes	No	No
Human activities	No	Yes	No	No	Yes
Tourist	No	Yes	No	No	Yes
Hatchery area	No	No	Yes	Yes	Yes

S1: Khurushkhull; S2: Laboni; S3: Dariyanagar; S4: Pechar Dip; S5: Inani

Results

Figure 3 shows the water quality parameters of the sampling locations. The water quality parameters were found significantly different ($p < 0.05$) among the locations. In additions, water quality parameters were also found significantly different ($p < 0.05$) on the basis of months in the same locations. Whatever, the pH, dissolve oxygen, alkalinity, nitrite etc. were found more or less similar in all the locations.

Table 5. summarizes microbiological loads of the selected sampling habitats during the experimental period-

Table 5. Microbiological loads of live feed habitat

Month	Total bacteria (CFU/mL)	*Vibrio* spp. (CFU/mL)	*Salmonella* (CFU/mL)	Total Coliform (CFU/mL)
October	1.01×10^3	12	29	1205
November	2.75×10^4	8	27	1530
December	2.49×10^3	3	25	926
January	2.21×10^3	-	19	876
February	2.76×10^4	98	53	418
March	2.51×10^3	13	32	760
April	3.22×10^4	137	26	1921
May	2.31×10^3	-	-	363
June	3.15×10^4	125	17	1394
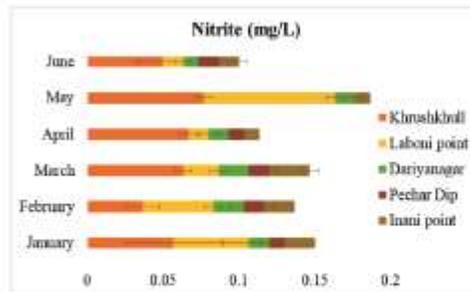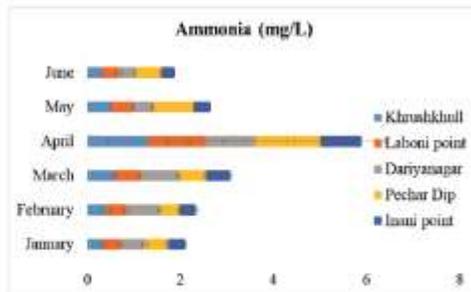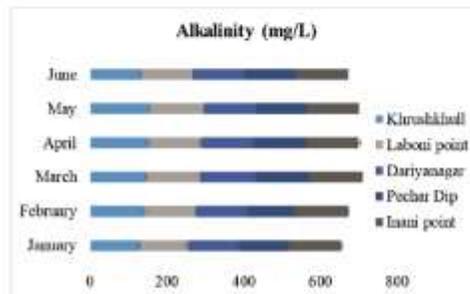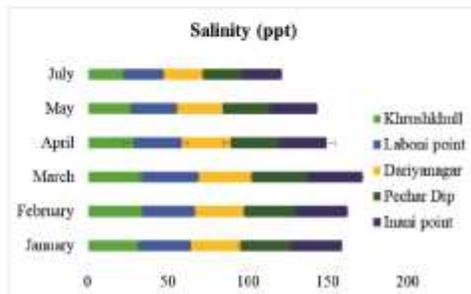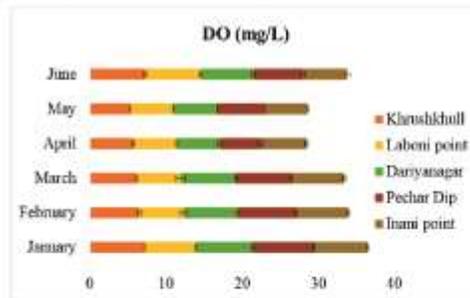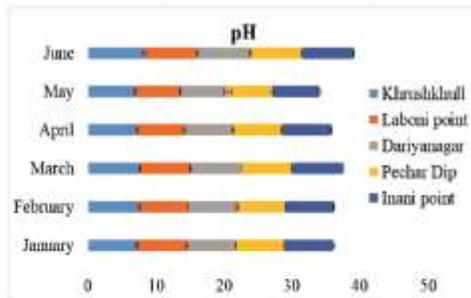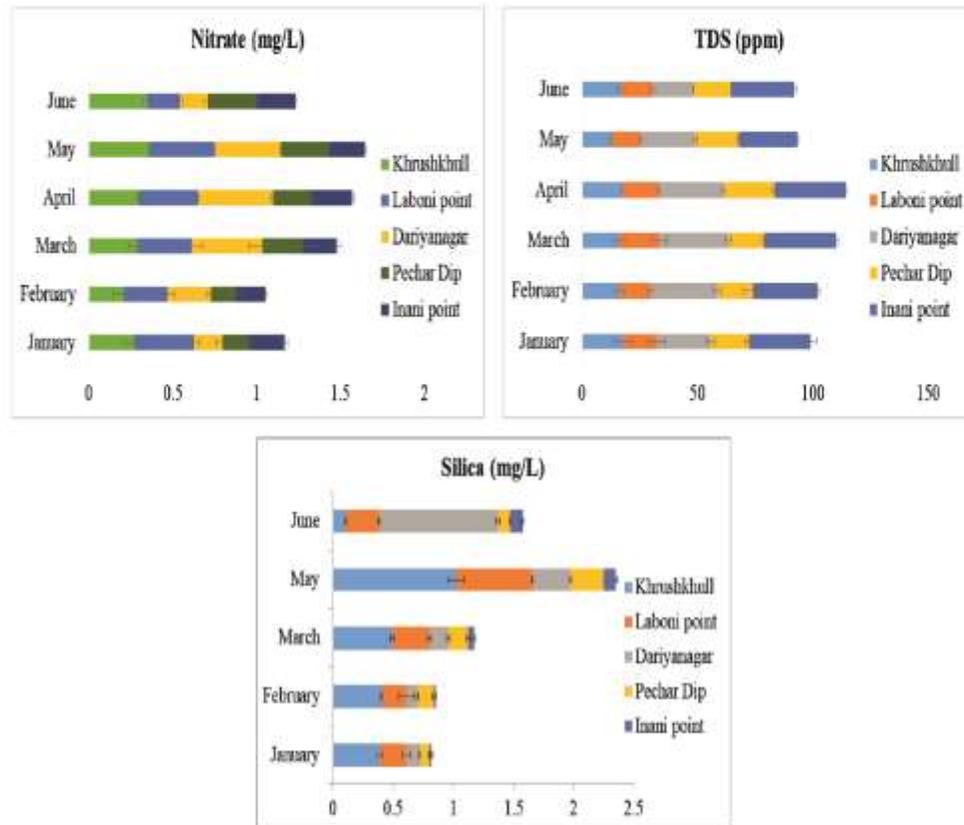
Figure 3. Monthly basis water quality parameters of the sampling locations

