ANNUAL REPORT
2023-2024

Bangladesh Fisheries Research Institute
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BFRI Annual Progress Report 2023-24

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2023-24

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Published by
Director General
Bangladesh Fisheries Research Institute
Mymensingh

Annual Report 2023-24. Bangladesh Fisheries Research Institute, Mymensingh.

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Bangladesh Fisheries Research Institute: An Overview

Fish and fisheries have a crucial role in the culture and heritage of Bangladesh. The sector has a substantial impact on nutrition, employment creation, and foreign exchange revenue. Considering the significant opportunities in the sector for improving nutrition and creating job opportunities, especially for the most disadvantaged, and the need to effectively utilize aquatic resources through science, the President of the People's Republic of Bangladesh was pleased to promulgate an Ordinance entitled "The Fisheries Research Institute Ordinance 1984" on 11 July 1984. In pursuance of this Ordinance, the Fisheries Research Institute (FRI) was established in July 1984. In 1997, the FRI has been renamed as Bangladesh Fisheries Research Institute (BFRI) through the amendment of the 1984 Ordinance.

The Institute was established in 1984 but started functioning in 1986 after employing the necessary employees while setting up first research facilities. Since then, the institute has been crucial in helping the nation realize the fisheries development target outlined in consecutive development plans.

Vision of the Institute

Development of need-based technology leading to increasing fisheries production of the country.

Mission of the Institute

To conduct research for the development of need-based technology on aquaculture and fisheries resource management of the country.

Mandate of the Institute

- To carry out basic and adaptive research for development and optimum utilization of all living aquatic resources and coordinate fisheries research activities in Bangladesh;
- To conduct experiment and standardize techniques for maximizing productions and better management of living aquatic resources;
- To identify new production opportunities and develop them to usable levels;
- To develop skilled research manpower through training;
- To transfer developed technologies to users through training of extension workers, planners, fish farmers and other stakeholders;
- To advise the Government in all matters relating to research and management of living aquatic resources.

Management of the Institute

The Institute (BFRI) is an autonomous research organization that is administratively affiliated with the Government of the People's Republic of Bangladesh through the Ministry of Fisheries and Livestock. The general direction, administration and supervision of the affairs of the institute is vested in the Board of Governors consisting as follows:

Board of Governors

Chairman	: Hon'ble Minister, Ministry of Fisheries and Livestock
Vice-chairman	: Secretary, Ministry of Fisheries and Livestock
Members	: Executive Chairman, Bangladesh Agricultural Research Council
	: Vice-chancellor, Bangladesh Agricultural University, Mymensingh
	: Member (Agriculture), Planning Commission
	: Director General, Department of Fisheries
	: Two Members of the Parliament to be appointed by the Govt.
	: Two persons to be appointed by the Govt. among the persons having interest in fisheries development
	: Two persons to be appointed by the Govt. engaged in research in BFRI
Member-Secretary	: Director General, BFRI

The Board of Governors has the authority to exercise all powers and accomplish all actions that the Institute is capable of. The Board can establish committees to aid in carrying out its duties as deemed essential. The Director General, as the Chief Executive of the Institute, implements its programmes according to the policies and directives set by the Board of Governors.

BFRI Organogram

The Headquarters of the Institute is located at Mymensingh. The Institute has five research stations and five sub-stations based on different aquatic ecosystems. The organogram of the institute is shown in next page.

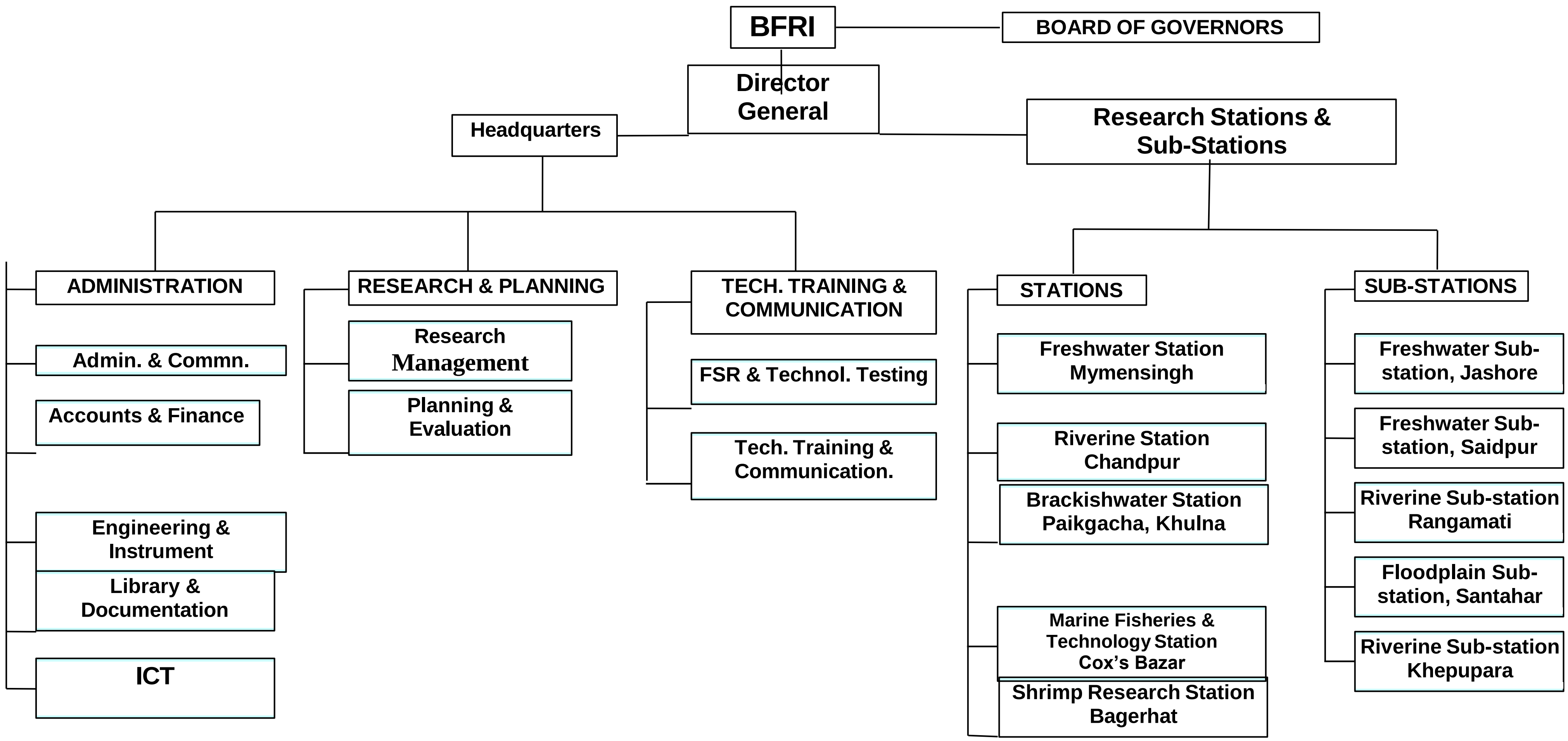
Stations and Sub-stations

Headquarters, Mymensingh

The Headquarters of the Institute is located at the south-west corner of the Bangladesh Agricultural University, Mymensingh, which is about 120 km north of the capital city, Dhaka. The Headquarter functions through its various divisions in respect of administrative development, coordination and operation of its research programs. The divisions are:

- Research and Management,
- Planning and Evaluation
- Technology Testing, Training and Communication,
- Administration and Common Service,
- Engineering and Instrument,
- Library, Documentation and Public Relations,
- ICT, and
- Accounts and Finance.

ORGANOGRAME
Bangladesh Fisheries Research Institute
Mymensingh



Freshwater Station (FS), Mymensingh

The largest station of the Institute, with an area of 40 ha is located at Mymensingh attaching to the BFRI Headquarters. The station has well established and sophisticated carp and prawn hatcheries. The station has as many as 118 drainable ponds consisting of 20 mini ponds; 52 nursery ponds (0.1 ha each), 47 rearing ponds (0.25 ha each) and 16 grow-out/brood stock ponds (1.6-2.6 ha each). Other physical facilities include a feed store, office buildings, residential quarters, a 35-bed dormitory, a community center and a 5-bed guesthouse. The station is actively involved in conducting research on hatchery management, fish genetics and reproduction, carp polyculture, integrated fish farming, fish feed and nutrition, pearl culture, fish disease, health management and socio-economic aspects. The various research activities of the station are implemented by the following divisions:

- Reproductive Physiology and Genetics,
- Aquaculture and Farming System,
- Nutrition, Food and Feed Technology,
- Fish Disease Diagnosis and Health Management,
- Soil, Water and Productivity Management,
- Fisheries Socio-economics.

Three sub-stations are attached to the Freshwater station. These are:

Floodplain Sub-Station, Santahar*:* To support the floodplain fisheries development program taken up by the Government, studies on the ecology, limnology and gear selectivity of floodplains are being undertaken at the Santahar Sub-station. The sub-station succeeds in breeding and culture of certain endangered fish species like., *Aspidoparia jaya*, *Neotropius atherinoides* etc.

Freshwater Sub-Station, Jashore: To support freshwater aquaculture farmers and hatchery operators of greater Jashore region, the Freshwater Sub-Station has been conducting research on breeding and culture of BFRI Super Tilapia, carp disease diagnostic services and also farming system research and development.

Freshwater Sub-Station, Saidpur*:* To support the fisheries development program in northern region of Bangladesh, a freshwater sub-station is established in Saidpur Upzilla under Nilphamari. The prime objective of the sub-station is to conduct need-based research to suit with the ecosystem of northern Bangladesh and to transfer technology to the farmers through effective training and demonstration. The sub-station succeeds in breeding and culture of certain endangered fish species like., *Barilius* spp., *Mystus bleekeri*, *Labeo dero*, *Labeo angra* etc.

Riverine Station (RS), Chandpur

The station is situated in the riverine port city of Chandpur, with an area of 17.2 ha and has 36 non-drainable ponds ranging in size from 0.12 to 0.37 ha each and with a total of 8.6 ha. water area. In addition, the station has one carp, one catfish and one prawn hatchery, two deep tube-wells, specialized laboratories, library, office buildings, residential quarters and an 8-bed guest house. One research vessel, one mechanized wooden boat equipped with research facilities, and three speed boats are available for undertaking riverine survey and studies relating research and management to hilsa and other riverine fisheries resources. The Riverine Station consists of 6 research divisions, which are as follows:

- Stock Assessment and Resource Dynamics,
- Fisheries Resource Management and Conservation,
- Culture-based Fisheries Management,
- Reproductive Biology of Riverine Species,
- Environment and Aquatic Pollution.

Two Sub-Stations are attached with the Riverine Station, and these are:

Riverine Sub-Station, Rangamati

To devise sustainable management and development strategies for the Kaptai lake fishery, Riverine Sub-Station (RSS) undertakes various adaptive research programs. Priorities are given on continuous monitoring of biological productivity, stock assessment, natural spawning, and population dynamics of various commercially important fishes and major carps, in particular. Recently, RSS has been introducing pen and cage aquaculture programs in the creeks and lagoons of Kaptai lake to culture fingerlings of major carp and thus to support artificial stocking of the lakes by Bangladesh Fisheries Development Corporation (BFDC), Kaptai lake project. Extension works are being carried out through adaptation of pen and cage aquaculture, installation of pens and cages in the creeks/coves in Kaptai lake on participatory basis.

Riverine Sub-Station, Khepupara, Patuakhali

The fish landing and wholesale center of BFDC at Khepupara Upazilla has been handed over to BFRI to develop as a Sub-Station and carry out research mainly on hilsa fishery. The old infrastructure has now been renovated by BFRI. Due to manpower, funds and logistic constraints, research is being conducted on hilsa in a limited scale. In addition to this, technical advice to the fish farmers is being provided and improved fish seeds are distributed to the local farmers time to time.

Brackishwater Station (BS), Paikgacha, Khulna

The station was established in 1987 with a view to undertake research and development activities on various aspects of coastal aquaculture and fisheries management. The station is located at Paikgacha Upazilla under Khulna and has an area of 30.56 ha. The station has got

53 drainable experimental bracksihwater ponds of different sizes ranging from 0.05 to 1.0 ha, an experimental hatchery for the production of prawn and commercially important brackishwater fin-fish seeds and a number of laboratories. The station has 5 research divisions, such as:

- Nutrition and Feed Technology,
- Disease Diagnostic and Health Management,
- Brackishwater Aquaculture,
- Estuarine Ecology and Environment,
- Soil, Water and Productivity Management.

This station is involved in conducting research on increasing productivity of coastal *ghers*, environment friendly shrimp culture development, crab seed production and fattening, seed production and culture of commercial finfishes, diseases management, aquatic environment monitoring etc. The research work undertaken so far by this station includes socio-economic studies on shrimp farming, survey and assessment of shrimp fry resources and its breeding ground, production potential of *gher* fishery (with improved management practices), polyculture of shrimp and mullet, breeding, culture and fattening of mud crab *(Scylla* spp.), breeding and nursing of *Macrobrachium rosenbergii*, improved method of shrimp farming, breeding and culture of brackishwater catfish and green back mullet etc.

Marine Fisheries and Technology Station (MFTS), Cox's Bazar

This station, with an area of 4 ha, was established at Cox's Bazar in 1991. The station is being equipped with a crab breeding hatchery, live feed laboratory, outdoor complex with 39 cisterns (200 m^2 each), residential buildings for officers and staff accommodation, service building and an 8-bed guest house. There is a new 7-storied laboratory cum office building now under construction.

The mandate of the station includes research on marine ecology, seaweeds culture, environmental studies, stock assessment and population dynamics of commercially important species, diseases diagnosis and control, development of processing and preservation technologies, socio-economic studies of marine and coastal fishers and quality control of marine products.

Shrimp Research Station (SRS), Bagerhat

The station was established on 2010 at Sadar Upazilla under Bagerhat with an area of 8.0 ha. The mandate of the station is to conduct research on enhancing shrimp production, shrimp health management, shrimp feed and nutrition, post-harvest handling and quality control of shrimp and shrimp products. The station consists of a 2-storied Office-cum-Laboratory building, 3-storied Staff dormitory, and 4-storied Training dormitory of the station. Moreover, a pond complex composing 9 experimental ponds of different sizes are being used for experimental purposes. The laboratories of the station are:

- Shrimp Health Management,
- Quality Control,
- Shrimp Feed and Nutrition,
- Water and Soil Quality Management.

Manpower

The manpower status of the Institute is highlighted in the following table:

Head	Approved posts			Filled up posts		Vacant posts	
	Officer	Staff	Total	Officer	Staff	Officer	Staff
Revenue	268	257	525	112	186	155	71

Development of Technologies

Regular research activities of the institute lead to generate various aquaculture and management technologies for better management of the resources and increase the fish production. Till 2023, the Institute has evolved more than 83 aquaculture and fisheries management technologies. Among them, 11 technologies have been developed during 2022-23 period and these are as follows:

- ✓ Induced breeding and seed production of endangered fish sp. loach, Balachata (*Somileptes gongota*)
- ✓ Breeding and seed production technology of Mud crab (*Scylla olivacea*)
- ✓ Induced breeding technology of Boirali (*Barilius barila)*
- ✓ Induced breeding technology of Angus (*Labeo angra)*
- ✓ Induced breeding technology of Kholisha (*Colisa fasciatus)*
- ✓ Induced breeding technology of Jatpunti (*Puntius saphore)*
- ✓ Pearl culture in freshwater Mussel
- ✓ Induced breeding technology of Kursha (*Labeo dero)*
- ✓ Induced breeding technology of Loitta tengra (*Mystus bleekeri)*
- ✓ Induced breeding and seed production technology of Dhela (*Osteobrama cotio)*
- ✓ Determination of standing biomass sustainable yield (MSY) of Hilsa (*Tenualosa ilisha*)

Technology transfer: Subsequent to development of technologies or management practices, the generated research results were transferred through various mechanisms. Different government agencies including Dept. of Fisheries, NGOs, farmers and entrepreneurs were offered training on research-evolved technologies. After successful maturation of technologies, printing materials like manuals, booklets, leaflets, posters etc. were published and distributed among the users.

On-Farm trials*:* Field trials of the on-station research findings were conducted for adaptation of technologies in on-farm conditions through government and non-government extension agencies, private entrepreneurs and NGOs.

Farmer's Advisory Services: The Institute through its different Stations and Sub-Stations provided advisory services to the farmers on improved fish farming technologies, water quality monitoring, feed quality, diseases control etc. Scientists of the institute also provided service on national crises related to fisheries and environmental issues as and when deemed necessary.

Training Programs

Training on different aspects of fisheries is utmost important for boosting -up of fish production and to ensure better management of aquatic resources. A series of well-structured training programs are organized by the Institute every year to disseminate the research evolved technologies to the end users. Moreover, effective transfer and dissemination of the technologies and management procedures such as training of extension workers both of Government and NGOs, teachers, students and Journalists are also organized by Institute. The training programs organized on different aspects are as follows:

- Improved fish culture and management
- Seed production and culture techniques of endangered fish species
- Pearl culture techniques in freshwater ponds
- Shrimp nursery, culture and management
- Crab fattening techniques
- Pen and cage culture techniques
- Fisheries and aquaculture research management
- Mud eel culture technique
- Seaweed culture and product development
- Effect of sanctuary on Hilsa production
- Culture technique of Mussels and Snails in Bangladesh

The Institute also conducts training on research methodology, financial management, office management, e-filing, e-GP and other research-oriented programs for researchers of the Institute to shine up their capability.

Training programs conducted: For boosting-up fish production and to ensure better utilization of aquatic resources, BFRI organizes series of training programs every year for farmers, entrepreneurs, unemployed youth, rural women and university students, extension workers both of Government and NGOs, teachers, journalists and LGED fisheries facilitators. The main objective of offering such type of need and opportunity-based training is to transfer and disseminate technologies among various stakeholders and end users. During July 2022-June 2023 a total of 146 training batches were completed and 3,090 nos. of people were trained up by the institute.

Institutional manpower development: For strengthening the capabilities of scientists, administrative and management personnel, the Institute organizes different in-country and overseas short-term and long-term training programs, study tour and experience-sharing visits. During 2020-22, a total of 13 scientist achieved overseas short-term and long-term training in 4 programs, besides, 14 different in-country training programs have been organized for the scientists and officers. 2 scientists have been awarded PhD from abroad

Workshop/Seminar organized: The Institute organized 8 numbers of National workshops and seminars in different disciplines to identify the problems and sharing and exchanging knowledge generated through research in this year. The Institute and its Stations and Sub Stations organize Regional and National workshops every year to review the research projects and to present the research progress of the Institute.

Public Relation and Publications

Public Relations (PR) division of BFRI provides information among different stakeholder of fisheries sector and so on. Public Relations (PR) also give information as well as latest research success to the Press. During 2019-20 a total of 145 news and reports have been published in different print and electronic media including the daily Ittefaq, the daily Prothom Alo, the daily Jugantor, the daily Kaler Kantho, the daily Star, The daily Financial Express, the daily Bangladesh Pratidin and so on. Besides, some well circulated agri news magazines also publish BFRI news for example: Monthly Krishi Surakkha, Krishi Projukti, Monthly Khamar etc. In addition, BTV, ATN Bangla, Ekushey TV, Jamuna TV, Channel i, Channel 24, DBC, Independent etc. also broadcast BFRI news and achievements.

The Institute publishes research findings, annual reports, newsletters, journals, workshop proceedings, training manuals, extension materials in the form of booklets, leaflets and posters. The publications are available at the Library and Documentation Center as well as at different regional stations and sub-stations of the Institute. The following publications were published during the reporting period:

ক্রমিক নম্বর	প্রকাশনার নাম	প্রকাশনার ধরণ
১.	বিলুপ্তপ্রায় মাছের প্রজনন ও চাষ প্রযুক্তি নির্দেশিকা	প্রযুক্তি নির্দেশিকা
২.	Bangladesh Journal of Fisheries Reasearch Vol. ১৯(১-২), ২০২০	জার্নাল
৩.	বৈরালি মাছের কৃত্রিম প্রজনন ও পোনা উৎপাদন কৌশল	লিফলেট
৪.	জাতপুটি মাছের কৃত্রিম প্রজনন ও পোনা উৎপাদন কৌশল	লিফলেট
৫.	আঙ্গুস মাছের প্রজনন ও পোনা উৎপাদন কৌশল	লিফলেট
৬.	খলিশা মাছের কৃত্রিম প্রজনন ও পোনা উৎপাদন কলাকৌশল	লিফলেট
৭.	স্বল্পমূল্যে বাণিজ্যিক গুরুত্বসম্পন্ন স্পিরুলিনা চাষ প্রযুক্তি কৌশল	লিফলেট
৮.	Fisheries Newsletter	নিউজলেটার

Institute gives special value to publication and documentation of aquaculture and management technologies for their wider adoption. For this reason, extension manuals, leaflets, posters, handouts etc. were well circulated to govt. and non-govt. extension agencies, farmers, entrepreneurs etc.

Library and Documentation

Bangladesh Fisheries Research Institute Library and Documentation Centre (FRILDOC) act as a repository of literature and technical information and provides latest information on scientific research and experimental development in all branches of fish and fisheries. The most of the FRILDOC collection backup on the subjects: aquaculture, brackish water aquaculture, mariculture, marine science, biology, ecology, environmental science, agriculture, life sciences, sea weeds, plankton, food processing, feeds, zoology, botany, geography, economics, marketing, geology, socioeconomics, rural development etc.

The library has 9108 technical and general books 186 titles of scientific periodicals 5120 miscellaneous publications. In addition to above collection, the library has kinds of reference books, academic dissertations, government and others departmental publications.

The FRILDOC is operating in fully automated environment. The various activities of the centre have been computerized using Library Management Information System (LMIS) software. The FRILDOC provides the following documentation services:

- Document Delivery Service
- Current Awareness Service
  i) Current Content Service
  ii) Monthly Accession list
  iii) Monthly News paper Articles
- Reference service
- Bibliographical service
- Abstracting service
- SDI (Selective Dissemination Information) Service
- Internet Service
- Photocopy Service
- ASFA (Aquatic Sciences and Fisheries Abstract) DVD Service
- TEEAL (The Essential Electronic Agricultural Library) Service
- Digital Library Service (BFRI in Aquatic Commons digital repository (http://aquaticcommons.org/view/issuing_agency/Bangladesh_Fisheries_Research_Institute.html).
- Hinari, AGORA, OARE, ARDI and GOALI **(**The Research4Life programme) Service

During the reporting period of July 2020 to June 2022, a number of books, Journals, periodicals etc. procured for the library. The library has also received a noticeable number of books journals, periodicals, proceedings, research reports, annual report, newsletters and magazines on complimentary and exchange basis. The library-maintained exchange programme with more than 75 leading national and International organizations. The category wise list is shown below:

Items	2022-2023
Books	185
Journals	17
Reports/Proceeding of seminars and workshops/papers	11
Newsletters/Bulletins/Reprints/Off prints	43
ASFA (Aquatic Sciences and Fisheries Abstract) DVD	up to 2017
TEEAL (The Essential Electronic Agricultural Library)	up to 2013

The library maintained free mailing of institutional publications to various research organizations, Universities, NGOs, entrepreneurs and farmers to keep the aware with the latest development in fisheries research.

Working Linkage

The institute conducted its research, training, and management endeavors in close collaboration and through connections with numerous national and international organizations and agencies. Additionally, the institute maintained close ties with public extension organizations and various non-governmental organizations operating in the country in order to disseminate technologies and solicit their feedback. BFRI maintained close liaisons with national institutions and engaged in collaborative efforts pertaining to fisheries research and development (R&D). The Department of Fisheries (DOF) is unquestionably the primary focus among national collaborators, followed by NARS institutions and joint research and development programmes with various NOGs.

Finance and Accounts

The sources of funds of the institute comprise grants from the government, and grants from different donor agencies. Government grant from the revenue budget is usually provided to meet only salaries and allowances of staff small protein of operational costs. The cost of development, maintenance and research is also borne by the government from its development budget provided in the form of development project.

Receipts and expenditure: The institute received an amount of Tk. 4274.50 lakh during the year 2022-23.

Research Progress 2023-24

Improved Germplasm Production of Carps, White Pangas and Pure-line Breeding of Kalibaus (*Labeo calbasu*)

Researcher (s)

Dr. Mohammad Ashaf-Ud-Doulah, SSO
Dr. Selina Yeasmine, SSO
Dr. Rakhi Das, SSO
Md. Amdadul Haque, SO

Objectives

1. To upgrade and produce quality seeds of Indian major carps, BFRI Shuborno Rui, Catfishes, and distribute among the farmers and/or hatchery owners.
2. To develop live gene bank with quality brood stocks through implementation of effective breeding plan.
3. To produce improved stocks of kalibaus through cross breeding and mass selection techniques
4. To evaluate the growth performance of selected breeds with non-selected breeds of kalibaus (generation to generation).

Achievements (2023-2024)

1. Production of F_1 base population of kalibaus (pure and cross breed line)

Collection and Domestication: Two wilds populations of Kalibaus (*L. calbasu*) viz., collected from Megna river and Haor source. All of two stocks are being separately reared in Freshwater Station pond complex until they are being used for the breeding program. At the age of 2 years or more of the fish, they are being individually selected and reared in brood ponds until being used those fish for the production of pure and cross breed lines following mass selection protocols.

Development of founder F_1 base population (Pure and Crossbreed lines)

At the age of maturity breeding of two different stocks of Kalibaus were initiated in July 2022. During the month of March 2022, at least 100 pairs of broods from each of the stock are being stocked in separate pond and taken more care for further maturing them. For production of F_1 base generation of Kalibaus, mature broods are being selected from each of the two crossbred lines viz., Megna (Female) X Haor (Male), Megna (Male) X Haor (Female) & two pure-line viz., Megna (Female) X Megna (Male), Haor (Female) X Haor (Male), respectively. Produced and matting are being followed through (1 X 1) pure-line and crossing technique.

Induced breeding and fertilization of eggs

Male and female fish were selected for breeding purposes. Matured males were identified by a slightly pointed genital papilla, and females by a swollen abdomen and a reddish swollen vent. The maturity of the female was confirmed by a slight pressure on the ventral side of the fish for oozing of eggs. Male and female fishes were placed in separate tanks for about 6 hours with gentle shower of water to induce spawning. Both male and female fishes were artificially induced by intra-muscular injection with pituitary gland (PG) extract with a dose of 2mg/kg and 6mg/kg body weight, respectively. After injection, the male and female broodfish were placed in spawning tanks. After 8 hours of PG extract administration, when ovulation was found to be almost completed, the broods were removed from the spawning tanks and placed on a blanket for stripping. Male and female fishes were stripped by gentle pressure on the abdomen to collect the milt and eggs in a plastic bowl. Milt and eggs were mixed thoroughly by using clean and soft poultry feather to accomplish fertilization and placement in well-aerated freshwater.

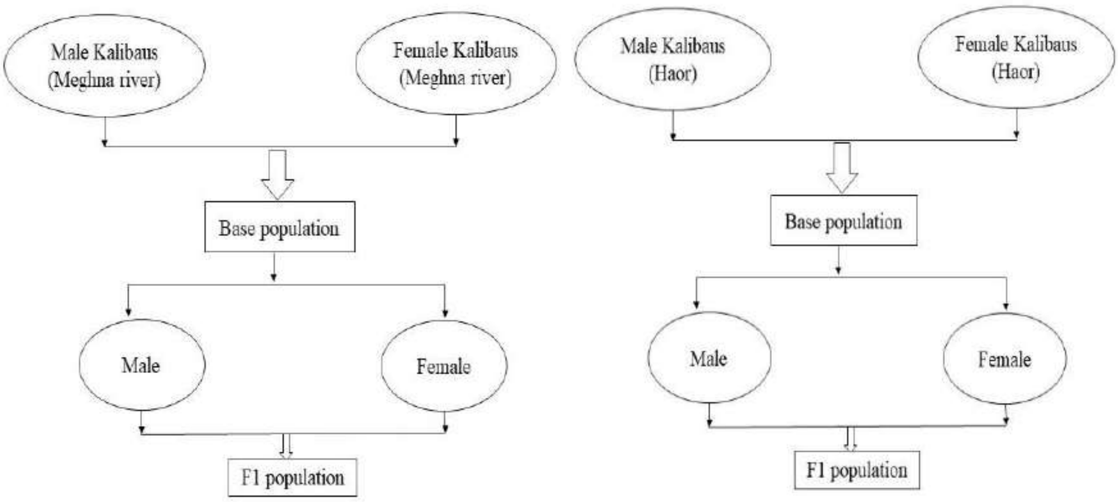


Fig.1: Schematic diagram for the production of F1 progeny (Pure-line) of Kalibaus

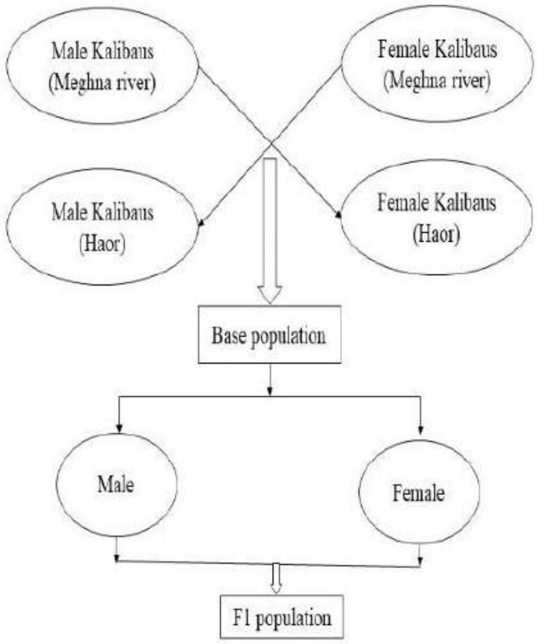


Fig. 2: Schematic diagram for the production of F1 progeny (Cross breed) of Kalibaus

From each group, 5000 fingerlings are being reared in rearing ponds and assuming 40% mortality therefore 3000 fingerlings are being available for stocking in grow-out ponds. For brood stock development, fingerling are being stocked at the rate of 10000 fingerlings/ha. During all phases of the growing period, the fish are being fed 25-28% protein rich feeds and at the age of 1.5 to 2 years at least 1000 to 1500 breeders are being ready for individual (mass) selection.

Table 1: Status of breeds of kalibaus

Sources	Length (cm)	Weight (g)	Number (Pairs)	Remarks
Meghna	40-60	800-1600	120	Would be used for breeding program
Haor	35-50	750-1300	150	

Fig 1. Breeds of kalibaus

Table 2: Status of F_1 base population.

Sources		Average Length (cm)	Average Weight (g)	Number fry
Group	Cross-breeds			
G_1	F_1 Male (M) × F_1 Female (M)	36.09±1.13	320.33±2.27	1000
G_2	F_1 Male (H) × F_1 Female (H)	29.15±1.28	269.42±2.13	1000
G_3	F_1 Male (M) × F_1 Female (H)	37.70±1.12	354.57±2.12	1000
G_4	F_1 Male (H) × F_1 Female (M)	32.42±0.91	289.52±2.22	1000

Fig 2. F_1 base population of kalibaus.

After 12 months rearing in grow-out ponds, best selected 20% fish from each separate stocks on the basis of individual growth performances and the fish are being transferred in to brood pond for further developments of broods. At the age of 2 years or more, randomly selected fish are being used for planned breeding for producing next generation's viz., F_2, F_3, F_4 and to be continued.

2. Evaluation of growth performance of cross breeds and pure lines of F_1 generation of kalibaus:

Experimental site:

The experiment was conducted at the pond complex in Freshwater Station, BFRI, Mymensingh over 150 days from January, 2023 to June, 2024. The trial was conducted in ponds. At first the pond was completely drained and all aquatic vegetation was manually removed. The pond was fenced with a synthetic net and surrounded by dykes for protection against unwater animals. Bottom racking was performed in order to remove toxic gases from the pond bottom. Diluted Lime ($CaCO_3$) was applied on the pond surface at the rate of 250 kgha^{-1}. The ponds were filled with water from deep tube-well at the pond complex one day prior to stocking. A number of four ponds were used which were further divided into three parts with the help of synthetic net in such a way that the area of each part was one decimal.

Experimental design:

The experiment was designed to evaluate the growth performance of four groups viz., Group-1 [F_1 Male (Meghna) × F_1 Female (Meghna)], Group-2 [F_1 Male (Haor) × F_1 Female (Haor)], Group-3 [F_1 Male (Meghna) × F_1 Female (Haor)], and Group-4 [F_1 Male (Haor) × F_1 Female (Meghna)] of F_1 kalibaus in captive condition. A number of 100 fingerlings were stocked in ponds with an area of one decimal. After stocking, fingerlings were fed 25-28% protein rich feed prepared with locally available ingredients (rice bran, wheat bran, fish meal, mustard oil cake etc.) at the rate of 10-3% body weight.

Table 3: Design of experiments to evaluate growth performance of F_1 kalibaus

	Groups of F_1 kalibaus			
	G-1	**G-2**	**G-3**	**G-4**
Descriptions	F_1 Male (Meghna) × F_1 Female (Meghna)	F_1 Male (Haor) × F_1 Female (Haor)	F_1 Male (Meghna) × F_1 Female (Haor)	F_1 Male (Haor) × F_1 Female (Meghna)
Stocking density (Fingerlings/decimal)	100			
Feeding (% protein)	25-28			
Feeding rate (%)	10-3			
Period of trial (day)	150			

*G indicates group

Growth monitoring and data collection:

Length and weight of 15 randomly selected fish from each experimental unit were measured using a digital balance and recorded fortnightly. At the end of the experiment, length and weight were measured to estimate the growth of F_1 kalibaus. The following formulas were used to calculate the growth of fish:

$$\text{Length gain (cm)} = \text{Mean final lenght} - \text{Mean initial length}$$

$$\text{Weight gain (g)} = \text{Mean final weight} - \text{Mean initial weight}$$

$$\text{Average daily weight gain} = \frac{\text{Mean final weight} - \text{Mean initial weight}}{T_2 - T_1}$$

$$\text{Specific growth rate (\%)} = \frac{\ln W_2 - \ln W_1}{T_2 - T_1} \times 100$$

Monitoring of water quality parameters:

Water quality parameters such as Temperature, Dissolved Oxygen (D. O.), pH, and Ammonia were measured using mercury thermometer (Saraan, Denmark), DO test kin (Biosol, AA Bioteck, India), pH test kit (CP-102, Thailand), and Total ammonia test kit (AQUA AM) respectively.

Results:

Growth performance:

Initial length and weight of fish did not differ significantly $(p > 0.05)$ among the four groups of F_1 kalibaus, whereas final length, final weight, length gain, weight gain, Average daily weight gain, and specific growth rate differ significantly $(p < 0.05)$ among the four groups of F_1 generations (Table 4). Group-3 [F_1 Male (Meghna) × F_1 Female (Haor)] showed highest weight gain (250.13±2.28[a]) g, length gain (16.34±1.20[a]) cm, average daily weight gain (1.66±0.02[a]), and specific growth rate (1.10±0.04[a]) followed by Group-4 [F_1 Male (Haor) × F_1 Female (Meghna)], group-1[F_1 Male (Meghna) × F_1 Female (Meghna)], and Group-2 [F_1 Male (Haor) × F_1 Female (Haor)] respectively (Table 4). Growth in fishes is not throughout the year and the fluctuations in the growth expressed it self on scales of the fish. The growth rate of fishes was not found in systematic order (increasing or decreasing order). The fluctuations in fish length indicate the fish growth, compensation. It is common in almost all natural stocks of fishes (fresh water, brackish water and marine water)

Table 4: Growth performance of F_1 generation of kalibaus (*L. calbasu*) over 150 days trial

Growth parameters	Groups of F_1 kalibaus			
	Group-1	**Group-2**	**Group-3**	**Group-4**
Mean initial length (cm)	20.11±0.43[a]	18.26±0.45[a]	21.16±0.40[a]	18.71±0.34[a]
Mean final length (cm)	36.05±1.12[b]	29.25±1.15[c]	37.50±1.16[a]	32.52±0.97[b]
Length gain (cm)	15.94±1.41[b]	10.99±1.21[c]	16.34±1.20[a]	13.82±0.94[b]
Mean initial weight (g)	97.80±0.61[a]	93.92±0.50[a]	104.67±0.48[a]	100.87±0.42[a]
Mean final weight (g)	320.33±2.27[b]	269.42±2.13[c]	354.54±2.12[a]	289.62±2.22[b]
Weight gain (g)	222.53±2.21[b]	175.50±2.54[c]	250.13±2.28[a]	188.25±2.33[b]
Average daily weight gain (g)	1.48±0.01[b]	1.17±0.02[c]	1.66±0.02[a]	1.25±0.02[b]
Specific growth rate (%bw/day)	0.98±0.04[b]	0.78±0.04[c]	1.10±0.04[a]	0.83±0.03[b]

[*]Values in the same row having different superscript letters indicate significant difference at $p < 0.05$

Water quality parameters:

Water quality parameters were monitored fortnightly. Several water quality parameters such as temperature ranged between 30.38±0.64 to 30.47±0.64℃, DO 6.09±0.36 to 6.31±0.22 ppm, pH ranged between ranged between 7.32±0.39 to 7.50±0.25, and ammonia ranged between 0.015±0.001 to 0.027±0.002 ppm in experimental units among the four groups of F_1

kalibaus (Table 5). Water quality parameters were not varied significantly $(p > 0.05)$ among the experimental units (Table 5). Water quality parameters in all experimental units were within the suitable range for fish growth.

Table 5: Water quality parameters of the experimental units

Parameters	Experimental units of four groups of kalibaus			
	Group-1	**Group-2**	**Group-3**	**Group-4**
Temperature (℃)	30.43 ± 0.80^{a}	30.47 ± 0.64^{a}	30.38 ± 0.64^{a}	30.44 ± 0.93^{a}
D. O. (ppm)	6.15 ± 0.20^{a}	6.21 ± 0.29^{a}	6.09 ± 0.36^{a}	6.31 ± 0.22^{a}
pH	7.32 ± 0.39^{a}	7.36 ± 0.33^{a}	7.50 ± 0.25^{a}	7.46 ± 0.31^{a}
Ammonia (ppm)	0.015 ± 0.002^{a}	0.015 ± 0.001^{a}	0.021 ± 0.003^{a}	0.027 ± 0.002^{a}

*Values in the same row having different superscript letters indicate significant difference at $p < 0.05$

3. Production and distribution of quality mass seeds of Indian major carps (Rui, Catla, Mrigal, Kalibaus), Chinese carps (Silver carp, Bighead carp), Silver Barbs (Rajpunti), and Catfsih (White pangas):

Keeping in line with production target, Carp hatchery of Freshwater Station running under the project produced about 557.800 kg of spawn comprising of Indian major carps, Chinese carps, Silver barbs, and White pangas (Table 6). Major contributions in total production of spawn came from the Indian major carps while kalibaus contributed least.

Table 6: Production of Improved Germplasm of Carps, Barbs, and Catfish

Species	Production		Breeding period
	Spawn (kg)	Fry/Fingerlings (Nos)	
BFRI Shuborno Rui	255.00	-	April-July
Catla	49.850	-	April-June
Mrigal	90.950	-	April-July
Kalibaus	15.625	-	April-July
Silver carp	3.000	-	March-July
Bighead carp	20.275	-	March-July
Silver barb (Rajpunti)	123.100	-	March-July
White pangas	7.000	-	April-July
Total	**557.800**	**-**	

The produced spawn was distributed and disseminated among the fish farmers, hatchery and nursery owners throughout the country. Along with spawn, a number of 30,000 fingerlings were produced which is used for further brood development or research purposes.

Development of YY GIFT Production using Marker-assisted Selection and Quality bi-sex Seed Production of GIFT Strain through Cohort Breeding

Researcher(s)

: Dr.Selina Yeasmine, SSO
: Dr. Mohammad Ashaf-ud-Doulah, SSO
: Md. Nahiduzzaman, SO
: Md. Moniruzzaman, SO

Objectives of the Project

i. to develop MAS-selected YY super-males of GIFT
ii. to produce of quality mass seed of GIFT strain using Rotational Breeding
iii. to produce improve brood stock of Pabda following cross breeding and mass selection protocol
iv. to produce quality seed of Pabda and distribute to the fish farmer and hatchery owners.

06. Achievements (2023-24)

Experiment 1 YY GIFT production using Marker-assisted selection

The following procedures were maintained for YY GIFT production

1. Pseudo female production

In the field complex of BFRI, FS, 9 large males and 9 large females from the brood stock population were selected from BFRI GIFT Tilapia. One male and one female (ready to spawn, showing pink to red and protruding genital papilla, fully opened genital pore) were then selected based on body size, body shape and reproductive status. One full-sib family (named GIFT-BFRI-F1) was developed by crossing these two individuals. In 9 hapas having 2.0 m^3 were set up in pond and single pair of mature GIFT tilapia was stocked. Fertilized eggs were collected and kept in the hatching jar for incubation. After yolk sac absorption, the 500 offspring of 9 full sib families transferred in 9 mini cisterns. Diethylstilbestrol (DES) hormone was used with feed @ 0.50g/kg and 1.0g/kg feed for fry feeding. The hormone treated feeds provided to the fry thrice a day up to 21 days. A total of 500 offspring of 3 full sib families each also transferred in another 03 cistern as control groups. After completed of hormone treatment, both groups of fry were transferred to hapas in pond condition. The swim-up fry were divided into three experimental groups of ~500 individuals and were reared in nursery cisterns. The fry from one tank were fed with normal food. Two different doses (0.5g and 1g per kg feed) of hormone Diethylstilbestrol

(DES) were used in food of GIFT fry to identify the optimum dose of Diethylstilbestrol (DES). The fry from the other two tanks (for sex-reversal treatment) were provided with feed treated with diethylstilbestrol (DES, Aladdin, China) at 0.5g and 1g per kg of feed. The DES were first dissolved with ethanol evaporation method (Mair and Santiago 1994) to prepare a stock solution, which were then thoroughly mixed with the feed. The dried pellets were provided to the fry twice a day from the first feeding stage up to three weeks. Each treatment had two replications. At 30 days, the fingerlings from each group were transferred to a pond (in hapa), respectively and were reared with the commercial pellets twice daily without hormone until sexed at the age of 120 days. At 120 days, the sex phenotype and body weight of each fish were recorded, and fin samples from each fish were also collected and kept in absolute ethanol for DNA extraction and genotyping.

Total 1500 fish from the three groups (Table1) were collected and sexually identified such as male or female fishes. In group one all individuals were female 77% and group two found 370 of the 500 individuals were phenotypically sex female. In contrast, the female rate was only 40% in the control group. In Treatment-1 (T_1) 82% female was produced where 1g/kg Diethylstilbestrol (DES) was used in feed and 74% female was produced in Treatment-2 (T_2) where 0.5mg/kg Diethylstilbestrol (DES) was applied (Table1). So the dose of 1g/kg Diethylstilbestrol (DES) was considered as the optimum dose where maximum female were produced. These female are called Pseudo females which are genetically male (XY). These females were used for the production of YY super male. Twenty five females of the treatment-1 were reared up to maturity and used as brood females in the next step. This hormone induced female brood stock contained both the sex reversed XY female and normal XX female. Variable growth rates of tilapia were found after trails (Table 2). Highest final weight gain (g) was found at T_1 (group 1). The survival rates (%) of fish were 82, 78, and 74 in T_1, T_2 and T_3 respectively (Table 2).

Table-1 Summary information on Sex Reversal of Tilapia after using Diethylstilbestrol (DES) hormone.

Group	DES (g)/feed (kg)	Individual number (n)	Female	Male	Intersex	Female rate (%)

T_1	1	500	410	90	0	82
T_2	0.5	500	370	120	10	74
Control (T_3)	N/A	500	230	270	0	54
	Total		1500			

Table 2 Growth Data and survival rate of Diethylstilbestrol (DES) hormone treated Tilapia in different treatments.

Sl no.	Treatment	Average Length (cm)	Average Weight (gm)	% of Survival
1.	T_1	24.68±0.91	256.9 ± 1.62	82
2.	T_2	20.01±0.66	198.6±1.78	78
3.	T_3	18.04±0.77	142.2±1.73	74

2. Identification/Confirmation of Pseudo female using microsatellite marker

i) Collection of Fin sample and isolation of genomic DNA

For pseudo female identification, approximately 30mg fin sample was collected from each of 10 hormone treated fry of Tilapia using sterile scissor (Figure-1). Collected fin sample were kept in absolute ethanol for DNA extraction and genotyping. The collected fin samples were cut into small pieces with a tissue grinder in a 1.5 ml eppendorf tube and then genomic DNA was extracted using extraction kit following ethanol precipitation method as described by (Islam and Alam, 2004).

Figure. 1 Fin sample collection for pseudo female identification

ii) Primer selection and PCR amplification

The Sex determination (SD) marker closely linked to sex trait located on chrLG23 (Chen *et al.*, 2019) (Forward primer: 5′-TCCCATTTAGACC ACCACACCTCAACAACA-3′; Reverse primer: 5′-GTCAGAAT GCACTTTAACACAGAGATACCA-3′) were used to genotype each individual. PCR amplifications were performed as per previously reported research (Gu et al., 2018) and were carried out in a 20-μL volume using 2× PCR master mix, 1 ng genomic DNA, and 0.5 μmol/L forward and reverse primers (Dongsheng, China) in a thermal cycler. The following program were applied (one cycle of 3min at 94°C, 38 cycles of 30 sat 94°C, 30sat 55°C and 30s at 72 °C, followed by a final extension of 5 min at 72 °C). When PCR was completed the PCR products were kept in a refrigerator (4^0c) for electrophoresis.

iii) Electrophoresis and visualization of PCR products

PCR products (6 μl) were confirmed by running through a 6% agarose gel containing 3μl ethidium bromide in 1×TBE buffer (Figure-2). DNA bands were observed under UV light on a transilluminator and photographed by a Gel Doc with digital camera. The resulting PCR products were detected by electrophoresis with 6% polyacrylamide gels and silver staining. Following confirmation of PCR amplification, 3μl of PCR product of each sample were electro-phoresed on a 6% denaturing polyacrylamide gel containing 19: 1 acrylamide: bis-acrylamide and 7M urea. The electrophoresis was accomplished by using the SequiGen GT sequencing gel electrophoresis system in Laboratories. After pre-heating, the PCR products were loaded between the teeth of the comb, and the DNA ladder was loaded on either side of the gel. The gel was run with the power set at 60 W and temperature set at 50°C for required length of time (1h 20 min) according to the size of the DNA fragment. After completion of electrophoresis, the gel was stained with silver nitrate following Promega (Madison, WI) silver staining protocols for visualization of the DNA fragments. The electrophoretic bands corresponding to particular allele at each locus were identified (Figure-3). The pseudo females (XY genotype), XX females and XY males were identified based on phenotypic sex traits and genotypic data.