Discussion

The contamination of coastal water can be due to some non-point discharges such as run off form agricultural, commercial, urban, industrial lands or run-off from naturally vegetate areas. It could also be attributed because of the increased frequency of human intervention. Due to this contamination intensity, the collected raw samples were maintained into a separate place which is named as isolation chamber. However, the seawater for laboratory use were treated and autoclaved properly to remove contaminations in the stock cultures.

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Tanu, M.B., Barman, A.C., Siddque, M.F., Sku, S., Hossen, M.N., Southgate, P.C., & Mahmud, Y. (2022). Impact of culture period on quality of image pearls produced by the freshwater mussel, Lamellidens marginalis, in Bangladesh. *Journal of Shellfish Research, 41*(1), 75–83.

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Md. Lipon Mia	Scientific Officer
Srebash Kumar Saha	Scientific Officer
Mousumi Akhter	Scientific Officer
Most. Aziza Begum	Scientific Officer
Mahmudul Hasan Mithun	Scientific Officer
Nasima Begum	Scientific Officer
Rafia Afrin	Scientific Officer
Saima Sultana Sonia	Scientific Officer
Al-Amin	Scientific Officer
Md. Reaz Morsed Ranju	Scientific Officer
Farjana Jannat Akhi	Scientific Officer
Md. Abu Kawser Didar	Scientific Officer
Md. Shahin Alam	Scientific Officer
Zahidul Islam	Scientific Officer
Md. Iqramul Haque	Scientific Officer
Mezbabul Alam	Scientific Officer
A N M Rezvi Kaysar Bhuiyan	Scientific Officer
Md. Ayemuddin Haque	Scientific Officer
Md. Rakibul Islam	Scientific Officer
Md. Abu Naser	Scientific Officer
Md. Masudur Rahman	Scientific Officer
Farhana Yasmin	Scientific Officer
Md. Aktaruzzaman	Scientific Officer
Tashrif Mahmud Minhaz	Scientific Officer

Administrative Staff

Name of Staff	Designation
Dr. Abdur Razzaque	Deputy Director
Shamsun Nahar	Deputy Director
Md. Asadur Rahman	Deputy Director
Sheikh Rasel	Deputy Director
Muhammad Kamrul Haque	Deputy Director
Md. Azizul Haque	Deputy Director
Mohammed Shahid Ullah	System Analyst
Md. Razibul Karim	Executive Engineer
Mahmuda Khandaker	Senior Librarian
Jannatul Ferdous Jhuma	Assistant Director (C.C.)
S. M. Sariful Islam	Publication Officer
Md. Mamunur Rashid	Assistant Enginner
Md. Ruhul Amin	Administrative Officer
Hosne Ara	Administrative Officer
Kazi Zohirul Kaium	Administrative Officer
Md. Abdul Kadir	Administrative Officer
Kazi Talim Uddin	Accounts Officer
Md. Abdus Samad	Security Officer