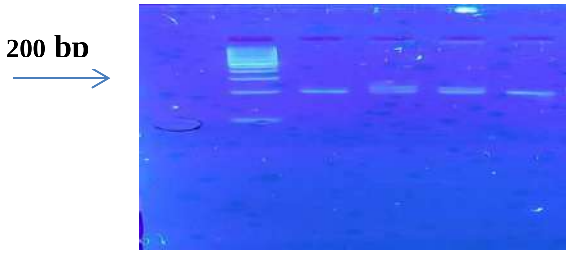


Figure-2 PCR amplification of T_1 and T_2 (M-Mother, F-Father, T1 & T2- Offspring) 1Kb: DNA ladder

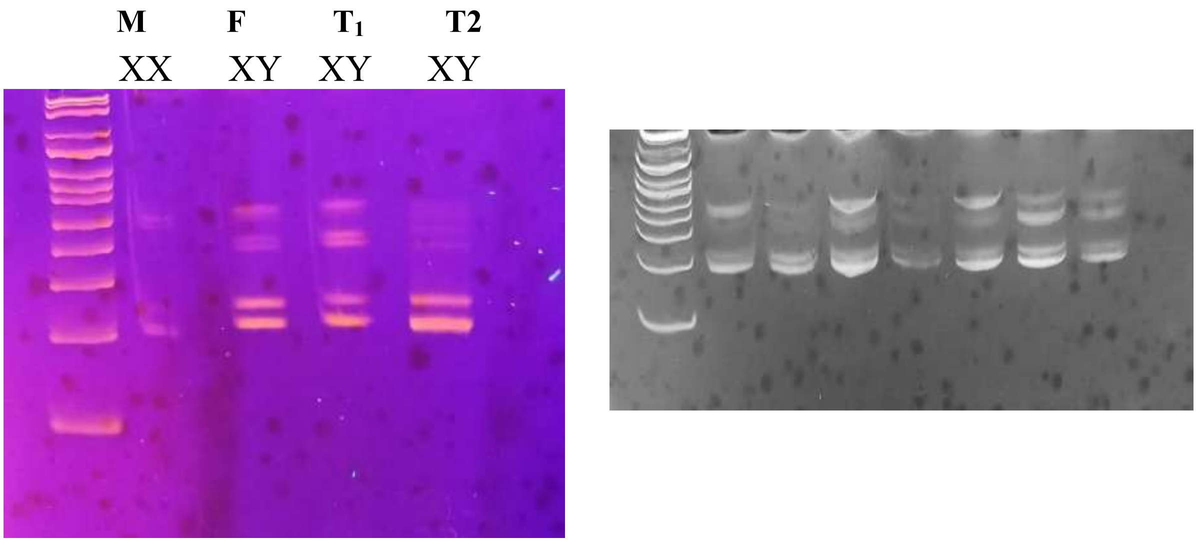


Figure 3 Genotyping of individuals during marker-assisted selection using the sex-linked marker SD (M-Mother, F-Father, T_1 & T_2- Offspring)

3. Supermale production through Cross breeding of Pseudo females (XY) with genetic male Tilapia (XY)

F-2 families were produced by crossing pseudo females (XY genotype) with genetic males (XY genotype). Three hapas having 2.0 m^3 were set up in breeding pond. Then single pair of Pseudo females (XY) tilapia with genetic male Tilapia (XY) was stocked in each hapa (Figure-4). After 12 days, fertilized eggs were collected and kept in the hatching jar for incubation. After yolk sac absorption, the offspring were transferred in mini cisterns. Fry were fed with normal nursery feed. After 21 days the mean weight of fry is 9.0g. After 120 days, the sex of each fish was recorded, and fin samples were collected and kept in absolute ethanol for DNA extraction and genotyping. The supermales (YY genotype) were identified based on phenotypic sex trait and confirmed through genotyping.

Breeding Hapa **Pseudo females (XY) and genetic male (XY**

Swim-up fry rearing **Offspring**

Figure. 4 Cross breeding of Pseudo females (XY) with genetic male Tilapia (XY)

Experiment 2. Cohort breeding program of GIFT strain for quality seed production

A total of 400 mature GIFT of F-13 strain were stocked in each pond for cohort breeding. The fish were fed with floating feed containing 28-30% crude protein at the rate of 4-8% on the basis of body weight after stocking. After that, 300 male and 100 females were selected from each pond for breeding.

Experiment 3: Production of F_1 base population of Pabda (*Ompok pabda*) through mass selection from wild sources

Collection and Domestication:

A total of 275 fingerlings of Pabda (*Ompok pabda*) were collected from natural sources and stocked in a previously prepared pond and reared the fishes with applying floating feed containing 28-30% crude protein at the rate of 4-8% on the basis of their body weight.

Molecular characterization of infectious diseases in commercially important fishes and development of their vaccines

Researcher(s):

: Dr. Md. Shirajum Monir, SSO
Farjana Jannat Akhi, SO

Objectives of the Project

i. To isolate and identify hypervirulent bacteria responsible for mass mortality of commercially cultured fishes.

ii. To develop feed-based inactivated whole-cells vaccines with the local isolated bacteria against the emerging fish diseases.

iii. To formulate the vaccination regimen and evaluate the efficacy of the newly developed feed-based whole-cells vaccines.

Achievements (July 2023 - June 2024):

a. Preparation of formalin inactivated whole-cells bacterial vaccines

The pathogenic bacterial strains of *Aeromonas hydrophila* and *A. veronii* were used to develop inactivated whole-cells vaccines for Pangasius. Briefly, the selected bacterial stocks of *Aeromonas hydrophila* and *A. veroni* were grown individually on TSA plates for overnight at 28 to 30 ^{o}C. Then, 10 colonies from each cultured bacteria were inoculated into two separate flasks of 500 mL containing brain heart infusion broth and cultured in an incubator at 130 rpm for 24 h at 30 ^{o}C. After incubation, 10-fold serial dilution and colony counts were utilized to determine each of the bacterial concentrations by following the standard method. The *A. hydrophila* and *A. veronii* bacterial cells were re-suspended separately in sterile PBS solution to keep the final bacterial concentration of 2.4×10^{9} CFU/mL. Each of the propagated bacteria was then inactivated through adding around 0.5% formalin and left at 4 ^{o}C for overnight. Afterwards, the inactivated bacteria were harvested by using a refrigerated centrifuge at 6000× *g* for 14 min and washed with sterile phosphate buffered saline (PBS). After that, the vaccine was prepared by combining both the inactivated whole-cells (FICs) of the monovalent *A. hydrophila* and *A. veronii* vaccines at a ratio of 1:1 and finally by adding of Freund's complete adjuvant (FCA).

Fig. 1: Inactivated whole-cells pellets of *Aeromonas* sp. after centrifuged and formulated vaccine with Freund's complete adjuvant (FCA).

b. Quality and safety tests of the developed vaccines

Prior to vaccination trials, the sterility and safety of the prepared vaccines were verified following the standard methods (Table 1). There was no indicative signs of diseases after vaccination in laboratory trials, which indicates the prepared vaccines are safe for immunization of Pangasius.

Table 1. Sterility & viability tests of prepared whole cells vaccine

Culture Media	**Incubation periods**		
	24 hours	48 hours	72 hours
TSA (Tryptic Soy Agar)	-	-	-
BHIA (Brain Heart Infussion Agar)	-	-	-
TSB (Tryptic Soy Broth)	-	-	-

Remarks : (-) negative as no contamination/none bacterial growth

c. Immunization of brood stock with inactivated vaccine and breeding

Before vaccination and blood sampling, the selected male and female Pangasius brood fish were anaesthetized. The brood fish were vaccinated by interperioneal (i.p.) injection with 0.4 mL/kg fish (2.4×10^9 CFU/mL). Besides on, the phosphate-buffered saline (PBS) group was a control group where the brood fish were injected with only PBS. All fish were clinically healthy after receiving the vaccines. The immunized male and female brood fish were allowed to breed in hatchery after one month vaccination. The larvae samples at 5, 10, 15, and 20 days after hatching were collected from the immunized and non-immunized groups.

Fig. 2: Immunization of brood Pangasius with inactivated whole-cells vaccines in Freshwater Station hatchery, BFRI and produced immunized fingerlings

d. Preparation of feed-based vaccines

The commercial pellets feed was ground with a blender to make feed powder. Then, the whole cells inactivated bacterin (WIB) with palm oil was suspended thoroughly in sterile PBS solution to achieve the concentration of 2.4×10^9 CFU/mL of each WIB at 1 L until a homogenous solution for preparing the monovalent or bivalent incorporate vaccine. After that, the prepared monovalent or bivalent vaccine was independently sprayed directly onto the 1 kg feed powder and finally, the individual WIB concentrations were obtained at 2.4×10^9 cells/g of feed. A feed mixer machine was used to equally disseminate and impregnate the monovalent or bivalent vaccine thoroughly onto the fish feed powder. In contrast, for the control group, only PBS with 10% palm oil was mixed onto the feed powder for preparing the unvaccinated fish feed. Finally, the vaccine mixed feed powder was loaded into a mini feed pellet machine to prepare the re-pellet feed, and the pellets were dried over night at 28 ^oC in a dry air oven. Then, both the monovalent and bivalent vaccinated feed pellets were left at 4 ^oC until use.

Fig. 3: Feed-based inactivated whole-cells oil-based vaccine preparation

e. Indirect ELISA for detection of serum and mucus antigen-specific IgM

The serum (Fig. 4a) and mucus (Fig. 4b) IgM levels of fish against *A. hydrophila* were measured by using indirect ELISA from weeks 0 - 9 post-vaccination. Following feed-based vaccination, all vaccinated groups showed significantly ($P < 0.05$) increasing IgM levels at week 1, as compared to the control group. After the single booster on week 2, the IgM levels for all immunized groups began to rise again and reached peak values at week 4, whereas only IgM levels responses of serum and mucus against *A. hydrophila* peaked in all vaccinated group up to 8 week.

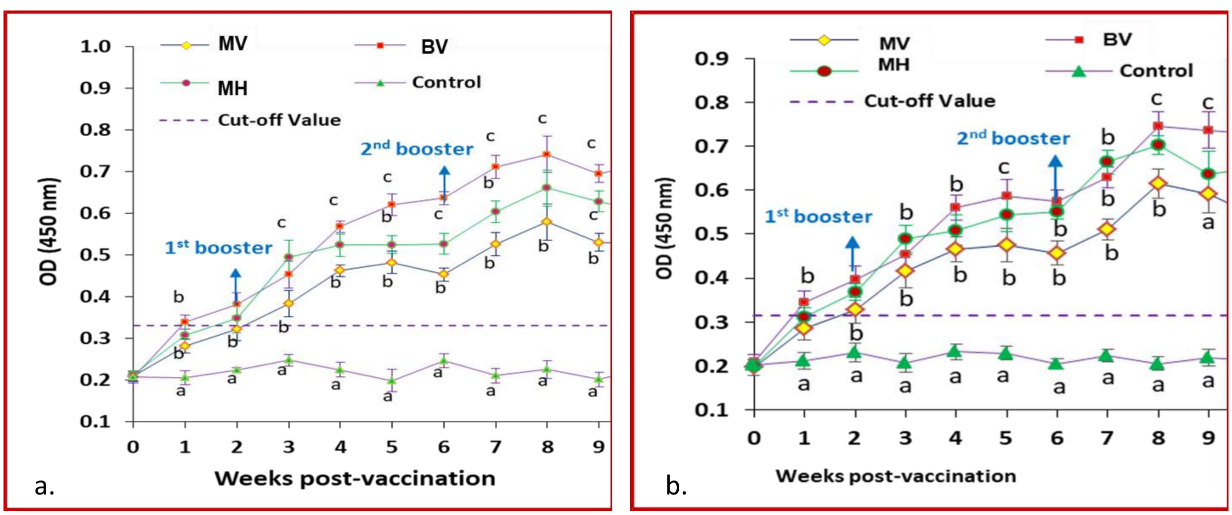


Fig. 4: Antibody titer of specific IgM in serum (a) & mucus (b) against *A. hydrophila* in Pangasius following feed-based vaccination

f. Indirect ELISA for detection of serum antigen-specific IgM after active (*i.p.*) vaccination

The IgM levels of the serum against *A. hydrophila* (Fig. 5) in vaccinated group was found to increase from the post-vaccination of week 1, as compared to the control group. After week 1, the levels of IgM in vaccinated group was enhanced until week 3, but declined gradually on week 4.

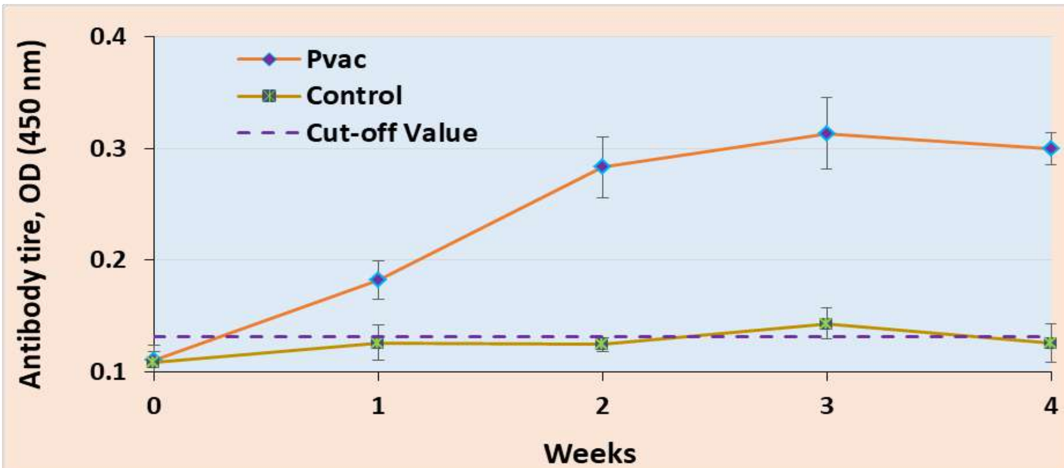


Fig. 5: Antibody titer of specific IgM in serum against *Aeromonas hydrophila* in brood Pangasius following i.p. vaccination

Development of Breeding Technique of Snakehead Fish

Researcher(s):

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: Maliha Khanom, SO
: Md. Saiful Islam, SO
: Nazia Nahin Nisheeth, SO

Objectives of the Project (2023-24):

- ✓ Effects of different stocking density on nursery performance of Striped Snakehead *Channa striata*
- ✓ Effects of different stocking density on growth and production performance of *Channa striata*

Achievements (2023-2024):
The following research works were carried out under the project from July 2023 to June 2024.

Experiment 1. Effects of different stocking density on nursery performance of Striped Snakehead *Channa striata*

The experiment was performed using the concrete tank in Freshwater Station, BFRI from September 2023 to November 2023.

Experimental design
The present experiment was conducted with three treatments namely T_1, T_2, and T_3 and each with three replications (Table 1). The experimental designs are given below:

Table 1. Experimental design for the nursery performance of *C. striata*

Treatments	Stocking size (cm)	Stocking density (nos./m^3)	Feed
T_1	0.01-0.02	300	Commercial feed (P: 50-43%) (50%) and live feed (50%) was supplied at 50-10% BW
T_2		450	
T_3		600	

Tank preparation
Tanks were prepared by draining out the water and it was filled up with water. The water depth was maintained to a maximum of 1.0m. To maintain water quality, tank water was changed at regular intervals using water from a deep tube-well supply.

Stocking
Required no. of fry was produced through induced breeding of fish. Fry were stocked 300, 450 and 600 in T_1, T_2 and T_3 treatments respectively. The mean initial length and weight of fry was taken before stocking in the tanks.

Feed and feeding
Commercial feed (P: 50-43%) (50%) and live feed (50%) was supplied at the rate of 50-10% BW thrice daily.
Growth sampling of fish
Fish were sampled every 15 days at intervals in the morning. The length and weight were

recorded by random sampling from each tank.

Fish harvesting

Harvesting was done in November 2023 by dewatering after the completion of the experiment.

Water quality monitoring

The Physicochemical parameters of the tank's water were monitored weekly and data recorded.

Analysis of experimental data

Growth parameters were recorded and analyzed such as length and weight gain, survival, Specific Growth Rate (SGR) and FCR of fish. The procedure of calculation is as follows:

- Length gain (cm) = Average final length (cm) – Average initial length (cm)
- Weight gain (g) = Mean final weight (g) − Mean initial weight (g)
- $\text{SGR } (\%\text{bw/d}) = \frac{(\ln \text{final weight} - \ln \text{initial weight})}{\text{culture period in days}} \times 100$
- Survival rate (%) = (No. of fish harvested/ No. of fish stocked) x 100

Results

Following 90 days of nursing, the fish were harvested. In the current experiment, stocking density had a direct impact on the final weight, survival, and FCR. The average initial weight (g) and length (cm) of *C. striata* fry were more or less same in all treatments (Figure. 1). The mean final lengths and weights of fry were 12.26±1.13 cm and 14.26±1.33 in T_1, 11.70±1.28 cm and 12.68±1.40 g in T_2, and 11.66±1.23 cm and 12.47±1.19 g in T_3, respectively. The ANOVA test results revealed that, in comparison to the T_2 and T_3 treatments, the fry from the T_1 treatment grew more rapidly and gained more weight. Among treatments, there was a significant difference ($p<0.05$) in the SGR. Mean SGR for T_1, T_2, and T_3 were 8.07±0.06, 7.94±0.08 and 7.92±0.09, respectively (Table 2). After 90 days of the trial period, it was noticed that the survival rates in T_1, T_2 and T_3 were 76.88±5.15, 66.22±4.42 and 63.87±4.59 respectively (Table 2). The survival rates varied significantly among the treatments ($p<0.05$). Low stocking density was more effective at bolstering survival rates. At the end of the experiment, it was found that the FCR in T_1, T_2 and T_3 was 3.52±0.09, 3.69±0.07 and 3.78±0.08, respectively (Table 2). The FCR varied significantly among the treatments ($p<0.05$).

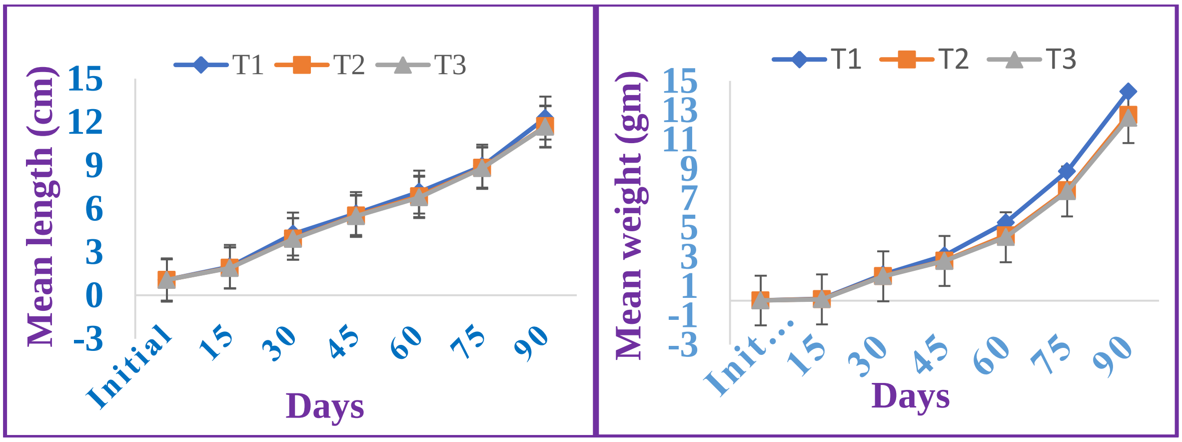

Figure 1. Bi-weekly variation in A) Mean length (cm) and B) Mean weight (g) of *C. striata*

Table 2. Growth performance, survival (mean±SE) and FCR of *C. striata* fry in different stocking density

Parameters	Treatments		
	T_1	T_2	T_3
Initial weight (g)	0.01±0.001[a]	0.01±0.002[a]	0.01±0.002[a]
Initial length (cm)	1.06±0.04[a]	1.06±0.05[a]	1.09±0.04[a]
Final weight (g)	14.26±1.33[a]	12.68±1.40[b]	12.47±1.19[b]
Final length (cm)	12.26±1.13[a]	11.70±1.28[b]	11.66±1.23[b]
ADWG (g)/day	0.15±0.004[a]	0.14±0.003[b]	0.14±0.004[b]
Weight gain	14.25±1.02[a]	12.67±1.11[b]	12.46±0.98[b]
SGR (%/day)	8.07±0.06[a]	7.94±0.08[b]	7.92±0.09[b]
FCR	3.52±0.09[c]	3.69±0.07[b]	3.78±0.08[a]
Survival (%)	76.88±5.15[a]	66.22±4.42[b]	63.87±4.59[b]

Water parameters such as temperature, pH, DO, ammonia, and total alkalinity were measured at biweekly intervals. Table (3) shows the results of the water quality parameters. The water quality parameters were found to be more or less similar and all of the parameters were within the suitable ranges for fish culture. Water temperature and total alkalinity did not differ significantly ($p>0.05$) among treatments (Table 3). However, significant variations ($p< 0.05$) in pH, DO, and NH_3 among the treatments were found.

Table 3. Mean values of the water parameters during the experiment (Sep-Nov 2023)

Parameters	Treatments		
	T_1	T_2	T_3
Water temp. (°C)	27.13±1.53[a]	27.09±1.33[a]	27.11±1.18[a]
pH	7.79±0.16[a]	7.67±0.11[b]	7.82±0.14[a]
DO (mg/L)	5.29±0.39[b]	5.18±.47[c]	5.32±.51[b]
Alkalinity (mg/L)	117.40±5.8[a]	116.70±4.52[a]	116.90±4.7[a]
NH_3 (mg/L)	0.019±.008[a]	0.017±0.005[c]	0.020±0.007[b]

Experiment 2. Effects of different stocking density on growth and production performance of *Channa striata*

The experiment was conducted at Freshwater Station of Bangladesh Fisheries Research Institute (BFRI) from February to June 2024.

Experimental design

The present experiment was conducted with three treatments namely T_1 (50), T_2 (75), and T_3 (100) and each having three replications (Table 4). The experimental designs are given below:

Table 4. Experimental design for growth performance of *Channa striata*

Treatments	Stocking size (cm)	Stocking density (nos./dec)	Feed
T_1	15-20	50	Commercial feed (P: 45-40%) 50%, trash fish and live feed 50% are being supplied at 10-03% BW twice daily.
T_2		75	
T_3		100	

Pond preparation

The ponds were prepared by draining out the water and exposed to sunlight for about 2 weeks. Lime was applied at the rate of 250 kg/ha. Two days after liming, the ponds were filled up with water. The water depth was maintained to a maximum of 1.2m using inlet and out let system in each pond. The research ponds were fenced by nylon net with the bamboo sticks to make more replicable ponds as shortages of the pond's facilities. Urea (50 kg/ha) and TSP (50 kg/ha) were applied uniformly into the ponds after 5 days of liming. To maintain water quality, the pond water was changed at regular intervals using water from a deep tube-well supply.

Stocking of fish

Fish was stocked 50, 75, and 100 nos per decimal which was considered as T_1, T_2 and T_3, respectively. Stocking was done when the water temperature remained low. The initial average length and weight of fish was taken before stocking.

Feed preparation and feeding

Commercial feed (P: 45-40%) 50%, trash fish and live feed 50% were supplied @ 10-03% BW twice daily.

Sampling

Fish was sampled every 15 days at intervals in the morning. The length and weight was recorded by random sampling.

Harvesting

Harvesting was done in June 2024 by dewatering after the completion of the experiment.

Water quality monitoring

The Physicochemical parameters of water such as water temperature, pH, dissolved oxygen, total alkalinity and ammonia were monitored weekly and data recorded.

Analysis of experimental data

Growth parameters were recorded and analyzed such as length and weight gain, survival, Specific Growth Rate (SGR) and FCR of fish.

Result

Growth performances and production

Throughout the study period, the following fish parameters were recorded: mean initial weight, final weight, weight gain, specific growth rate (SGR%/day), survival rate, and FCR. These data are compiled in Table 5. Initial fish weight and length wasn't different significantly ($p>0.05$) among the treatments, but final fish weight, weight gain, SGR, and FCR significantly varied on different stocking density (Figure 2). The mean initial length (cm) and weight (g) were 15.46±1.42 and 27.95±2.44, 15.39±1.55 and 27.87±2.37, and 15.44±1.49 and 27.91±2.51 in T_1, T_2 and T_3, respectively. Both length and weight showed noticeable variations ($p<0.05$) after 150 days of stocking of fish. The T_1 treatment showed faster growth rates compared with T_2 and T_3. The value of weight gain (%) in T_1 (1404.70±8.57) was significantly higher ($p<0.05$) than T_2 (1247.21±9.98) and T_3 (1120.97±11.41) due to higher stocking density in T_2 and T_3. Specific growth rate (SGR %/day) in T_1 (1.81±0.03) was significantly higher ($p<0.05$) than T_2 (1.73±0.05) and T_3 (1.63±0.06). The highest SGR (%/day) value was observed in T_1 on the other hand lowest was observed in T_3. The survival rate (%) of *C. striata* in T_1 (79.0±1.82) was significantly higher ($p<0.05$) where the stocking density was lower than T_2 (68.5±1.99) and T_3 (63.5±2.79) but no significant variation ($p>0.05$) between T_2 and T_3.

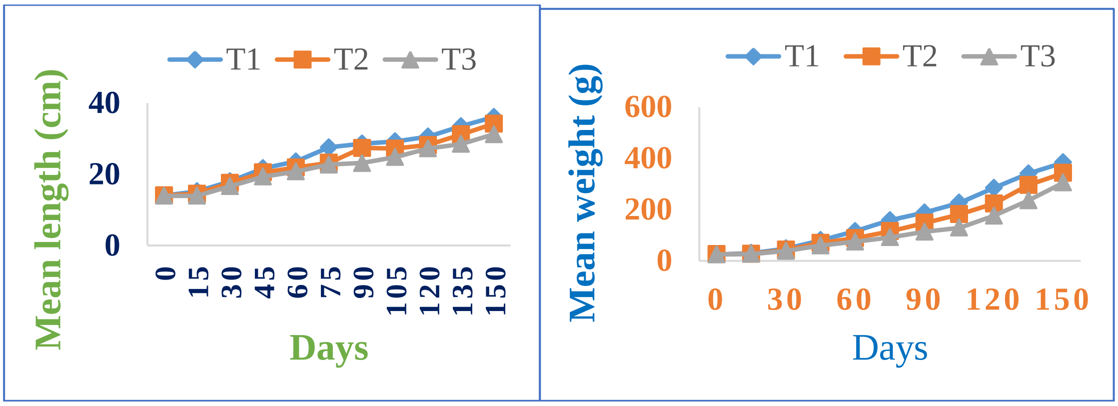

Figure 2. Bi-weekly variation in A) Mean length (cm) and B) Mean weight (g) of *C. striata* under different stocking density

The production of *C. striata* in T_3 varied significantly higher ($p<0.05$) than T_1 and T_2. Fish production of *C. striata* were found higher in T_3 followed by T_2 and T_1 where the stocking density of *C. striata* was 8000 piece/6000L water in tank (Table 5).

Table 5. Growth performance, survival rate and FCR of C. striata under different stocking density

Parameters	T_1	T_2	T_3
Initial weight (g)	25.52±3.68^{a}	25.46±3.71^{a}	24.48±3.36^{a}
Initial length (cm)	14.07±0.82^{a}	14.03±0.78^{a}	13.97±0.77^{a}
Final weight (g)	384.29±22.48^{a}	343.60±32.42^{b}	305.7±38.44^{c}
Final length (cm)	36.1±2.41^{a}	34.2±2.30^{b}	31.26±1.42^{c}
Weight gain (%)	1404.70±8.57^{a}	1247.21±9.98^{b}	1120.97±11.41^{c}
Daily growth rate (%)	2.38±0.04^{a}	2.12±0.06^{b}	1.87±0.05^{c}
SGR (%/day)	1.81±0.03^{a}	1.73±0.05^{b}	1.63±0.06^{c}
Survival rate (%)	79.0±1.82^{a}	68.5±1.99^{b}	63.5±2.79^{b}
FCR	2.13±0.08^{c}	2.43±0.11^{b}	2.58±0.14^{a}
Production/dec.(kg)	15.18±0.28^{c}	17.67±0.39^{b}	19.41±0.48^{c}

The mean values of the physico-chemical parameters that were measured in the experimental tanks are listed in Table 6. All of the water quality measures were determined to be within ranges that are appropriate for fish culture, and they were found to be quite similar. There was no significant variation ($P>0.05$) in the water quality parameters of water temperature and NH_3 throughout the treatments; however, there were significant differences ($P<0.05$) in the parameters of pH, dissolved oxygen, and total alkalinity among the treatments (Table 6).

Table 6. Water quality parameters of the experimental tanks (February-March 2024).

Parameters	Treatments		
	T_1	**T_2**	**T_3**
W. temperature (°C)	28.43±2.53[a]	28.52±2.42[a]	28.47±2.33[a]
pH	7.80±0.14[a]	7.69±0.11[b]	7.78±0.16[a]
DO (mg/L)	5.29±0.39[a]	5.18±0.47[b]	5.31±0.51[a]
Alkalinity (mg/L)	118.40±5.8[a]	117.70±4.52[a]	116.90±4.7[a]
NH_3 (mg/L)	0.021±.008[a]	0.019±0.005[a]	0.02±0.007[a]

Genetic improvement of major carps (Rohu and Catla) and DNA-barcoding of Fisheries resources in Bangladesh

Researchers

: Dr. Jonaira Rashid, SSO

: Md. Amdadul Haque, SO

Objectives of the project

i. To improve Rohu and Catla stocks through selective breeding and justify with molecular techniques
ii. To identify fisheries resources at the species level based on DNA barcoding data

Achievements:

Expt. 1 Communal rearing of 'BFRI Subarno Rui' (5^{th} generation) at grow-out pond

A total of 30 fingerlings from each of the selected 60 families (total 1800 fishes) were stocked in communal grow out pond. Fishes were fed with locally available commercial feed containing about 35% protein at the rate of 3-5% body weight daily. Thus, an average of 1800 fish (#30 from 60 families) are being reared communally in rearing pond to raise brood fishes following all scientific management measures. Growth performances of F_5 Rohu during communal rearing are shown in table 1. The results of the present study on growth performances in terms of mean length and mean weight gain of F_5 Rohu are 34.57±1.42 (cm) and 506.25±49.92 (g) respectively.

Table 1. Growth performance of BFRI F4 improved generation of Rohu in communal grow out pond

Parameters	Initial status (November 2023)	Present status (June 2024)
Length (cm)	18.50±1.61	34.57±1.42
Weight (g)	41.02±11.30	506.25±49.92

Expt. 2 Evaluation of growth performance of BFRI Shubarno Rui (F_5) at on station management

BFRI F_5 generation of Rohu (*L. rohita)* was produced from the F_4 generation on the basis of family selection protocol. On the other hand, a non-selected control group of F_5 generation of Rohu (*L. rohita)* was produced to compare growth performances between them. To conduct this experiment, an on-station trial was carried out for a period of five months. Upgraded stocks of Selected F_5 generation of Rohu (*L. rohita)* were stocked in Treatment-I while, non-selected control group of Rohu (*L. rohita)* were stocked in Treatment-II. After stocking, commonly available supplementary feed was applied. Fishes were sampled at monthly interval to assess growth performances and to adjust feeding ration. Water quality parameters such as temperature, DO, pH, alkalinity, ammonia and transparency of the experimental ponds were monitored fortnightly. After 5 months of rearing, the fish will be harvested and data were analyzed through one way ANOVA Table 2.

Table 2. Growth performance of Improved BFRI Rohu (F_5) after five months of rearing

Treatment	Final weight (g)	Survival (%)	Production (Kg/ha)
Treatment-I BFRI Rohu (F_5)	401.63±38.53*	95	574.30±38.25*
Treatment-II Control	321.45±41.78	92	446.80±32.53

*Significant at 0.005% level

Expt. 3 Genetic variability study of Rohu and Catla stocks using DNA markers

To conduct this experiment fish samples were collected from the BFRI Improved Rohu and the wild source Rohu. The hatchery samples were collected from the local carp hatchery, Mymensingh. To perform DNA fingerprinting for genetic characterization, a total of 30 fish samples were taken randomly from each stock. In addition, Catla fish samples were collected from wild sources (Halda & Jamuna) during September-October, 2023. A total number of 30 fish samples were taken randomly from each stock. Genomic DNA was isolated using a commercially available kit. To analyze the genetic variability of Rohu and Catla stock. PCR reactions were performed on each DNA in a 10 µl reaction mix containing 1 µl of 10× *Taq* polymerase buffer, 0.2µl of each primer, 1 µl of dNTPs, and 1 unit of *Taq* DNA polymerase and 150 ng µl of genomic DNA. The PCR products were stored at -18°C. Electrophoresis will be performed at 100 V for 35 mins with 1.5% agarose where a clear DNA band was found (Fig. 1).

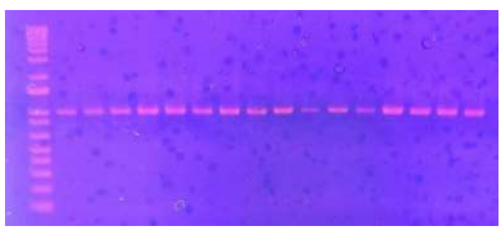

Fig.1 Gel electrophoresis of PCR amplified product. Ladder 1 kb+.

Expt. 4 Development of Base population of Catla broodstocks for genetic improvement

The stock improvement of Catla fishes was conducted using wild stocks. The collected wild stocks (The River Halda and Jamuna stocks) were reared in earthen ponds with an average depth of 1 m located in the pond complex of Freshwater Station, BFRI, following all scientific management practices, including fertilization, liming, supplementary feeding, and water management. Fishes were fed with supplementary feed containing 28-30% protein at 5-3% body weight daily. For stock improvement, collected Catla fishes from the River Halda and the River Jamuna were stocked in a pond having an area of 50 decimal separately. The stocked fishes are being fed with commercially available carp feed at the rate of 5-3% body weight once daily. The results of the present study on growth performances in terms of mean length and mean weight gain of Catla (Halda origin) 61.18±5.12 (cm); 3.43±2.37 kg and the River Jamuna origin are 55.80±3.14 (cm); 2.88±3.14 kg, respectively.

We have designated the interstock crosses to produce the base population (fig. 2) and the groups were acronymed as H♂ X J♀, Ha ♀X H♂, J♂ X H♀, J ♂ X Ha♀, Ha♂X H♀, and Ha♂ X J♀ (table 3).

Table 3. Di allele cross to produce the base population

Male	**Female**		
	H	**J**	**Ha**
H	**-**	**x**	**x**
J	**x**	**-**	**x**
Ha	**x**	**x**	**-**

a b

Fig. 2 Breeding program to develop base population of Catla a) egg collection b) hapa nursing

Growth performances of different groups of Catla base popoulations are shown in Table 4. After one month of nursing, the highest growth was found in group 1(H♂ X J♀) and the lowest was found in group 4 (J ♂ X Ha♀).

Table 4. Present status of Catla base population after one month of hapa nursing

Group	**Present status (June 2024)**	
	Length (cm)	**Weight (g)**
1 (H♂ X J♀)	2.44±0.73	0.09±0.06
2 (Ha ♀X H♂)	2.01±0.26	0.06±0.03
3(J♂ X H♀)	2.02±0.35	0.06±0.01
4(J ♂ X Ha♀)	1.02±0.80	0.04±0.03
5(Ha♂X H♀)	2.37±0.87	0.07±0.04
6(Ha♂ X J♀)	2.21±0.17	0.08±0.02

Expt. 5 Identification and characterization of fisheries resources based on DNA barcoding data

A total of 25 freshwater fish samples and 10 samples of horseshoe crab were collected from different regions of Bangladesh for DNA barcoding. COI gene was successfully amplified using Fish F1 and Fish R1 primer (Fig.3). The PCR products were purified using a PureLink™ PCR purification kit for sequencing and data analysis. thermal cycling conditions: initial denaturation at 95 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 35 sec, primer annealing at 52 °C for 30 sec and primer extension at 72 °C for 40 sec, and final extension for 5 min at 72 °C. Amplified products of COI was separated on 1.5% agarose gel, stained with Ethidium bromide, followed by gel elution using a spin column-based gel extraction kit per the manufacturer's instructions.

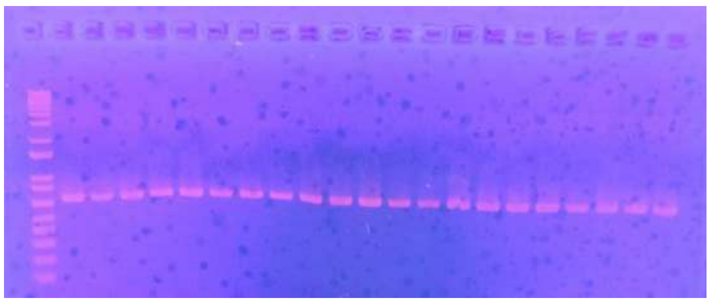

Fig.3 Gel image of PCR amplified product. Ladder 1 kb+

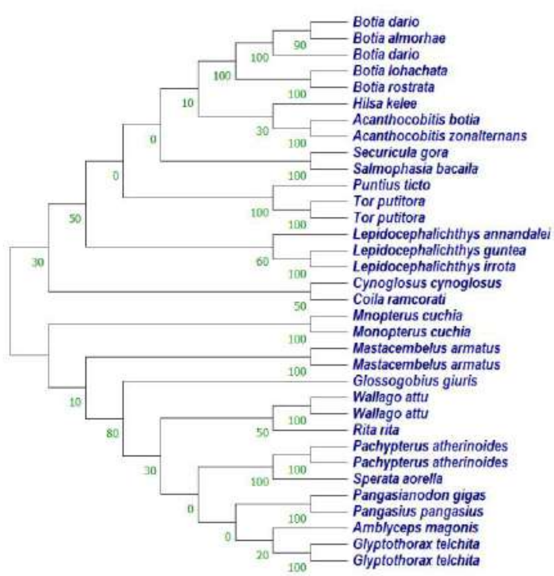

Fig.4 Phylogenetic tree of identified fish species

A total of 25 threatened freshwater fish species were identified and phylogenetic tree was reconstructed to reveal the phylogenetic position of the fishes (Fig.4). Two species of horseshoe crab *Tachypleus gigas* and *Carcinoscorpius rotundicauda* were identified and maximum likelihood phylogenetic tree was reconstructed (Fig.5) and two distinct population of *Carcinoscorpius rotundicauda* was identified from Cox-bazar and Sundarban.

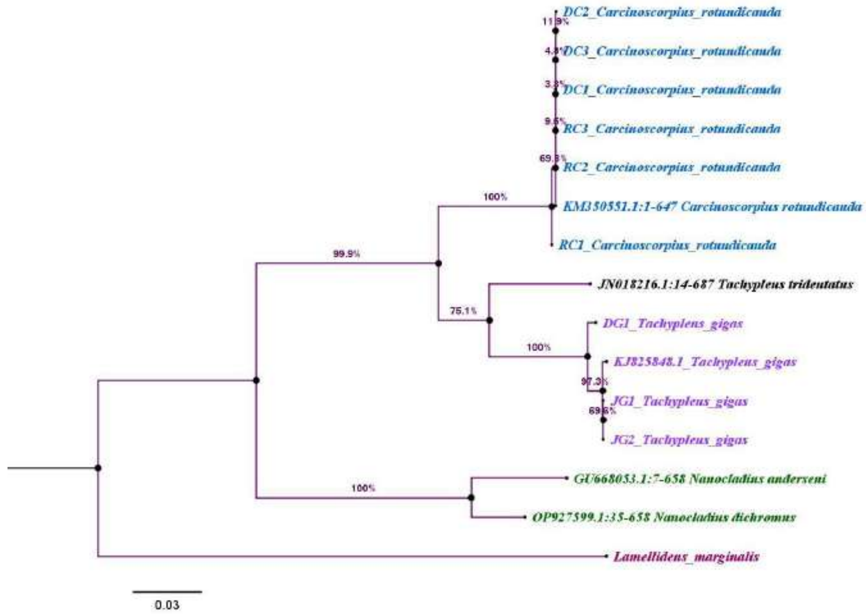

Fig.5 Maximum likelihood phylogenetic tree of Horseshoe crab

Breeding biology of commercially important freshwater mollusk and development of culture technique with fish

Researcher(s):

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Objectives of the Project

i. To investigate breeding biology of commercially important mollusk (mussel and snail) available in Bangladesh
ii. To develop breeding technique and culture system of mollusk with fish in confined condition and pond ecosystem

06. Achievements (2021–2022):

Exp. 1: Culture technique development of pond snail (*Viviparus bengalensis*)

This experiment was set to determine the culture technique of freshwater pond snail (*Viviparus bengalensis*). One control was taken with five different treatments with different stocking density of pond snail. Pond was prepared following the standard procedure (drying, liming and fertilization). After pond preparation pond snail and multi-species of fish were stocked at the last week of March, 2024. Fish are feeding with commercial feed at the rate of initially 10% and down to 3% of the total biomass of the fish twice daily. Organic and inorganic fertilizers are applying fortnightly at the rate of 0.5 kg, 0.1 kg T.S.P and 0.1 kg urea per decimal. Lime also applying fortnightly at the rate of 0.5 kg/decimal. Growth and survival of the snail and fishes are observing on monthly basis and at the end of the experiment data will be compiled and analyzed.

Result:

Table 1: Average present weight of fishes in different treatment

Treatment	**Species combination**				
	Density of snail (*Viviparus bengalensis*)	**Rohu (*Labeo rohita*)**		**Mrigal (*Cirhinus mrigala*)**	
		Average Initial weight (g)	**Average Present weight** (g)	**Average Initial weight** (g)	**Average Present weight** (g)
T_1	2.0 kg	56.81±2.56	163±0.56	41.32±1.89	150±0.32
T_2	2.5 kg		167±0.32		155±0.54
T_3	3.0 kg		170±0.87		158±0.26
T_4	3.5 kg		169±0.57		152±0.24
T_5	4.0 kg		166±0.44		153±0.58
Control	-		167±0.25		158±0.78

After 3 months of observation, from the above table it can be implies that, the average weight *of Labeo rohita* were 163±0.56, 167±0.32, 170±0.87, 169±0.57, 166±0.44 and 167±0.25 in T_1, T_2, T_3, T_4, T_5 and control respectively. In terms of *Cirhinus mrigala* the average weight were found 150±0.32, 155±0.54, 158±0.26, 152±0.24, 153±0.58 and 158±0.78, respectively. Highest growth was observed in T_3.

Exp. 2: Assessment of gametogenic stage of pond snail (*Viviparous bengalensis*)

Gonad maturation stages of pond snail were measured by following the standard procedure of histological process of mollusk one pond was selected in Freshwater Station, BFRI. Ponds were dried and limed at the rate of 1kg per decimal and filled with water. After pond preparation about 15kg of year-round collected snail from different aquatic habitat were stocked. Both organic and inorganic fertilizer and commercial feed was applied fortnightly for ensuring sufficient feeds for snail. Six individual once a week was brought to laboratory and processed for histological study within 24 hrs. Snail height, length and weight were measured with vernier calipers and electric balance and 12 month data was recorded. Sample specimen was dissected for gonadal tissue collection and fixed in Bouin fluid for 48 hrs. After that the sample was preserved in 70% ethyl alcohol until histological procedure. Following steps were followed for histological study: Step 1: Slicing, Step 2: Dehydration, clearing and infiltration, Step 3: Embedding, Step 4: Trimming, Step 5: Sectioning, Step 6: Staining, Step 7: Mounting, Step 8: Microscopic observations Developmental stages of male and female gametic cells were differentiated, as described by Peredo and Parada (1984), Tair-Abbaci, and Garric, (2012). Development of ova and spermatozoa were summarized as mentioned by Chatchavalvanich *et al.,* 2006, Gamarra-Luques *et al.,* 2013.

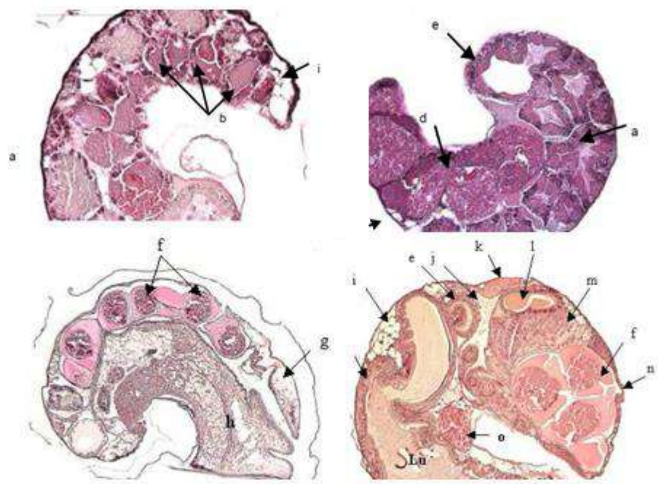


Figure 1: The different gonad maturity stages of female *Viviparous bengalensis* a) digestive gland; b) 1st order secondary oocyte; c) secondary mature oocyte; d) germinating center; e) digestive tube; f) embryos; g) mantle; h) foot; i) albumen gland; j) kidney; k) nephridial gland; l) oviduct; m) mucus gland; n) embryonic sac; o) mature oocyte; arrow: primary oocyte; Lu: light of digestive tube

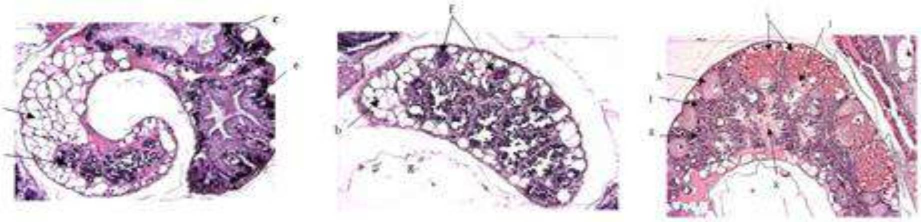


Figure 2: Different gonad maturity stages and internal organization of different organs of observed in male *Viviparous bengalensis*

a) acini; b) albumen gland; c) digestive tube; d) light of digestive tube; e) digestive gland; f) primary oocytes; g) acini covered with spermatogonia; h) 1st order secondary oocytes; i) secondary mature oocytes; j) germinating center; k) efferent canal covered with spermatozoids; l) germinating spermatogonia; m) spermatozoids; n) different maturity stages of spermatogonia, with light increasing along with maturity

a. Early Development stage

In the female, oocytes remained in attached condition inside the follicles. In the male, spermatozoa were observed in attached condition inside the follicles.

b. Late Development stage

In this stage, most oocytes and spermatozoa were ready to release within the follicles.

c. Ripe stage

In the female, most oocytes were free within the follicles, but some oocytes were attached to the follicle wall. In the male, follicles filled by spermatozoa arranged in characteristic bands.

d. Spawning

In the female, large spaces inside the follicles and between free oocytes were present. Some follicles were completely devoid of oocytes. In the male, a marked decrease in the quantity of spermatozoa was observed in the follicles. Large spaces inside the follicles occurred. In some follicles, only a few residual spermatozoa were present.

e. Spent

At this stage, most oocytes and spermatozoa were spawned and only some observed unspawned within follicles.

During the study period, the highest ripe ova were found in March followed by April while, highest percentage of spawning ova was found in May followed by June. The highest percentages of spent gonad were found in September. According to the histological observation the peak breeding season was identified for *Viviparous bengalensis* from April to August.

Table 2: Male and female ratio of pond snail during the study period

Month	Sample	Male	Female
July	20	12	8
August	20	12	8
September	20	8	12
October	20	12	8
November	20	8	12
December	20	13	7
January	20	8	12
February	20	12	8
March	20	8	12
April	20	14	6
May	20	10	10
June	20	12	8
Male: female (1:1.053)			

On the other hand after the histological identification the male female ratio was found 1:1.053 (Table 2). Sex ratios are among the most basic of demographic parameters and provide an indication of both the relative survival of females and males and the future breeding potential of a population. So it can be said that male female ratio was in more or less in balanced condition.

Expt. 3: Estimation of Condition Factor (CF) of pond snail (*Viviparous bengalensis*)

The condition factor (k) of the snails was determined from the relationship between the shell length and weight measurements using the equation (Gayanilo and Pauly, 1997);

$$\text{Condition Factor} = \frac{100W}{L^3}$$

Where:

K = Fulton's condition factor, W = Weight of shell in grams (g), L = Total length of shell in centimeters (cm)

Achievement: K value of less than 1 is an indication of a poor condition. But in this result from the table we can say that all the value are above one and near about one so the snail was in good condition during the reporting period. Mollusk condition strongly influenced by the condition of abiotic and biotic environment it was obtained and could be used as a tool to assess the ecosystem health.

Table 3: Condition factor of pond snail (*Viviparous bengalensis*)

Month	**Total length of shell (cm)** ±SD	**Body weight (g)** ±SD	**Condition Factor (CF)** ±SD
July	2.85±0.20	4.58±0.49	1.00±0.10
August	2.51±0.19	3.01±0.53	1.00±0.09
September	2.87±0.19	4.60±0.97	1.00±0.08
October	2.64±0.29	3.61 ±0.97	1.00±0.09
November	2.82±0.23	2.21±0.85	0.95±0.06
December	2.56±0.16	2.06±0.41	0.91±0.06
January	3.11±0.20	2.28±0.94	0.95±0.04
February	2.70±0.25	2.70±0.90	0.99±0.07
March	2.55±0.15	3.10±0.49	1.00±0.05
April	2.83±0.19	3.45±0.33	1.00±0.09
May	3.03±0.25	3.94±0.41	1.00±0.06
June	3.23±0.23	3.99±0.35	1.00±0.06

Exp. 4: Identification of specific species of freshwater mussels through DNA Barcoding analysis

Mussel samples were collected from freshwater habitats. Immediately after the collecting the specimens, tissue samples were stored in absolute ethanol. Approximately 100 mg of white muscle tissue from each specimen were preserved in 95% ethanol for genomic DNA isolation. Total genomic DNA was extracted from the muscle tissue, using commercially available DNA extraction kit. For DNA barcoding, partial 5' region of COI gene is amplified in a final volume of 50 µl with final concentration of 1X reaction buffer (10 mM Tris-HCl, 50 mM KCl), 2.0 mM $MgCl_2$, 0.2 mM of dNTP mix, 10 pmol of forward and reverse primer, 2U of Taq DNA polymerase and 100 ng of template DNA.

The primers for amplification of LCO1490 5' GGTCAACAAATCATAAAGATATTGG 3' and HCO2198 5' TAAACTTCAGGGTGACCAAAAAATCA 3' (Folmer et al. 1994) for sequencing of barcode region. Each reaction included a negative control (no template DNA) and was carried out in a thermal cycler under following thermal cycling conditions: initial denaturation at 95 °C for 4 min, followed by 35 cycles of denaturation at 95 °C for 35 sec, primer annealing at 48 °C for 30 sec and primer extension at 72 °C for 40 sec and final extension for 5 min at 72 °C. Amplified products of COI was separated on 1.5% agarose gel.

DNA extraction - PCR amplification- Sequencing- Data processing- Species discovery

Achievement:

Table 5: Some identified species of mussel after DNA analysis

>1 TAGCATTGTGGTCTGGATTGATTGGATTAGCTTTGAGACTTT TGATTCGAGCTGAGTTAGGGCAACCAGGTAGTTTGTTAGGG GACGATCAGTTATATAATGTTATTGTAACGGCTCATGCGTTT ATAATAATTTTTTTCTTGGTGATGCCAATGATGATTGGTGGG TTTGGAAATTGGTTGATTCCTTTGATGATTGGGGCTCCTGAT ATAGCTTTTCCTCGATTAAATAACTTAAGATTTTGATTGCTTG TACCTGCTTTGTTTTTGTTGTTGAGATCTTCTTTGGTGGAGAG GGGTGTTGGGACTGGTTGAACGGTTTATCCACCTTTGTCAGG AAATGTAGCTCATTCTGGGGCTTCTGTTGATTTAGCTATTTTT TCTTTGCATCTTGCCGGTGCTTCTTCCATTTGGGGGCTATTAA TTTTATTTCTACTGTGGGAAATATGCGGTCTCCGGGTATAGT TGCTGAGCGAATTCCGTTATTTGTTTGGAGTGTTACTGTAAC GGCTATTTTATTAGTAGCTGCTTTGCCTGTGTTAGCTGGTGCT ATTACTATGCTACTAACTGATCGTAATTTAAATACATCTTTTT TTGACCCTACTGGGGGAGGTGATCCAATTTTATATATACACT TGTTTTGATTTTTGG	*Lamellidens marginalis* 98.88% 
>2 AAGATATTGGAACTTTATATTTGCTGTTAGCCCTGTGATCTG GGTTAATTGGGTTAGCTTTAAGGCTTTTGATTCGAGCTGAGT TAGGTCAGCCTGGAAGGTTATTAGGGGATGATCAGCTCTATA ATGTAATTGTAACGGCTCATGCTTTTATGATAATTTTCTTTTT AGTTATGCCAATGATGATTGGGGGATTTGGCAATTGGTTAAT TCCTTTAATGATTGGGGCTCCTGATATGGCTTTCCCTCGATTA AATAACTTGAGATTTTGGTTGCTTGTGCCAGCTTTATTCTTGT TGCTGAGGTCTTCTTTAGTGGAAAGGGGTGTTGGGACTGGAT GGACGGTTTATCCCCCTTTGTCTGGGAATGTGGCCCATTCGG GAGCTTCTGTTGATTTGGCAATTTTTTCTTTACACCTTGCCGG TGCTTCTTCCATTCTGGGGGCTATTAATTTTATTTCAACTGTT GGTAATATGCGGTCTCCGGGCTTGGTGGCTGAGCGTATTCCT TTGTTTGTGTGAAGGGTAACTGTGACTGCTGTGTTGTTAGTG GCCGCTTTGCCCGTGCTAGCCGGTGCTATTACTATATTACTT ACTGATCGTAATTTAAACACATCATTTTTTGACCCTACGGGG GGAGGTGATCCTA	*Parreysia rakhinensis* 97.95% 
>3 AAGATATTGGTACGTTGTATTTGTTGTTGGCTTTGTGGTCTGG GTTAATTGGCTTGGCTTTGAGGCTTCTTATTCGGGCGGAGCT GGGACAGCCTGGTAGGTTGTTGGGGGATGATCAGTTGTATA ATGTAATTGTTACAGCGCATGCTTTTATGATGATTTTCTTTTT AGTGATGCCCATGATAATTGGTGGTTTTGGGAATTGGCTTAT TCCTCTTATGATTGGGGCTCCTGATATAGCTTTTCCACGGTTG AATAACTTAAGGTTTTGGTTGCTTGTGCCTGCTCTTTTTTTAT TATTAAGATCTTCTTTGGTTGAGAGGGGTGTTGGTACTGGCT GAACTGTGTATCCGCCATTGTCTGGGAATGTGGCCCATTCAG GGGCTTCGGTTGATTTAGCTATTTTTTCTTTACATCTTGCTGG TGCCTCTTCTATTTTGGGGGCTATTAATTTTATTTCAACAGTT GGAAATATGCGATCTCCTGGGCTAGTTGCTGAGCGGATTCCA TTGTTTGTGTGGGCTGTAACAGTAACAGCGATTTTGTTGGTT GCAGCTTTACCTGTGTTGGCTGGTGCTATTACTATGTTGCTTA	*Pilsbryoconcha exilis* 100% 

CTGATCGTAATTTAAATACCTCGTTTTTTGACCCCACTGGAG GTGGTGATCCA	

Exp. 5: Risk assessment of micro plastics in the aquatic ecosystem of Buriganga river.

Selection of study sites: Ten potential sites were selected for the sampling preferably at the opening of the city drains. Spatial sampling was conducted at 1-2 km from each site.

Sample collection: Water samples were collected in plastic bottles from each sampling site. Prior to sampling, all bottles were rinsed with deionized water, followed by washing with the sampling water from the individual sampling point. Then, water samples were taken in the sampling bottle (500 ml) and 1 ml concentrated HNO_3 was added for acidification. Sediment samples were collected using a clean stainless-steel scoop. Collected sediment samples were wrapped with aluminum foil paper and were placed in a cold box to transport to the laboratory. Samples of fish, mussels, snails, and crabs were collected by the assistance of local fishermen. Collected specimens were placed in ice box and transported to the laboratory for further analysis.

Extraction of micro plastics: In the laboratory, collected samples were digested and extracted for the identification of MPs following the standard procedures.

Achievement: Different types of micro plastic were found in gut and muscle content of fish in Buriganga river.

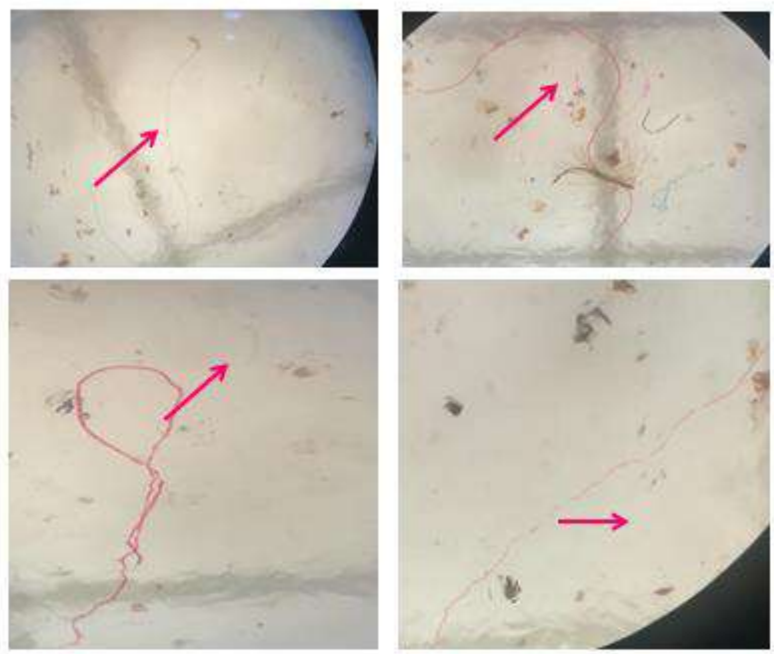


Figure 3: Presence of micro plastic in gut content and muscle in different fish of Buriganga

Production of High Valued Catfish Using Bottom Clean Aquaculture System in Tank

Researchers : A.K.M. Saiful Islam, SSO
: Md. Rabiul Awal, SO
: Md. Moshiur Rahman, SSO
: Asma Jaman, SO

Objectives of the Project (2023-24):

- Production performance of Stinging Catfish (*Heteropneusteus fossilis*) in tank bottom clean aquaculture system
- Production potentials of Gulsha (*Mystus cavasius*) in tank bottom clean aquaculture system

Achievements (2023-2024):

The following research works were carried out under the project from July 2023 to June 2024.

Experiment 1. Production performance of stinging catfish (*Heteropneusteus fossilis*) in tank bottom clean aquaculture system

The experiment was conducted at Freshwater Station of Bangladesh Fisheries Research Institute in plastic indoor tanks with volume of 6000L where water capacity is 8000L for the period of 120 days from February to June 2024. There were three treatments and stocking density was 4000 (T_1), 6000 (T_2), and 8000 (T_3) nos. per 6000L water (Table 1). The design of experiment is given below:

Table 1. Experimental design for *H. fossilis* in bottom clean system

Treatments	Stocking density (Nos./6000L/Tank)
T_1	4000
T_2	6000
T_3	8000

Tank preparation and management

First of all, the diameter and height of the tanks were measured. Then a cemented basement was made according to the size of the tank. After that, round shaped iron frame was made. Inside of the tank was covered by Tarpaulin. 6 elbows (4 bottom & 2 upper side) were placed in tank at 45-degree angle for water circulation. An outlet was set up in the middle of the bottom to let out the wastages. Outlet was covered with a cage so that fish cannot be passed with that outlet. Outlet pipe was attached to a mechanical filter. Water was filtered in that filter and goes to the pond. A motor was used to lift water from the attached pond. Oxygen blower was set up for aeration in the tank water (Figure 1). Finally, fish was stocked in the tanks.

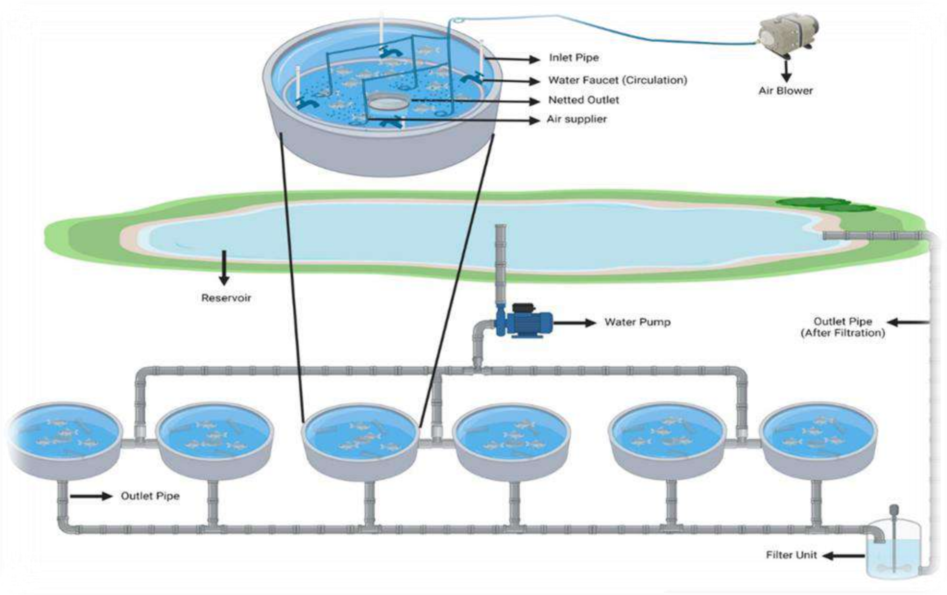


Figure 1. Different unit of bottom clean aquaculture in tank

Fish stocking and management

The stinging catfish, *H. fossilis* was stocked according to design of experiment which mentioned in previous Table (1). After stocking of fish, commercial feed (37-35% crude protein) was supplied twice a day @ 10-3% or estimated body weight.

Assessment of water quality parameters

Water parameters such as temperature, pH, dissolved oxygen and total ammonia nitrogen in the tanks during the growth test was monitored and measured on biweekly basis.

Harvesting of Fish

After 120 days of rearing, fish was harvested from the tank by daring out of water. After harvesting, comprehensive data was recorded. Then growth, survival and production was estimated. Cost benefit analysis was also be done to know the economic viability of tank bottom clean system. Moreover, which species more suitable in tank bottom clean system that information was also be generated.

Analysis of experimental data

Growth parameters was recorded and analyzed such as length and weight gain, survival, Specific Growth Rate (SGR) FCR and BCR of fish.

Results

Water quality parameters

The mean values of physic-chemical parameters recorded during the experimental tanks were recorded and presented in Table 2. The water quality parameters were found to be more or less similar and all of the parameters were within the suitable ranges for fish culture. The water quality parameters of water temperature was not significantly differ ($P>0.05$) among the treatments but significantly difference ($P<0.05$) were found in pH, dissolved oxygen, total alkalinity and ammonia (Table 2).

Table 2. Physico-chemical parameters of water in the experimental tanks during the experimental period (Feb-Jun/2024)

Parameters	T_1	T_2	T_3	Suitable range
Water temperature (^{0}C)	28.41±2.38[a]	28.37±2.31[a]	28.32±2.29[a]	25-31
p^H	7.55±0.26[a]	7.62±0.20[a]	7.86±0.25[b]	6.50-8.50
DO (mg/L)	6.51±0.66[a]	6.37±0.73[b]	6.23±0.57[c]	4.50-6.8
Alkalinity(mg/L)	117.57±6.17[b]	117.45±5.04[b]	119.3±6.33[a]	70-190
Ammonia(mg/L)	0.01±0.009[b]	0.02±0.010[a]	0.02±0.015[a]	0.00-0.04

Growth performances and production

The mean initial weight, final weight, weight gain, specific growth rate (SGR%/day), survival rate and production of fishes were recorded during the study period and summarized in Table 3. There was no significant variation in initial weight of fish among the treatments but in case of final weight, weight gain, SGR and production of *H. fossilis*, varied on different stocking densities (Figure 2). The mean final weight of *H. fossilis* in T_1 (22.13±10.98g) was significantly higher ($p<0.05$) than T_2 (19.58±6.99g) and T_3 (19.46±8.62g) but no significant variation between T_1 and T_2. The mean final weight gain of *H. fossilis* in different treatment was 21.44±0.26g, 18.90±0.29g and 18.79±0.32g in T_1, T_2 and T_3, respectively. The value of weight gain in T_1 was significantly higher ($p<0.05$) than T_2 and T_3 due to higher stocking density in T_2 and T_3. Specific growth rate (SGR %/day) in T_1 (2.89±0.04) was significantly higher ($p<0.05$) than T_2 (2.80±0.05) and T_3 (2.81±0.07) but no significant variation ($p>0.05$) between T_2 and T_3. The survival rate (%) of *H. fossilis* in T_1 (90.83±1.81) was significantly higher ($p<0.05$) where the stocking density was lower than T_2 (88.30±2.32) and T_3 (87.48±2.40) but no significant variation ($p>0.05$) between T_2 and T_3. Feed Conversion Ratio (FCR) was recorded 1.16±0.09, 1.21±0.11 and 1.23±0.14 in T_1, T_2 and T_3, respectively. The lowest FCR was recorded in T_1 where the lowest number of fingerlings was reared which indicated the best feed utilization. FCR in T_1 is significantly differ ($p<0.05$) from T_2 and T_3. The mean gross productions of H. fossilis were 80.39±4.51, 103.73±5.22 and 136.20±7.89 kg/6000L water/120 days in T_1, T_2 and T_3, respectively. The production of *H. fossilis* in T_3 varied significantly higher ($p<0.05$) than T_1 and T_2. Fish production of *H. fossilis* were found higher in T_3 followed by T_2 and T_1 where the stocking density of *H. fossilis* was 8000 piece/6000L water in tank.

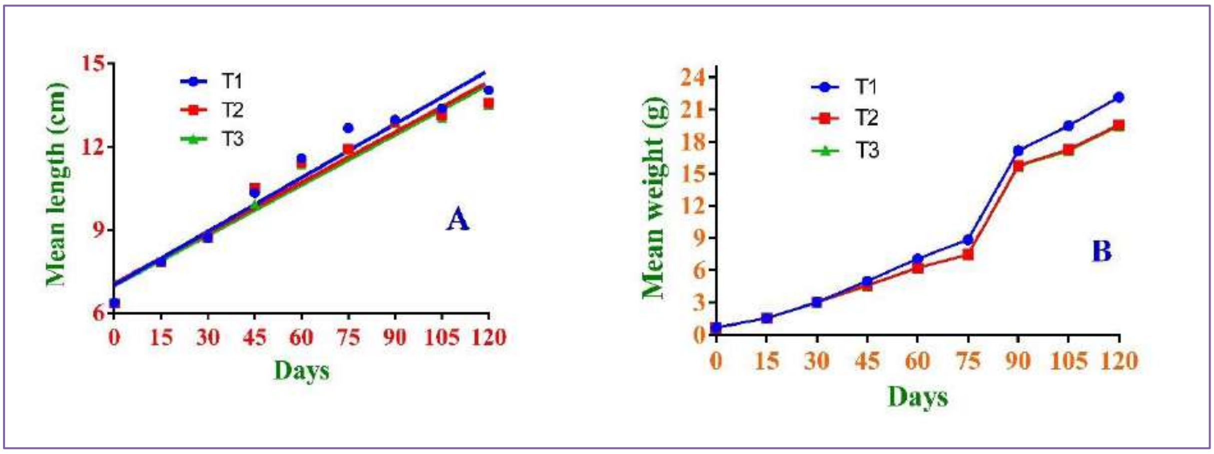


Figure 2. Bi-weekly variation in growth of *H. fossilis* under different stocking density

Table 3. Growth performances and production of shing (*H. fossilis*) under the three treatments during the culture period

Parameters	Treatments T_1	T_2	T_3
Initial length (cm)	6.37±0.86[a]	6.34±0.78[a]	6.36±0.8[a]
Initial weight (g)	0.69±0.24[a]	0.68±0.2[a]	0.67±0.2[a]
Final length (cm)	14.04±2.24[a]	13.57±1.62[b]	13.52±1.92[b]
Final weight (g)	22.13±10.98[a]	19.58±6.99[b]	19.46±8.62[b]
Weight gain	21.44±0.26[a]	18.90±0.29[b]	18.79±0.32[b]
Weight gain (%)	3107.25±44.4[a]	2779.41±54.87[b]	2804.48±65.98[b]
ADLG (%)	6.39±0.04[a]	6.03±0.06[b]	5.97±0.09[b]
ADWG (%)	17.87±0.05[a]	15.75±0.07 [b]	15.66±0.10[b]
Survival rate (%)	90.83±1.81[a]	88.30±2.32[b]	87.48±2.40[b]
FCR	1.16±0.09[b]	1.21±0.11[a]	1.23±0.14[a]
SGR (%)	2.89±0.04[a]	2.80±0.05[b]	2.81±0.07 [b]
Final Biomass (kg/tank)	80.39±4.51[c]	103.73±5.22[b]	136.20±7.89[a]

Economic analysis

A simple benefit-cost analysis (BCA) was done to know the profit level. The total cost of production (Tk./tank) was consistently lower in T_1 (16,010) than those in T_2 (19,791) and T_3 (24,352) (Table 4). Highest net benefit (Tk./tank) was obtained in T_3 (13,784) followed by T_2 (9,253) and the lowest in T_1 (8,107). The highest BCR was found in T_3 (1.57) and lowest was found in T_2 (1.48).

Table 4. Cost and profit from analysis of Shing (*H. fossilis*) culture at tank bottom clean aquaculture system

Item	Amount (TK)/tank/120 days			Remarks
	T_1 (BDT)	T_2 (BDT)	T_3 (BDT)	
A. Cost				
Tank preparation (Tarpolin, Iron cage, plastic pipe, etc)	3,150	3,150	3,150	Made for 10 years where there will be 20 crops
Price of fingerlings	2,400	3,600	4,800	@TK. 0.60/Piece
Feeds	7,460	10,041	13,402	
Human labour cost	1,000	1,000	1,000	
Miscellaneous	2,000	2,000	2,000	Electricity, medicine etc
Total Cost	16,010	19,791	24,352	
B. Return				
Shing	24,117	29,044	38,136	Market price related with size (TK. 300, 280 and 280/kg of shing in T_1, T_2 and T_3, respectively)
Total return	24,117	29,044	38,136	
Net Profit (B-A)	8,107	9,253	13,784	
BCR	**1.51**	**1.48**	**1.57**	

Experiment 2. Production potentials of Gulsha (*Mystus cavasius*) in tank bottom clean aquaculture system

The experiment was conducted at Freshwater Station of Bangladesh Fisheries Research Institute in plastic indoor tanks with volume of 6000L where water capacity is 8000L for the period of 120 days from February to June 2024. There were three treatments and stocking density was 4000 (T_1), 6000 (T_2), and 8000 (T_3) nos. per 6000L water (Table 5). The design of experiment is given below:

Table 5. Experimental design for *Mystus cavasius* in bottom clean system

Treatments	Stocking density (Nos./6000L/Tank)
T_1	4000
T_2	6000
T_3	8000

Tank preparation and management

First of all, the diameter and height of the tank was measured. Then a cemented basement was made according to the size of the tank. After that, round shaped iron frame was made. Inside of the tank was covered by Tarpaulin (Figure 3). 6 elbows (4 bottom & 2 upper side) were placed in tank at 45-degree angle for water circulation. An outlet was set up in the middle of the bottom to let out the wastages. Outlet was covered with a cage so that fish cannot be passed with that outlet. Outlet pipe was attached to a mechanical filter. Water was filtered in that filter and goes to the pond. A motor was used to lift water from the attached pond. Oxygen blower was set up for aeration in the tank water. Finally, fish was stocked in the tanks.

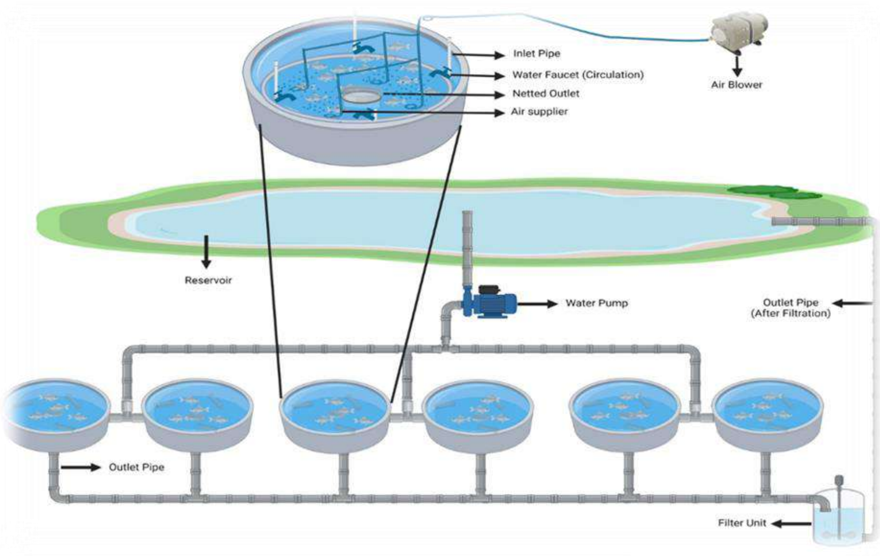


Figure 3. Different unit of bottom clean aquaculture in tank

Fish stocking and management

The Gulsha *M. cavasius* was stocked according to design of experiment which mentioned in previous Table (5). After stocking of fish, commercial feed (37-35% crude protein) was supplied twice a day @ 10-3% or estimated body weight.

Assessment of water quality parameters

Water parameters such as temperature, pH, dissolved oxygen and total ammonia nitrogen in the tanks during the growth test was monitored and measured on biweekly basis.

Harvesting of Fish

After 105 days of rearing, fish was harvested from the tank by daring out of water. After harvesting, comprehensive data was recorded. Then growth, survival and production was estimated. Cost benefit analysis was also be done to know the economic viability of tank bottom clean system. Moreover, which species more suitable in tank bottom clean system that information was also be generated.

Analysis of experimental data

Growth parameters was recorded and analyzed such as length and weight gain, survival, Specific Growth Rate (SGR) FCR and BCR of fish.

Results

Water quality parameters

The mean values of physic-chemical parameters recorded during the experimental tanks were recorded and presented in Table 6. The water quality parameters were found to be more or less similar and all of the parameters were within the suitable ranges for fish culture. The water quality parameters of water temperature was not significantly differ (P>0.05) among the treatments but significantly difference (P<0.05) were found in pH, dissolved oxygen, total alkalinity and ammonia (Table 6).

Table 6. Physico-chemical parameters of water in the experimental tanks during the experimental period (Feb-Jun/2024)

Parameters	T_1	T_2	T_3	Suitable range
Water temperature (^{0}C)	28.37±2.35^a	28.30±2.24^a	28.24±2.21^a	25-31
p^H	7.55±0.26^a	7.62±0.20^a	7.86±0.25^b	6.50-8.50
DO (mg/L)	6.51±0.66^a	6.37±0.73^b	6.23±0.57^c	4.50-6.8
Alkalinity(mg/L)	117.57±6.17^b	117.45±5.04^b	119.3±6.33^a	70-190
Ammonia(mg/L)	0.01±0.009^b	0.01±0.010^b	0.02±0.015^a	0.00-0.04

Growth performances and production

The mean initial weight, final weight, weight gain, specific growth rate (SGR%/day), survival rate and production of fishes were recorded during the study period and summarized in Table 7. There was no significant variation in initial weight of fish among the treatments but in case of final weight, weight gain, SGR and production of *M. cavasius*, varied on different stocking densities (Figure 4). The mean final weight of *M. cavasius* in T_1 (15.33±5.03g) was significantly higher (p<0.05) than T_2 (14.52±3.94g) and T_3 (14.39±4.69g) but no significant variation between T_2 and T_3. The mean final weight gain of *M. cavasius* in different treatment was 14.84±0.03g, 14.04±0.05g and 13.89±0.07g in T_1, T_2 and T_3, respectively. The value of weight gain in T_1 was significantly higher (p<0.05) than T_2 and T_3 due to higher stocking density in T_2 and T_3. Specific growth rate (SGR %/day) in T_1 (3.28±0.02) was significantly higher (p<0.05) than T_2 (3.24±0.04) and T_3 (3.20±0.05). The highest specific growth rate (SGR %/day) was observed in T_1 whereas lowest was observed in T_3. The survival rate (%) of *M. cavasius* in T_1 (96.55±2.32) was significantly higher (p<0.05) where the stocking density was lower than T_2 (94.97±2.86) and T_3 (94.72±2.98) but no significant variation (p>0.05) between T_2 and T_3. Feed Conversion Ratio (FCR) was recorded 1.04±0.04, 1.11±0.07 and 1.13±0.10 in T_1, T_2 and T_3, respectively. The lowest FCR was recorded in T_1 where the lowest number of fingerlings was reared which indicated the best feed utilization. FCR in T_1 is significantly differ ($p<0.05$) from T_2 and T_3 while no significant variation was observed in T_2 and T_3. The mean gross productions of *M. cavasius* were 58.61±3.23, 83.17±4.76 and 109.78±7.88 kg/tank/120 days in T_1, T_2 and T_3, respectively. The production of *M. cavasius* in T_3 varied significantly higher ($p<0.05$) than T_1 and T_2. Fish production of *M. cavasius* were found higher in T_3 followed by T_2 and T_1 where the stocking density of *M. cavasius* was 8000 piece/6000L water in tank.

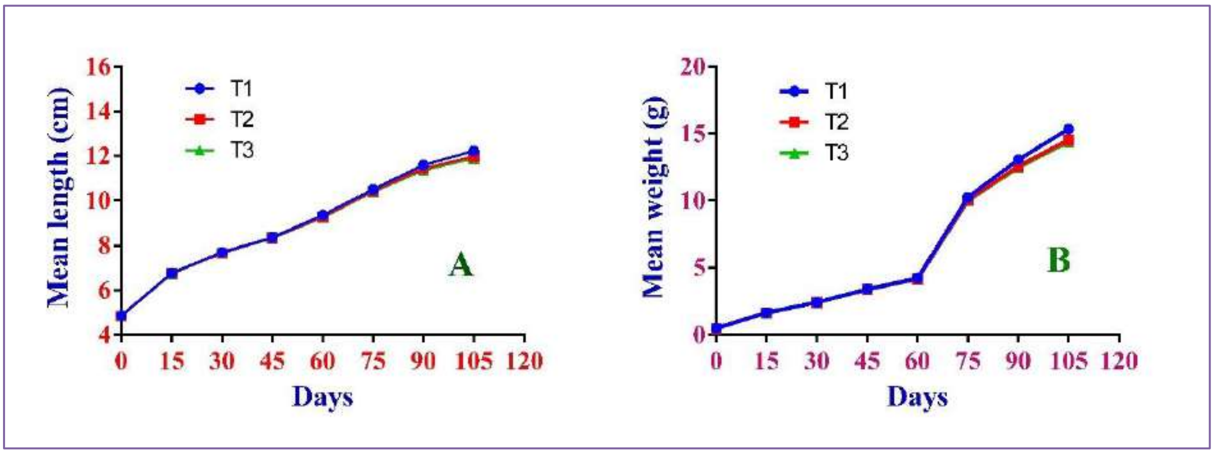


Figure 4. Bi-weekly variation in growth of *M. cavasius* under different stocking density

Table 7. Growth performances and production of Gulsha (*M. cavasius*) under the three treatments during the culture period

Parameters	Treatments		
	T_1	T_2	T_3
Initial length (cm)	4.86±0.52[a]	4.85±0.54[a]	4.84±0.71[a]
Initial weight (g)	0.49±0.19[a]	0.48±0.18[a]	0.50±0.20[a]
Final length (cm)	12.22±1.26[a]	11.97±1.12[b]	11.89±1.31[b]
Final weight (g)	15.33±5.03[a]	14.52±3.94[b]	14.39±4.69[b]
Weight gain	14.84±0.03[a]	14.04±0.05[b]	13.89±0.07[b]
Weight gain (%)	3180.00±45.07[a]	3071.25±64.34[b]	2916.90±74.98[c]
ADLG (%)	7.01±0.05[a]	6.78±0.06[b]	6.71±0.08[b]
ADWG (%)	14.13±0.04[a]	13.37±0.07[b]	13.23±0.08[b]
SGR (%)	3.28±0.02[a]	3.24±0.04[b]	3.20±0.05[c]
Survival rate (%)	96.55±2.32[a]	94.97±2.86[b]	94.72±2.98[b]
FCR	1.04±0.04[b]	1.11±0.07[a]	1.13±0.10[a]
Final Biomass (kg/tank/ 6000L water)	58.61±3.23[c]	83.17±4.76[b]	109.78±7.88[a]

Economic analysis

A simple benefit-cost analysis (BCA) was done to know the profit level. The total cost of production (Tk./tank) was consistently lower in T_1 (14,265) than those in T_2 (18,288) and T_3 (22,344) (Table 8). Highest net benefit (Tk./tank) was obtained in T_3 (21,568) followed by T_2

(14,980) and the lowest in T_1 (9,179). The highest BCR was found in T_3 (1.96) and lowest was found in T_2 (1.64).

Table 8. Cost and profit from analysis of Gulsha (*M. cavasius*) culture at tank bottom clean aquaculture system

Item	Amount (TK)/tank/105 days			Remarks
	T_1 (BDT)	**T_2 (BDT)**	**T_3 (BDT)**	
A. Cost				
Tank preparation (Tarpolin, Iron cage, plastic pipe, etc)	3,150	3,150	3,150	Made for 10 years where there will be 20 crops
Price of fingerlings	2,400	3,600	4,800	@TK. 0.60/Piece
Feeds	5,485	8,308	11,164	
Human labour cost	1,000	1,000	1,000	
Miscellaneous	2,230	2,230	2,230	Electricity, medicine etc
Total Cost	14,265	18,288	22,344	
B. Return				
Gulsha	23,444	33,268	43,912	Market price related with size (TK. 400/kg of Gulsha in all treatments)
Total return	23,444	33,268	43,912	
Net Profit (B-A)	9,179	14,980	21,568	
BCR	**1.64**	**1.81**	**1.96**	

Production Performance of Hairy River Prawn (*Macrobrachium rude)* with Feed and Fertilizer in Pond Condition

Researcher(s)

: Maliha Khanom, SO
: Md. Shahin Alam, SO
: Flura, SSO

Objectives of the Project (2023-24):

- To identify growth developmental stages of *Macrobrachium rude* in tank condition
- To assess suitable partial harvesting period of gura chingri in poly- culture Techniques
- To identify *Macrobrachium* spp. at species level based on DNA barcoding from different region of Bangladesh
- To produce quality post larvae (PLs) of *Macrobrachium rosenbergii*

06. Achievemnts (2023-2024)

Experiment 1: Identification of growth developmental stages of *Macrobrachium rude* in tank condition

The experiment was carried out in the research tanks of Galda Hatchery Complex at Freshwater Station of BFRI, Mymensingh, from July 2023 to June 2024. Each tank had a volume of 150 ml, and following thorough cleaning, it was filled with water, with the water depth maintained at a specified level.

Design of the experiment

The experiment was conducted into five distinct treatments (T_1, T_2 $T_{3,}$ T_4 and T_5), each replicated three times. The stocking densities for small prawns were maintained according to the Table1. A detailed depiction of the experimental arrangement was presented in Table 1.

Table 1. Experimental layout of assessment of growth developmental stages

<table>
<tr><th>Treatments</th><th>Average Stocking Size</th><th>Replications</th><th>Stocking density of Prawn (nos./L.)</th><th>Feed</th><th>Rearing periods</th></tr>
<tr><td>T_1</td><td>≤ 0.05g</td><td rowspan="5">3</td><td rowspan="5">1</td><td rowspan="5">Formulated feed will be applied @ 05-03%</td><td rowspan="5">12 months</td></tr>
<tr><td>T_2</td><td>0.06-0.15g</td></tr>
<tr><td>T_3</td><td>0.16-0.30g</td></tr>
<tr><td>T_4</td><td>≥ 0.35g</td></tr>
<tr><td>T_5</td><td>Spent</td></tr>
</table>

Stocking and management

After stocking the prawns, formulated feed were being applied in tanks T_1, T_2, T_3, T_4 and T_5, at a rate corresponding to 5-3% of the estimated body weight. Regular monitoring and maintenance of water quality parameters were conducted, accompanied by sampling endeavors to evaluate prawn growth performance, maturation, spawning timing and identify the time period for each stage of growth. Sampling was done at two months interval to know the prawn growth performance and maturation time etc.

Figure 1: Prawn was reared in tank condition

Results

Gura chingri was reared during twelve months in tank condition. Prawn staying in alive condition for twelve months. Mature gravid females released eggs but no Zoea was produced in tank condition. (Table 2)

Table 2. Assessment of growth in tank condition during July- June / 2024

Treatments	Initial Mean wt. (g)	1st sampling Mean wt. (g)	2nd sampling Mean wt. (g)	3rd sampling Mean wt. (g)	4th sampling Mean wt. (g)	5th sampling Mean wt. (g)	6th sampling Mean wt. (g	Mortality %	Comments
T_1	0.05 ±0.01	0.09 ±0.03	0.25± 0.03	0.25± 0.04	0.55± 0.04	0.61±0.09	0.78±0.03	18	* Mature gravid females released eggs but no Zoea were produced in tank condition. 
T_2	0.11±0.02	0.18 ±0.03	0.20± 0.03	0.30± 0.02	0.67± 0.02	0.69±0.05	0.85±0.02	15	
T_3	0.23±0.03	0.32 ±0.04	0.42± 0.04	0.58± 0.05	0.73± 0.05	0.81±0.07	0.98±0.05	22	
T_4	0.35±0.05	0.44 ±0.07	0.54± 0.07	0.65± 0.07	0.85± 0.07	1.14±0.16	1.02±0.08	41	
T_5	1.15±0.12 (Full Gravid)	1.08±0.09 (Partial Spent)			1.02±0.06 (100% Spent)			53	

Water quality parameters:

Fortnightly monitoring of water quality parameters in tanks, including temperature, pH, transparency, alkalinity, and dissolved oxygen (DO), are being carried out at 9:00 am throughout the experimental period and recorded data were shown in the following table (3).

Table 3. Water quality parameters of experimental tanks during July 2023 - June 2024

Parameters	T_1	T_2	T_3	T_4	T_5
Water Temp. (°C)	28.66 ± 2.16	28.71 ± 2.11	28.17 ± 2.18	28.47 ± 2.20	28.35 ± 2.21
pH	8.55 ± 0.31	8.70± 0.29	8.35± 0.34	8.26± 0.30	8.14± 0.16
DO (mg/L)	6.32 ± 0.57	6.55 ± 0.51	6.15 ± 0.36	6.29 ± 0.33	6.23 ± 0.36
NH_3 (mg/L)	0.02±0.0012	0.02±0.0015	0.014±0.001	0.01±0.001	0.014±0.001

Experiment 2: Assessment of suitable partial harvesting period for gura chingri in Poly- culture techniques

The experiment was carried out in three experimental ponds in Freshwater Station, BFRI for a period 180 days from January 2024 to June 2024. Each pond covers an area of three (03) decimal. Preparation of the ponds involve water drainage, followed by the application of lime at a rate of 250 kg/ha. Subsequent to lime treatment, the ponds are refilled with water, maintaining a water depth of 1.2 m.

Design of the experiment

The present experiment was conducted with three treatments namely T_1, T_2 and T_3 each with three replications. The stocking density of Hairy river prawn, BFRI Subarno rui, Catla and Silver carp fishes will be 1.5 kg/decimal, 15,4 and 12 nos per decimal in $T_{1;}$ 2 kg/decimal, 15, 4 and 12 nos per decimal in $T_{2;}$ 2.5 kg/decimal, 15, 4 and 12 nos per decimal in $T_{3.}$ The experimental layout is shown in Table 4.

Table 4. Experimental layout of poly-culture of small prawn, BFRI Subarno rui, Catla and Silver carp fish in pond

Treatments	Replications	Stocking density			
		Prawn (kg/dec.)	Subarno Rui (nos./dec)	Catla (nos./dec)	Silver carp (nos./dec)
T_1	3	1.5	15	4	12
T_2		2			
T_3		2.5			

Stocking and management

After stocking prawn and fish species, formulated feed was applied in treatments T_1, T_2, and T_3 at rates of 5-3% of the estimated body weight, respectively. Monthly sampling was conducted to assess the growth performance of both the prawns and fishes.

Habitat Management:

Special habitats for Gura chingri had been provided with bamboo and some submerged vegetation so that gura chingri can take shelter.

Partial Harvesting:

Partial harvesting was conducted according to partial harvesting time found from the previous year result.

Water quality parameters:

Water quality parameters of the ponds, including temperature, pH, transparency, alkalinity, and dissolved oxygen (DO), were monitored fortnightly at 9:00 AM throughout the experimental period for all treatment groups.

Harvesting:

At the end of the experimental period, harvesting of prawn and fishes were conducted through pond drying.

Economic analysis:

Subsequently, Cost-benefit analysis was executed to quantify the profit attained from distinct treatments within the poly-culture system involving prawns and fishes in the ponds.

Growth performance and production of fishes

The mean initial weight (g), final weight (g), weight gain (g), specific growth rate (SGR, % per day), food conversion ratio (FCR) and survival rate of fishes during the study period will be recorded. The following parameters will be used to evaluate the growth performance of experimental fishes

Weight gain (g) = Mean final weight (g) - Mean initial weight (g)

SGR (% per day) = $\frac{Ln\ final\ weight - ln\ initial\ weight}{Number\ of\ experimental\ days} \times 100$

Food conversion ratio (FCR) = $\frac{Feed\ fed\ (dry\ matter)}{Live\ weight\ gain}$

Survival rate = $\frac{No. of\ fish\ harvested}{No. of\ fish\ stocked} \times 100$

Results

Pond preparation

The experiment was conducted in nine earthen ponds in the freshwater Station of BFRI, Mymensingh for a period of 06 months from January to June 2024. The size of pond having three decimal each. The ponds were prepared by applying lime at the rate of 250 kg/ha. After liming, the pond filled up with water. The water level of the pond was maintained to

1.5 m. In order to provide a shelter for the Gura chingri the root particles of the bamboo trees were used at the bottom of the pond. To maintain water quality, the pond water was changed at regular intervals using water from a deep tube-well supply. After water filling of pond, inorganic fertilizers were used. Three days after fertilizer application, when the water turns green, prawns and fishes were stocked in nine ponds accordingly (Table-4) for development of poly-culture technique of Gura chingri, with suborn rui,catla and silver carp fishes.

Figure 2: Pond prepared for polyculture of small prawn

Collection of Hairy river prawn and fishes

Hairy river prawns were collected from the old Brahmaputra River and different beels, Mymensingh from the month of December 2024 to January 2024 and transported to the experimental sites through plastic bags with proper aeration. The collected species then kept in cistern for conditioning. Carp fishes were collected from different hatchery in Mymensigh region.

Figure 3: Prawn and Fish stocking

Stocking and management

Chingri have been collected for two months before stocking in culture ponds. The stocking density of Hairy river prawn, Suborno rui, Catla and Silver carp fishes were 1.5 kg/decimal, 15, 4 and 12 nos per decimal in $T_{1;}$ 2 kg/decimal, 15, 4 and 12 nos per decimal in T_2 and 2.5 kg/decimal, 15, 4 and 12 nos per decimal in T_3 respectively. Hairy river prawn and carp fishes have been collected and are stocked at the pond in the last week of December 2023 month. The initial average weight of hairy river prawn, rui, catla and silver carp were 0.16 ± 0.04g, 300.10 ± 5.49g, 370.71 ± 5.22g, 407.51 ± 6.35g respectively for $T_{1;}$ in T_2 prawn rui, catla and silver carp are 0.16 ± 0.04g, 302.12 ± 6.15g, 373.27 ± 5.16g, 410.18 ± 6.08g respectively and finally for T_3 prawn, rui, catla and silver carp are 0.16 ± 0.04g, 306.04 ± 6.82g, 371.62 ± 5.56g, 408.42 ± 6.17g respectively. After stocking of prawn and fish species, diets containing protein (30.53%) were supplied to the small prawns and fishes with a mixture of raw materials such as fish meal, mustard oil cake, rice bran, soyabean meal and wheat bran @ 05-03% of estimated body weight as per the experimental design. For increasing the primary productivity of water, 100g TSP and 50g urea, MOC and rice bran were applied at fortnightly interval. Monthly sampling was done regularly to know the growth performance of prawn and fishes.

Harvesting:

First partial harvesting was done in april 2024 after three month of culture period and then one-month interval havesting was conducted till six-month culture period. Final harvesting was done in July month by dewatering after completion of the experiment**.**

Results

Growth performance and production of fishes

The mean initial weight (g), final weight (g), weight gain (g), specific growth rate (SGR, % per day), food conversion ratio (FCR) and survival rate of fishes during the study period were recorded and presented in Table 5.

Table 5. Growth performances of prawn and fishes over a culture period of six months

Parameters	Species	T_1	T_2	T_3
Initial mean weight (g)	*M.rude*	0.16±0.04^a	0.16±0.04^a	0.16±0.04^a
	L. rohita	300.10±5.49^a	302.12±6.15^a	306.04±6.82^a
	L. catla	370.71±5.22^a	373.27±5.16^a	371.62±5.56^a
	H.molitrix	407.51±6.35^a	410.18±6.08^a	408.42±6.17^a
Final mean weight (g)	*M.rude*	1.03±0.12^c	1.10±0.10^a	1.06±0.11^b
	L. rohita	802.47±32.07^c	828.68±24.61^a	814.09±35.47^b
	L. catla	1496.28±35.10^c	1538.80±35.72^b	1589.91±34.19^a
	H.molitrix	1584.31±16.44^c	1612.15±13.26^b	1668.83±15.13^a
Mean weight gain (g)	*M. rude*	0.87±0.09^c	0.94±0.10^a	0.91±0.08^b
	L. rohita	502.37±24.58^b	526.56±18.53^a	508.05±28.65^b
	L. catla	1125.57±32.12^b	1165.53±21.92^b	1218.29±26.41^a
	H.molitrix	1176.81±28.04^c	1201.97±12.81^b	1260.41±13.85^a
Specific growth rate (%/day)	*M. rude*	1.03±0.15^c	1.07±0.14 ^{a}	1.05±0.16^b
	L. rohita	0.55±0.03^b	0.56±0.04^a	0.53±0.05^b
	L. catla	0.77±0.05^a	0.78±0.07^a	0.81±0.08^a
	H.molitrix	0.75±0.05^a	0.76±0.02^a	0.78±0.06^a
Survival rate (%)	*M. rude*	52.35±1.37^c	58.21±1.34^b	63.38±0.51^a
	L. rohita	98.26±0.34^a	98.7±0.41^a	98.8±0.52^a
	L. catla	98.39±0.23^a	98.5±0.65^a	97.9 ±1.17^b
	H.molitrix	97.88±0.13^b	98.82±0.49^a	98.85±0.83^a
FCR		1.69±0.10^a	1.64±0.14^b	1.62±0.11^c
Species-wise Production	*M. rude*	360.45±24.69^c	458.28±19.52^b	635.08±16.13^a
	L. rohita	2575.22±25.16^b	2658.71±28.21^b	2815.12±27.51^a

(kg/ha/6 month)	*L. catla*	1108.53±25.16^{b}	1139.66±28.21^{b}	1570.83±23.34^{a}
	H.molitrix	3912.48±28.81^{c}	4380.21±31.65^{b}	4531.95±37.15^{a}
Total production (kg/ha/6 month)		7,957	8,637	9,552

Values in the same row having the same superscript are not significantly different ($p > 0.05$)
After 06 months of culture, the highest final mean weight of prawn, subarno rui were found to be 1.10±0.10g, 828.68±24.61g respectively, in T_2 and catla and silver carp fishes were found to be 1589.91±34.19g, 1708.83±15.13g, respectively, in T_3 treatment. Subarno rui exhibited significantly lower growth performance in T_1 compared to T_2 and T_3. On the other hand, chingri showed significantly higher ($p < 0.05$) growth performance in T_2 than in T_1 and T_3. The food conversion ratio was significantly lowest in T_3 and highest in T_1. Furthermore, the highest specific growth rate (SGR) values were observed for prawn, subarno rui at 1.07±0.14, 0.56±0.04 respectively, in T_2 and catla and silver carp fishes were found to be 0.81±0.08, 0.78±0.06, respectively, in T_3 treatment At the end of the experiment, species-wise production of prawn, rui, catla and silver carp showed significantly different among the treatments. The production of chingri recorded significantly higher ($p < 0.05$) in T_3 than in T_1 and T_2. The total highest productions of prawn and carp fishes were recorded in T_3 (9,552 kg/ha/6 months), followed by T_1 (7,957 kg/ha/6 months) and T_2 (8,637 kg/ha/6 months).


Figure 4: Harvested chingri and fishes from the experimental pond

The economic benefit analysis of Poly-culture system:

A simple cost-benefit analysis analysis in polyculture system of Gura chingri with carp fishes from one-hectare ponds over a culture period of 6 months was done to estimate the return against investment and profitability that had been generated proper combination and stocking densities in polyculture were summarized in Table 3. The total costs of farming (BDT/ha) were lower in T_1 (11, 46, 252) than those of T_2 (12, 47, 860) and T_3 (13, 35, 758). The net benefits were calculated from three treatments as BDT 6,77,863; 7,74,786 and 9,97,612 per hectare for T_1, T_2 and T_3, respectively. The highest BCR was found to be 1.75 in T_3 treatment and lowest values were observed at 1.59 in T_1 treatment.

Table 6: Cost and benefits analysis of polyculture of prawn with Indian major carps fishes in one-hectare ponds over a 6 months culture period

Item wise expenditure / Operational costs		**T_1**	**T_2**	**T_3**
A.Cost				
1. Pond lease value for 6 months		40,000	40,000	40,000
2. Price of fingerlings				
2.a	Prawn @ TK 700/kg	2,59,350	3,45,800	4,32,250
2.b	Bfri Subarno rui @ TK 60.00/piece	2,22,300	2,22,300	2,22,300
2.c	Catla @ TK 50.00/piece	49,400	49,400	49,400
2.d	Silver carp @ TK 40.00/piece	1,18,560	1,18,560	1,18,560
3. Feeds		4,01,642	4,16,800	4,18,248
4. Lime, fertilizer etc.		5,000	5,000	5,000
5. Human labor, Transport etc.		50,000	50,000	50,000
Total cost		11,46,252	12,47,860	13,35,758
B. Incomes				
Prawn (950 tk/ kg)		3,31, 614	4,21,360	5,84,274
Bfri Subarno rui (220 tk/ kg)		5,66,548	5,84,916	6,19,350
Catla (200 tk/ kg)		2,21,706	2,27,932	3,14,166
Silver carp (180 tk/kg)		7,04,247	7,88,438	8,15,580
Total return		18,24,115	20,22,646	23,33,370
Net Profit (B-A)		6,77,863	7,74,786	9,97,612
BCR		1.59	1.62	1.75

Water quality parameters

Physico-chemical parameters of pond water such as temperature, pH, transparency, alkalinity, and DO of all treatments were monitored at the fortnightly interval and recorded data were shown in the following table (4). Water temperature of different ponds was more or less same. The mean temperature was 30.59 ± 2.26 ^oC, 30.36 ± 2.11 ^oC and 30.17 ± 2.18 ^oC in T_1, T_2, and T_3 respectively. The mean values of pH were 7.94 ± 0.32, 8.21± 0.29 and 8.53± 0.34 in T_1, T_2 and T_3, respectively. The mean values of DO were 5.39 ± 0.57, 5.47 ± 0.51 and 5.64 ± 0.36 in T_1, T_2 and T_3, respectively. The highest mean values of alkalinity were recorded in T_2 (135.34 ± 3.16) and the lowest was T_1 (132.40 ± 3.21). The highest mean values of ammonia were recorded in T_2 (0.002±0.0015) and lowest was in T_3 in polyculture.

Table 7. Water quality parameters of experimental ponds during January-June /2024

Water Temp. (^{o}C)	30.59 ± 2.26 [a]	30.36 ± 2.11 [a]	30.17 ± 2.18 [a]
pH	7.94 ± 0.31 [a]	8.21± 0.29 [a]	8.53± 0.34 [a]
DO (mg/L)	5.39 ± 0.57 [a]	5.47 ± 0.51 [a]	5.64 ± 0.36 [a]
Total Alkalinity (mg/L)	132.40 ± 3.21 [a]	135.34 ± 3.16[a]	134.62 ±3.09 [a]
NH_3 (mg/L)	0.019±0.0012 [a]	0.002±0.0015 [a]	0.0014±0.00 [a]

*Figures in the same row having the same superscripts are not significantly different ($p \geq 0.05$)

Experiment 3: Identification of *Macrobrachium* spp. at species level based on DNA Barcoding from different location in Mymensingh region

Sample collection and DNA isolation

Prawn samples were collected from freshwater habitats, fish landing centers, fish markets or from the local fishermen during January 2024 to June 2024. Immediately after the collecting the specimens, tissue samples were stored in absolute ethanol. Approximately 100 mg of white muscle tissue from each specimen were preserved in 95% ethanol for genomic DNA isolation. Total genomic DNA were extracted from the muscle tissue or fin tissue, using commercially available DNA extraction kit. Total collected Sample 40. This process will be contined.

DNA extraction - PCR amplification- Sequencing- Data processing- Species discovery

DNA barcoding: After sample collection, further lab research for DNA barcoding were done

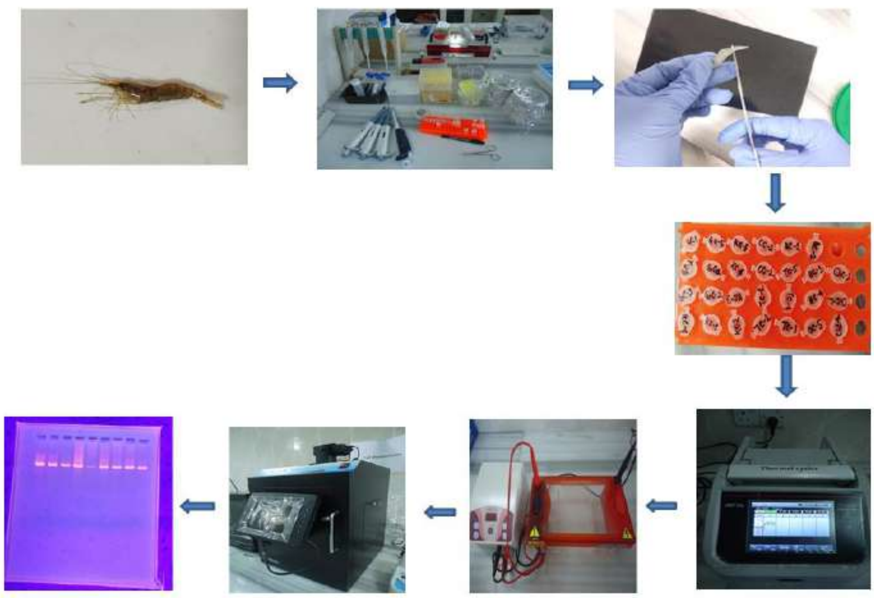
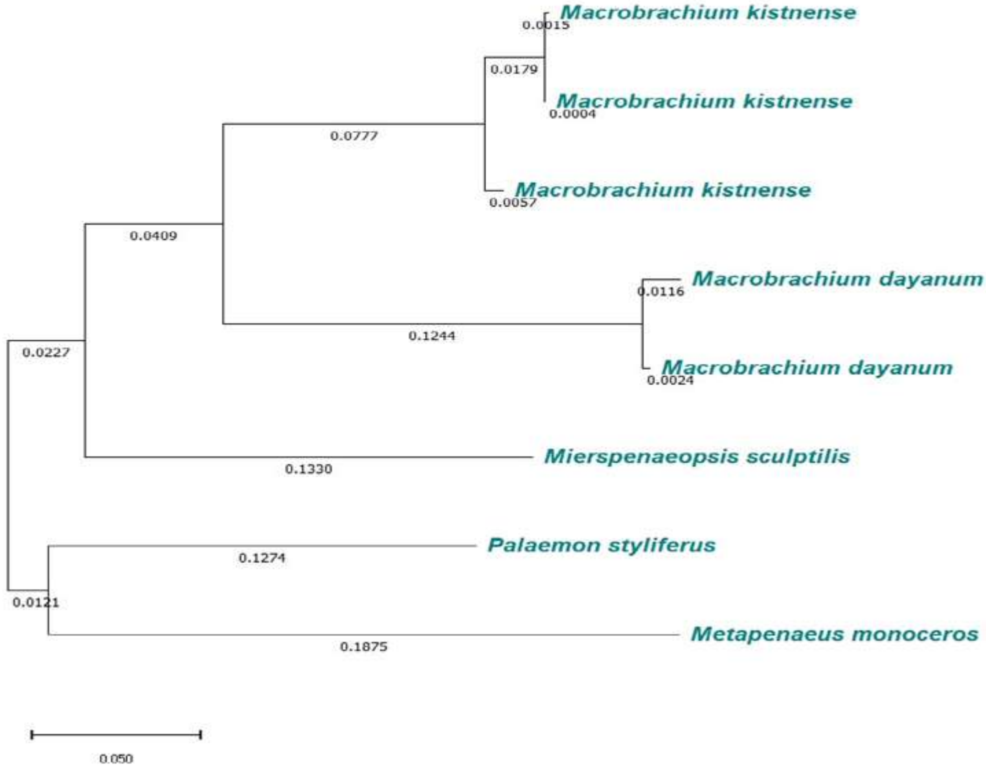


Figure: Diagram of DNA Barcoding process



Production of post larvae (PLs) of *Macrobrachium rosenbergii* at the hatchery

Collection of brine water and Brood Golda: Total 10,000 Liter Brine water (140 ppt) were collected from Mognama, pekua, Cox's Bazar in March/2024. This brine water was used to produce Post larvae of Galda. Berried females were collected from Kocha river Phirojpur.

Figure 5: Collection of brine water and brood female

Hatchery activities:

- Production of post-larvae (PLs) of *M. rosenbergii* commenced in May 2024.
- The larvae were fed twice daily with a mixture of *Artemia* nauplii, Black algae & spirulina powder.
- More than 80 thousands post-larvae were produced.

Figure 6: Activities of Post- larvae of Galda production

Improvement of Breeding and Culture Technique of Cuchia, *Monopterus cuchia*

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: Sharmin Sultana, SO

Objective of the project

i. To develop induced breeding technique of cuchia, *M. cuchia* using hormones;
ii. To improve fry to fingerling of *M. cuchia* rearing technique using different types of feed;
iii. To improve brood management technique for breeding of *M. cuchia*.

Achievements (2023-24)

Expt.1. Improvement of fry rearing technique of *M. cuchia* using different types of feed

An experiment was conducted to improve rearing technique of *M. cuchia* at the cistern (size: $2.76m^2$) complex of Freshwater Station, BFRI, Mymensingh for 90 days. Nine cisterns were selected, dried and cleaned with lime at the rate of 250 kg/ha and then water were supplied from a deep tube well and filled up to the depth of 1 meter. Baby eel or cuchia fry were stocked at the rate of $50/m^2$ in all treatments. In treatment 1, cuchia fry were fed commercial eel feed (50%) comprising with fish meal (50%). In treatment 2, cuchia fry were fed commercial eel feed (70%) and earthworm (30%). In treatment 3, cuchia fry were fed commercial eel feed (50%) and earthworm (50%). Vermi or earthworm was produced in the Vermi Compost Unit. Vermi or earthworm were used as feed ingredients and applied 10 % of total feed utilization. Initial weight of all treatment was 5.43±1.12g, 5.27±1.38g, 5.35±1.82g, respectively. After 90 days of rearing the final weight were 37.81±6.39g in Treatment-1, 56.00±6.59g in Treatment-2, 50.00±4.54g in Treatment-3. Survival rate in treatments were 86.53±0.06%, 90.92±0.08% and 88.76 ±0.04%, respectively.

Table-1 Effects of different types of feed on growth and survival of fry of *M. cuchia*

Treatments	Initial Weight (g)	Final weight (g)	Survival (%)
T_1 {Com. eel feed (50%) +Fish meal (50%)}	5.43 ± 1.12	37.81 ± 6.39	86.53 ± 0.06
T_2 {Com. eel feed (70%) +Earthworm (30%)}	5.27 ± 1.38	56.00 ± 6.59	90.92 ± 0.08
T_3 {Com. eel feed (50%) +Earthworm (50%)}	5.35 ± 1.82	50.00 ± 4.54	88.76 ± 0.04

Fig.1. Initial weight of cuchia fry

Fig. 2. Final weight of cuchia fry

Expt.2. Development of induced breeding technique for Cuchia, *M. cuchia*

The experiment was conducted in cistern ecology during 2nd week of March and continued up to the middle of April. Before hormone application cistern was prepared with soil. Brood were collected from natural sources and matured male and female broods acclimatized for 3-4 days in cemented cisterns. Brood were selected based on visual examination of the sexual characteristics *i.e.*, abdomen and genital opening. Brood treated with different types of hormone (PG, LHRH, gonopro-FH and buserelin). Hormone was injected in the abdomen, in front of the ovary, into each fish body cavity in a single injection. Males were injected with only half the female dose. After hormone administration, the fish were stocked in cisterns for breeding at a 1:1 ratio (Female: male).

Table 2: Different doses of hormone application

Hormone	**Applied doses**	
	Female	**Male**
Trial: 01		
PG	T1: 50.00 mg/kg	25.00 mg/kg
	T2: 60.00mg/kg	30.00mg/kg
	T3: 70.00 mg/kg	35.00 mg/kg
	T4: 80.00 mg/kg	40.00 mg/kg
	T5: 90.00 mg/kg	45.00 mg/kg
Trial: 02		
LHRH	T1: 100 µg/kg	50 µg/kg
	T2: 150 µg/kg	75 µg/kg
	T3: 200 µg/kg	100 µg/kg
	T4: 300 µg/kg	150 µg/kg
	T5: 400 µg/kg	200 µg/kg
Trial: 03		
Gonopro-FH	T1: 0.80 ml/kg	0.40 ml/kg
	T2: 0.90 ml/kg	0.45 ml/kg
	T3: 1.00 ml/kg	0.30 ml/kg
	T4: 1.00 ml/kg	0.50 ml/kg
	T5: 1.50 ml/kg	0.75 ml/kg
Trial: 04		
Buserelin	T1: 100 µg/kg	T1: 50 µg/kg
	T2: 150 µg/kg	T2: 75 µg/kg
	T3: 200 µg/kg	T3: 100 µg/kg
	T4: 250 µg/kg	T4: 125 µg/kg
Trial: 05		
LHRH (1st dose)	T1: 50 µg/kg	25 µg/kg
	T2: 100 µg/kg	50 µg/kg
	T3: 150 µg/kg	75 µg/kg
PG (2nd dose)	T1: 10 mg/kg	5 mg/kg
	T2: 15 mg/kg	7.5 mg/kg
	T3: 20 mg/kg	10 mg/kg

After hormone application cuchia were stocked in the cistern and observed up to 7 days. No mortality showed in treated male and females. Ovulation occurred after 52 hours of trial 5 application but no hatching shown.

Fig. 3. Hormone application with different doses

Expt. 3. Improvement of brood management technique for breeding of *M. cuchia*

The experiment was conducted from July to June. Pond preparation was done at the end of February by setting glass nylon net. Soil was removed around $0.19/m^2$ from the bottom of the pond and then glass nylon net was placed. Removed soil was further used on the glass nylon net. Deep tube well water was provided in pond. For the experiment, control diet (T_1) was formulated using fish paste+ fish meal+ wheat flour and another experimental diet (T_2) was prepared using same ingredients with vitamin A, E and minerals. The proximate composition of two diets was done from nutrition lab of FS, BFRI.

Table 3: Proximate composition of diets (dry weight basis)

No.	Sample	Proximate Composition (dry weight basis)			
		Protein (%)	Lipid/Fat (%)	Ash (%)	Moisture (%)
01.	Diet 1 (T_1)	36.47	4.62	13.56	45.35
02.	Diet 2 (T_2)	41.15	5.79	13.22	39.84

Stocking density of brood cuchia was maintained 45/decimal where male and female ratio maintained as 1:1. Water hyacinth was provided as shade and shelter. Two Treatments each with two replications was maintained and diets were treated as treatment.

About 1224 nos./decimal from treatment-1 and 1350 nos./decimal baby eel collected from treatment-2. Baby eel has been found strong and good in treatment-2 than treatment-1.

Fig. 4. Baby eel collection

Upgradation of pearl quality using different techniques in freshwater mussel

Researcher(s):

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: Mohammad Ferdous Siddique, SSO
: Sonia Sku, SSO
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Objectives of the Project

- To improve pearl quality by using different techniques in lab condition
- To reduce mortality rate of operated freshwater mussel by using different treatment
- To expand the technology through field trial of image pearl culture

Achievements (July– June 2024):

Experiment 1: Post harvest treatment of cultured pearl to increase the pearl quality

Experiment Chamber

A treatment unit with 4 chambers were used for post-harvest treatment. In this treatment unit the temperature were kept in a fixed range for treating the pearl. Temperature was kept in fixed range by using incoming heat and outgoing heat from the chambers.

Methodology

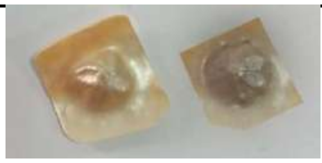
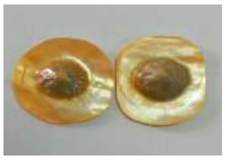
Different image pearls were cleaned with 10% ethanol and then dried. Then pearls were placed in vessels of hydrogen peroxide of different concentrations (50%, 55%, 60%, 65% and 70%). The dried pearls were kept 99% alcohol for 20h and then again kept in new 99% alcohol for another next 20h. After that a liquid solvent mixture of ethanol, methanol, distilled water (6:3:1) at 0.15mg/ml were mixed with 0.5ml of Tween 80. After mixing the chemicals, color reagent was mixed with 0.10mg/ml Mixture was placed under strong fluorescent lights (1,700-11,900 lumens) where they were kept for as long as 6 month at 33-40^0C under intense light and controlled temperature.

Achievement:

A success was found after six month of observation. After six months of chemical treatment image pearl was turned into qualified one. According to table 1, before the chemical treatment the luster of image pearl was 22±0.42, 21±0.22, 21±0.43, 20±0.35 and 22±0.39 lux for, T_5 respectively. While after chemical treatment the luster was increased to 58±0.68, 58±0.87, 61±0.65, 62±0.18 and 64±0.76 for T_1, T_2, T_3, T_4, T_5 respectively. Highest luster was found in T_4 and T_5 comparing to the other treatment, T_1, T_2, T_3 respectively. Shelf life of the treated pearls is monitoring to observe the longevity of the luster of the treated pearls through keeping on open Petri-dishes as long as for a period of 1 year.

Table 1: Image pearl after chemical treatment

Treatment	H_2O_2 concentration (%)	Luster of Before treatment (Lux)	Luster of pearl After treatment (Lux)
T_1	50%	22±0.42 	58±0.68 

T_2	55%	21±0.22 	58±0.87 
T_3	60%	21±0.43 	61±0.65 
T_4	65%	20±0.35 	62±0.18 
T_5	70%	22±0.39 	64±0.76 

Experiment 2: Medicinal treatment to decrease operated mussel mortality during pearl culture

Pond preparation

Ponds were selected for stocking and rearing of collected mussels. Pond was prepared by following standard procedure. Ponds was totally drained out and dried. After drying, lime and salt was applied at the rate of 1kg and 0.25kg per decimal to remove the insect and earthworm. After 6-7 days of liming, pond were filled with water. After pond preparation mussels were collected from different freshwater habitats of Bangladesh. Collected mussels were reared in stocking ponds with foods and fertilizers. After one months of rearing the reared mussels were brought to laboratory before two hours of operation and then mussel were operated for pearl culture. In this experiment *Lamellidens marginalis* and acrylic made powder made image were used. Species were collected from different freshwater regions.

Medicine treatment

Just after operation mussels were given to treatment for 7 days with different medicine in aquarium with the stocking density was 6 mussels /ft^3. Medicine was applied twice a day and water changed regularly. After 7 days of treatment the operated mussels were transferred to the culture pond.

Culture Method

Operated mussel are culturing in pond under net bag hanging method, stocking density of mussels are 120 mussel/decimal. For stimulating and maintaining the growth of natural plankton, organic, inorganic and fertilizers are applying fortnightly to the pond at the rate of 5kg organic manure, 0.125kg T.S.P. and 0.1kg urea per decimal respectively. Lime/$CaCO_3$ powder is applying 0.5kg per decimal respectively. Water temperature, pH, ammonia, DO and Ca^{2+} parameters are monitoring fortnightly. All the data will be compiled and analyzed after the completion of experiment

Table 2: Survival rate during the study period

Treatment	Medicinal ingredient	No. of operated mussels	Average survival rate of operated mussel (%)	Comment
T_1	Chlortetracycline Hydrochloride (Antibiotic) 1gm/3L	500	70	Experiment going on
T_2	Povisep (antiseptic) 1ml/3L	500	67	
Control	No medicine	500	52	

Achievement:

Operated mussels were medicine treated for 7 days in treatment 1 and treatment 2 (table 2) in aquarium with the stocking density of 6 mussels /ft^3 while in control condition no medicine were applied. Medicine was applied twice a day and water changed regularly. After 7 days of treatment the operated mussels were transferred to the culture pond. After three month of observation the survival rate was found in pond condition 70%, 67% and 52% in T_1, T_2 and in control respectively. During the study period highest survival was found in T_1. During the culture period water quality parameter was monitored fortnightly and data recorded. According to current record data water quality parameter was found in suitable range for pearl culture. Water quality parameter was found for temperature 26.55±0.15^{o}C, DO 5.14±0.35 mg/L, pH 7.05±0.36, ammonia 0.04±0.001mg/L and alkalinity 180.00±13.20mg/L.

Experiment 3: On farm trial of BFRI image pearl production techniques

Three different regions of Mymensingh Division were selected for on farm trial of BFRI image pearl production techniques. All the materials and operated mussel were supplied for the farmer in March 2024. At the end of the experiment produced pearl will be collected. After collection complete data will be recorded.

Table 3: Experimental design of on-farm trial

SL	Farmer's name and address	Culture period	Supplied materials	Pictorial view
1	Shuvo Ahmed Upazilla: Fulbaria District: Mymensingh	Pearl quality will be observed after 11 Months of culture	400 *Lamellidens marginalis* mussels inoculated with calcium image and net bag, float,	
2	Shahporan			

	Upazilla: Kolmakanda District: Netrokona		rope, fertilizer	
3	Ridoy Mondol Upazilla: Jamalpur sadar District: Jamalpur			

Achievement: After three month of observation the details about on farm trial is given in table 4

Table 4: On farm trial details

SL no	Location of Farm	Average water quality parameter		Survival
01	Fulbaria, Mymensingh	Temp	30.22±2.33	85%
		pH	7.65±0.44	
		DO	4.735±0.33	
		Ammonia	0.02±0.00	
		Alkalinity	135±7.07	
02	Kolmakanda, Netrokona	Temp	30.15±2.33	89%
		pH	7.71±0.26	
		DO	4.86±0.48	
		Ammonia	0.02±0.01	
		Alkalinity	137.5±4.74	
03	Jamalpur Sadar	Temp	30.5±2.33	88%
		pH	7.56±0.05	
		DO	5.075±0.95	
		Ammonia	0.02±0.00	
		Alkalinity	135±2.21	

Breeding, Seed Production and Culture of Endangered Fish Species in Bangladesh

Researcher(s)

: Dr. Md. Moshiur Rahman, SSO
: Md. Shahin Alam, SO
: Al-Amin, SO

Objectives of the Project: (2023-2024)

- ✓ To collect and domesticate Koitor poa (*Johnius coitor*), Garua (*Clupisoma garua*) and Mohashol (*Tor tor*)
- ✓ To study reproductive biology of the Koitor poa (*Johnius coitor*)
- ✓ To conduct the breeding trial of Ghaura (*Clupisoma garua*)
- ✓ To develop control breeding technique of Shal Baim *(Mastacembelus armatus*)
- ✓ To develop nursery technique of Shal Baim, (*Mastacembelus armatus*) in pond condition
- ✓ To collect indigenous freshwater fish species from different regions for live gene-bank
- ✓

Achievements (2023-24)

Expt.1. Collection and domestication of Koitor poa (*Johnius coitor*), Garua (*Clupisoma garua*) and Mohashol (*Tor tor*)

Fish species were collected from different natural sources like river, haor, beel etc. Collected fish was transported to Freshwater Station using oxygenated bag or drum. After transportation, the fishes were acclimatized in a pond for 1 hour. After acclimatization, the collected fish were stocked in a pond at Freshwater Station, BFRI Mymensingh having an area of 10-30 decimal according to table 1. During stocking, initial length and weight of the collected fish were recorded. The stocked fish rearing is continued by supplying commercial floating feed @ 8-3% body weight once daily. Monthly sampling were done for feed adjustment data.

Table 1. Total collected fish species until June 2024

Collected fish species			Feed
Johnius coitor	***Clupisoma garua***	***Tor tor***	
1500	250	03	Supplementary feed @ 08-03% body weight

Expt. 02. Study of the reproductive biology of the Koitor poa (*Johnius coitor*)

Collection of fish samples

The experiment was conducted from July 2023 to June 2024 for a period of 12 consecutive months, in the Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh. Total number of 10 female and 10 male of Koitor poa (*Johnius coitor*) were collected from Brahmaputra River of Mymensingh in each month, through the fishermen for the determination of fecundity and Gonado-Somatic Index.

Laboratory studies

Individual fish was measured for total length to the nearest cm with a measuring scale and body weight to the nearest g by an electronic balance.

Gonado-Somatic- Index (GSI)

Total body weight and gonad weight of collected fishes in each month was considered to calculate the mean Gonado-Somatic Index (GSI). Gonado-Somatic Index (GSI) was calculated according to Gravimetric method:

$$\text{GSI} = \frac{\text{Weight of gonad}}{\text{Weight of fish}} \times 100$$

Data of Gonado-Somatic Index (GSI) for female and male Koitor poa (*Johnius coitor*) were recorded during study period and month-wise changes in mean GSI values of female and male were collected according to the table 2.

Table 2: Details data and GSI values of female Koitor poa (*Johnius coitor*) for July 23-June 24

Month	No. of fish examined	Body Length of fish (cm)	Body wt. of fish (g)	Ovary wt. (g)	GSI (%)
July	10	12.8–15.4	22.0–68.0	0.24–0.49	0.38
August	10	13.3–15.0	23.0–73.1	0.72–0.83	0.79
September	10	13.7–15.3	25.0–78.2	1.24–2.43	2.02
October	10	14.1–18.2	28.0–88.1	2.72–3.89	3.46
November	10	14.8–19.0	33.0–101.0	3.03–4.97	4.39
December	10	14.7–16.5	38.0–96.0	4.03–6.68	5.89
January	10	14.5-16.3	28.0-70.0	3.29 - 4.12	3.45
February	10	13.56-15.2	23.0-66.0	2.03-3.17	2.71

March	10	13.0-15.0	20.1-50.2	1.41-2.97	1.92
April	10	14.5-16.3	28.0-70.0	0.79 - 1.12	0.87
May	10	13.56-15.2	23.0-66.0	0.43-0.97	0.72
June	10	13.0-15.0	20.1-50.2	0.29-0.59	0.53

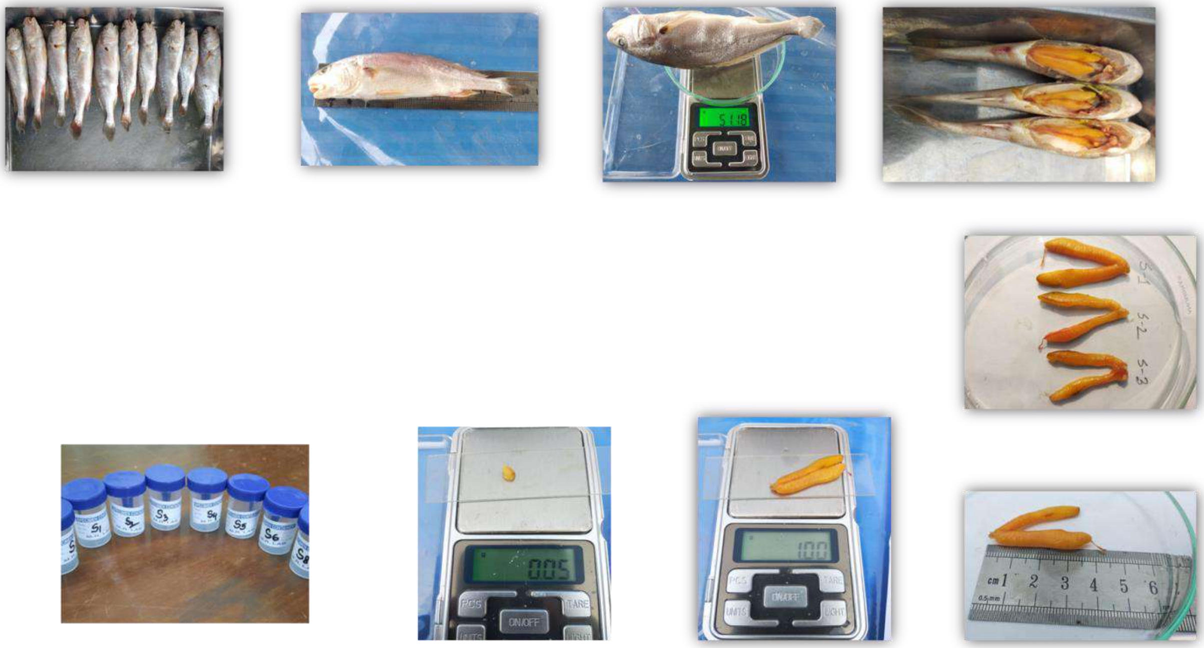


Figure: Procedures of calculating GSI values of female Koitor poa (*Johnius coitor*)

Table 3: Details data and GSI values of male Koitor poa (*Johnius coitor*) for July 2023-June 2024

Month	No. of fish examined	Body Length of fish (cm)	Body wt. of fish (g)	Ovary wt. (g)	GSI (%)
July	10	9.80-12.62	16.20-35.32	0.32-1.36	0.75
August	10	12.40-14.23	19.70-39.62	0.35-0.98	0.86
September	10	12.81-13.45	22.34-43.23	0.32-1.36	1.20
October	10	12.86-14.65	23.12-52.29	0.48-2.14	1.68
November	10	14.61-16.34	28.03-49.41	1.02-2.87	2.56
December	10	15.52-17.29	29.57-65.75	1.32-3.56	3.05
January	10	14.85-16.43	27.14-74.42	1.26-2.97	2.52
February	10	14.10-15.98	24.29-61.32	0.62-1.73	1.35
March	10	11.62-13.54	22.19-36.23	0.46-1.21	1.07

April	10	15.67-17.21	29.45-73.32	0.26-0.98	0.78
May	10	13.18-16.75	26.43-68.46	0.29-0.99	0.61
June	10	15.92-16.61	24.38-49.79	0.19-0.87	0.55

Figure: Procedures of calculating GSI values of Male Koitor poa (*Johnius coitor*)

Table 4. Details of hormone doses on Koitor poa (*Johnius coitor*) and corresponding data on ovulation, fertilization, hatching and survival rates during July 2023- June 2024

Treatments	Mean Body weight (g)		1st Injection dose (mg/kg)		Ovulation period (hr)	Ovulation rate (%)	Fertilization rate (%)	Hatching period (hr)	Hatching Rate (%)	Incubation Temp. (°C)	Remarks
	Male	Female	Male	Female							
T_1	23.30 ±0.09	34.50 ± 0.05	2.0	4.0	-	-	-	-	-	-	
T_2	23.40 ±0.02	32.40 ± 0.19	3.0	6.0	-	-	--	--	-	-	No Ovulation
T_3	25.10 ±0.07	36.00 ± 0.12	4.0	8.0	-	-	-	-	-	-	

Expt. 3: Performing breeding trial of Ghaura (*Clupisoma garua*)

Induced breeding trials of Ghaura (*Clupisoma garua*) will be conducted using PG and other synthetic hormone during June 2024 to August 2024. Hormonal dose will be applied in three treatments according to the table 3.

Table 3: Different amount of hormone doses will be used

Species	Expected time	Type of Hormone	Treatments	Dose of Injection (mg /kg BW)		
				Female		Male
				1st	2nd	
Clupisoma garua	May-August	Synthetic	T_1	0.5	-	1.0
			T_2	1.0		
			T_3	1.5		
		PG	T_1	2.0	10	6.0
			T_2	3.0	14	
			T_3	4.0	16	

Expt. 4: Development of control breeding technique of Shal Baim *(Mastacembelus armatus*)

The experiment were carried out to develop control breeding technique of *M. armatus* in Freshwater station in a controlled environment during February 2024 to August 2024. This experiment were conducted in three treatments with three replications. During pond preparation, 1.0-1.5 feet bottom soil will be removed from mini ponds and then filter net hapa (10m×6m×3m) were placed on the bottom using bamboo poles. After setting the filter net, removed soil were replaced on the net at 1 ft depth. After adding water, aquatic plants like water hyacinth were provided as a shelter. Matured shalbaim (100-250g) were stocked according to table 4. After stocking, fish paste and live prawn were supplied at the rate of 2-3% of estimated body weight. The physico-chemical parameters of experimental hapa such as water temperature, pH, dissolved oxygen and total hardness etc. were observed at monthly interval.

Table. 4: Stocking density of shalbaim (*M. armatus*)

Treatments	Brood shalbaim (pair)	Feed
T_1	10	Fish paste and live prawn
T_2	15	
T_3	20	

Figure: Produced fry of Shalbaim in controlled environment

Expt. 5: Development of nursery technique of Shal Baim, (*M. armatus*) in pond condition

The experiment were designed with 3 treatments, each with 3 replications. Induced bred of 7-day old larvae were used in the experiment. The experimental larvae were divided into three treatment groups and fed with different feeding treatment according to table 5. The experiment will be conducted in hapa (10m × 6m × 3m) for a period of 60 days in pond ecology. During pond preparation, 1.0-1.5 feet bottom soil will be removed from mini ponds and then filter net hapa will be placed on the bottom using bamboo poles. After setting the filter net, removed soil will be replaced on the net at 1 ft depth. After adding water aquatic plants like water hyacinth were provided as a shelter. Spawn were stocked 100 /m^3 in all the treatments. The larvae were fed 5 times a day at 08.00 hr, 12.00 hr, 15.00 hr, 18.00 hr and 21.00 hr upto satiation. The larvae were inspected regularly to record mortality, if any

Table 5. Different feed combination in different treatments

Treatments	Stocking density (m^3)	Feed Combination
T_1	100	Poultry eggs and zooplankton
T_2		Fish paste and live prawn
T_3		only zooplankton

Figure: Nursery Development of *M. armatus*

Improving Feed Formulation and Quality from Conventional and Non-conventional Feed Ingredients Supplementation with Amino Acids for Commercially Important Fish Farming

Researcher(S)

: Dr. Rakhi Das, SSO
: Dr. Mritunjoy Paul, SSO
: Md. Nahiduzzaman, SO
Saymuna Tarin Lupa, SO

Objectives

(i) To evaluate the effect of limiting amino acids in the formulated diets for commercially important Gulsha Tengra (*Mystus bleekeri*) species;

(ii) To develop feed formulation and quality from feed ingredients for Gulsha Tengra (*Mystus bleekeri*) in pond condition

(iii) To improve and validate the *Spirulina platensis* culture technique as fish feed ingredients;

(iv) To evaluate the supplementation of *Spirulina* in the formulated diets for improving growth of Gulsha Tengra (*Mystus bleekeri*) species;

(v) To recommend the potential limiting amino acids and *Spirulina* as feed additives in the formulated diets.

Achievements (2023-24):

A series of feeding trials were conducted to develop quality feeds with supplementation of synthetic amino acids in plant protein-based diets for *L. rohita*. Two feeding experiments on the supplementation of synthetic amino acids in plant protein-based diets (trial-1) consisting of 05 experimental diets and supplementation of a spirulina-based diet (trial-2) consisting of 04 experimental diets of *L. rohita* were conducted in an indoor rearing system of Freshwater Station, BFRI, consisting of a series of cylindrical fiberglass tanks (70-L each) for 60 days. The follow-up best &2nd best diet group of trial-1 in pond conditions was conducted to develop and optimize feeds with supplementation of synthetic amino acids in plant protein-based diets (trial-4). The follow-up diet group of trial-2 with 2% supplementation of Spirulina-based diet showed highest growth and also challenged with *Aeromonas hydrophilla* ($1\text{-}3\times10^5$cfuml-^{1}) of *L. rohita* in lab condition will be monitored for 15 days. The result of trial-2 transferred in pond conditions to develop and optimize Spirulina-based diet (trial-6). To continuously produce Spirulina in low-cost papaya skin medium for the formulation of a spirulina-based diet is another continuous experiment where pure stock of *S. platensis* in kosaric medium (KM) and papaya skin powder medium (PSPM) was maintained in the laboratory. Details of the technical progress of the feeding trials are described below briefly.

Expt. 1: Effects of amino acids in plant protein-based formulated diets for carp species

Experimental procedure and design:

The feeding trial is being carried out in a static indoor rearing system of Freshwater Station, BFRI, consisting of a series of cylindrical fiberglass tanks (70-L each) for 60 days. The same aged and uniform size of *L. rohita* is randomly distributed (1.8 ± 0.05 g) of 20 fish per 70-L fiberglass tank. Aeration is used to maintain an adequate level of dissolved oxygen in each test tank for the study. The initial wt. of fish was taken before the commencement of the feeding trial, and 7-day intervals were used to adjust the daily feed ratio. Water quality parameters such as temperature, pH, dissolved oxygen, and total ammonia are monitored through weekly sampling. At the beginning of the experiment, 5 fish were sacrificed to determine the initial whole-body composition. At the end of the feeding trial, all fish will be weighed, and the survival rate will be determined. Five to seven fish will be removed from each tank to determine whole body carcass composition. Five experimental diets (iso-nitrogenous and iso-energetic) were formulated to contain 30% crude protein and 6% lipid. Before diet preparation, the proximate composition of different feed ingredients, viz. fish meal, mustard oil cake, soybean meal, starch, and rice bran, were analyzed. The carcass composition of the experimental fish, *L. rohita* fingerling, for all the diets was analyzed.

1.1 Proximate composition of the experimental diets

The proximate composition of the five (05) iso-nitrogenous and iso-lipidic experimental diets used for feeding *L. rohita* fingerling in the present study is shown in Table 01.

Table 01: Proximate composition of the experimental diets (% dry weight)

Parameters	**[1]Diets**				
	T_1	T_2	T_3	T_4	T_5
Dry matter (%)	90.25	90.75	90.11	90.28	90.43
Crude Protein (%)	29.98	30.13	30.07	30.18	30.10
Ether Extract (%)	7.08	7.16	7.07	7.15	7.06
Crude Fibre (%)	3.35	3.38	3.52	3.67	3.79
Nitrogen Free Extract (%)	51.38	51.14	51.19	50.58	50.67
Total Ash (%)	8.21	8.19	8.15	8.43	8.38

[1]Diets: T_1 (Control diet); T_2: Fully plant protein-based without EAA (lysine and methionine); T_3: partially replaced with plant protein without EAA; T_4: partially replaced with plant protein adding EAA; T_5: Fully plant protein-based with EAA.

1.2. Physico-chemical parameters of water

The estimated average water quality parameters of the experimental tank were within suitable ranges for the culture of *L. rohita,* where water temperature ranged from 24.40 to 31.34^{o}C and at the same time air temperature ranged from 26.00 to 32.00^{o}C, dissolved oxygen 2.52 to 2.96 mg L^{-1}, ammonia-nitrogen 0.02 to 0.04 mg L^{-1}, total alkalinity 128-141 mg $L^{-1}$and pH 7.8 to 8.05.

1.3. Nutrient utilization, growth, and body indices

In the present study, One-way ANOVA analysis revealed that five dietary treatments significantly *(p* <0.05) affected the growth performance parameters of *L. rohita* fingerling (Table 2). Diet-5, a fully plant-based protein with limiting amino acids (Lysine and methionine), showed the highest wt. gain, wt. gain (%), SGR and lowest FCR among the five dietary groups, whereas diet-4, partially replaced with plant protein with amino acid, showed similar growth patterns.

Table 02: Growth performance of *Labeo rohita* reared in indoor rearing system for the period of 60 days

[1]Diets	Growth Parameter						
	[2]IW(g)	[3]FW(g)	[4]WG(g)	[5]WG (%)	[6]SGR (%/day)	[7]FCR	Survival (%)
T_1	3.22±0.08	8.66^{a} ±0.26	5.44^{b} ± 0.35	168.72^{a} ± 4.61	1.65^{b} ±0.07	1.95^{c} ±0.04	96%
T_2	3.40±0.07	8.19^{a} ±0.07	4.79^{a} ± 0.17	140.61^{a} ± 1.78	1.46^{a} ±0.08	2.23^{d} ±0.03	94%
T_3	3.25±0.01	8.42^{a} ±0.17	5.16^{ab} ±0.18	158.30^{a} ± 5.86	1.58^{b} ±0.04	1.99^{c} ±0.02	98%
T_4	3.16±0.07	10.86^{b} ±0.11	7.54^{c} ± 0.36	219.35^{b} ± 3.87	1.93^{c} ±0.03	1.53^{b} ±0.07	98%
T_5	3.19±0.01	11.11^{b} ±0.05	7.80^{c} ± 0.61	246.61^{b} ± 2.46	2.07^{c} ±0.06	1.43^{a} ±0.02	97%
***p*-value**	0.353	<0.001	<0.001	<0.001	<0.001	<0.001	

Data are expressed as mean ± SE, n=3 (One-way ANOVA) [1]Diets: T_1 (Control diet); **T_2**: Fully plant protein-based without EAA (lysine and methionine); **T_3**: partially replaced with plant protein without EAA; **T_4**: partially replaced with plant protein adding EAA; **T_5**: Fully plant protein-based with EAA; **[2]** IW, Initial weight; [3]FW, Final Weight; [4]WG, Weight gain; [5]WG (%), Weight gain percentage; [6]SGR, Specific growth rate; [7]FCR, feed conversion ratio

1.4. Whole body composition of *Labeo rohita*

According to a one-way ANOVA analysis, there is no significant effect on the body composition of *L. rohita* among five experimental diets (Table 3).

Table 03: Whole body composition (on % wet weight basis) of *Labeo rohita* reared in an indoor rearing system for 60 days

[1]Diets	Body composition
	Initial whole-body composition

	Moisture	Crude protein	Lipid	Total ash	Total carbohydrate
	61.36 ±0.57	29.46±0.45	5.33±0.05	3.4±0.02	1.36±0.05
	Final whole-body composition				
T_1	59.35±0.49	28.36±0.05	4.38±0.06	5.15±0.03	1.95±0.04
T_2	60.72±0.36	28.08±0.32	4.51±0.11	5.34±0.13	2.08±0.05
T_3	61.21±0.54	26.01±0.14	4.76 ±0.25	5.81±0.42	2.24±0.08
T_4	59.32±0.21	26.30 ±0.52	4.68±0.23	5.66±0.33	2.19±0.07
T_5	60.57±0.11	27.24±0.05	4.89±0.09	5.38±0.07	1.87±0.09
***p*-value**	0.123	0.23	0.20	0.08	0.47

Data are expressed as mean ± SE, n=3 (One-way ANOVA);[1]Diets: T_1 (Control diet); T_2: Fully plant protein-based without EAA (lysine and methionine); T_3: partially replaced with plant protein without EAA; T_4: partially replaced with plant protein adding EAA; T_5: Fully plant protein-based with EAA

Expt. 2: Effects of amino acids in plant protein-based formulated diets for Carp polyculture in pond condition

Experimental procedure and design:

Nine experimental ponds (400 m^2 area) situated in the pond complex of Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh, have been selected for the feeding trial. The water depth in each pond has been maintained to a maximum of 1.3 meters. So, all the ponds are being dried and renovated to maintain water depth properly. The follow-up feeding trial was conducted in pond conditions based on results from previous studies. Three experimental diets (iso-nitrogenous and iso-energetic) were formulated using locally available fish feed ingredients such as fish meal, soybean meal, mustard oil cake, corn flour, wheat flour, and rice bran in different combinations to contain 30% crude protein and 7% lipid. The limiting amino acids, (i) Lysine and (ii) Methionine, were added to the diets according to feed formulation. The bite-sized (2.0-4.0 mm) pellet feeds are made from a semi-auto pellet machine.

Table 4: Growth performance of carp species (Rui, Catla, Mrigal, Silver carp, and koi) cultured in polyculture systems after 80 days

[1]Diets	Species	Initial weight	Final weight	Weight gain	Weight gain (%)
T_1	Rui	57.20± 3.35	389.14^a± 5.35	330.24^a± 1.35	580.34^a± 1.35
	Catla	58.54± 3.35	453.66 ^{a} ± 2.35	392.34^a± 3.35	634.00^a± 3.5
	Mrigal	28.30± 5.35	253.66^a± 2.35	225.3^a ± 2.35	802.45^a± 2.35
	Silver carp	77.30± 4.35	219.30^a± 3.35	140.21^a± 1.9 5	185.34^a± 1.35
	Koi	15.46± 1.35	97.41^a± 2.35	80.41^a± 1.67	525.23^a± 3.5
T_2	Rui	56.33± 2.35	392.40^b± 3.35	336.10^b± 2.15	596.40^b± 5.05

	Catla	58.54± 4.35	481.30 ^{b} ± 3.35	422.76^{b} ± 4.05	719.24^{b} ± 1.39
	Mrigal	28.30± 6.35	271.30^{b} ± 3.35	241.23^{b}± 2.05	857.76^{b}± 1.34
	Silver carp	77.30± 4.35	297.30^{b}± 2.35	227.3^{b}± 1.05	286.40^{c}± 5.05
	Koi	15.46± 1.35	125.40^{b}± 3.35	107.40^{b}± 1.35	719.24^{b} ± 1.39
T_3	Rui	57.43± 3.35	408.54^{c}± 3.35	350.21^{c} ± 1.39	613.00^{c}± 2.36
	Catla	58.54± 2.35	475.45 ^{b} ± 4.35	412.86^{b}± 3.15	712.45^{b}± 1.57
	Mrigal	28.30± 4.35	265.45^{b}± 4.35	240.23^{b}± 5.35	845.87^{b}± 3.59
	Silver carp	77.30± 4.35	289.30^{b}± 3.35	212.93^{b}± 4.15	273.43^{b}± 2.36
	Koi	15.46± 1.35	130.69^{c}± 1.35	732.45^{c}± 1.57	732.45^{c}± 1.57

Data are expressed as mean ± SE, n=3; [1]Diets: T_1 (Control diet); **T_2**: partially replaced with plant protein adding EAA; **T_3**: Fully plant protein-based with EAA

Table 5: Yield attributes of carp species (Rui, Catla, Mrigal, Silver carp and koi) cultured in polyculture systems after 60 days

[1]Diets	Average Initial Biomass (kg)	Average Final Biomass (kg)	Average Net Biomass (kg)	Total Feed Given (kg)	[2]FCR
T_1	6.57	35.94^{a}	29.76^{a}	66.36	2.23^{b}
T_2	6.55	42.23^{b}	36.02^{b}	71.33	1.98^{a}
T_3	6.57	41.88^{b}	35.69^{b}	71.03	1.99^{a}

Data are expressed as mean ± SE, n=3; [1]Diets: T_1 (Control diet); **T_2**: partially replaced with plant protein adding EAA; **T_3**: Fully plant protein-based with EAA; [2]FCR, feed conversion rate.

Expt.3. Culture of *Spirulina platensis* in papaya skin powder medium and Zarrouk medium.

The experiment is being carried out on the rooftop of the hatchery complex, Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh. The two types of media viz. papaya skin powder medium **(**PSPM) and Zarrouk medium (ZM) are being used to culture *S. platensis*. For PSPM, one treatment with 3 replications and for ZM with 3 replications is used. Papaya was collected from the local market of Mymensingh, and then the skin of the papaya was removed, dried under the sun for seven days, and then dried overnight in an oven at 50°C. After complete drying, papaya skin was powdered with an electric blender, and then the powder was sieved through a sieve (300µm) to achieve very fine particles. Six plastic jars have been taken, each with a 20-liter capacity. Then, 15 liters of tap water was kept in each jar. Among six jars, nine chemicals were added in three jars, treatment 1, and three chemicals ($NaHCO_3$, Urea, K_2HPO_4) were added in another three jars, treatment 2, with three replications. In treatment 2, 7.5 g papaya skin powder has been added in each jar. After adding prepared media *S. platensis* were inoculated and mixed well gently.

Treatment 1 with three replications has been prepared by adding the required amount of different chemical ingredients with water. After mixing, *S. platensis was* inoculated in the prepared media and mixed well gently. These cultured jars are continuously aerated using an electric aerator. Sampling was taken every alternative day from each bottle to observe *S. platensis* cell density and different water quality parameters of culture media. Sampling was done at every alternative day from each jar to observe *S. platensis* cell density and different water quality parameters of culture media. The pure stock of

S. platensis in kosaric medium (KM) and papaya skin powder medium (PSPM) was being maintained in the laboratory.

Results

Physico-chemical parameters of culture media

The physico-chemical parameters i.e. temperature was ranged 26.00-31.8^{o}C, pH: 9.1-9.03, dissolved oxygen (DO): 5.27-6.08mgL^{-1}, measuring the voltage between a pH sensitive glass electrode (MVPH): 123.70-146.07, total dissolved solid (TDS): 1020.00-3870.00, electric conductivity (EC): 1501.00-2576.00, hectopascal pressure unit (hpa%): 1011.00-1471.00 and salinity: 0.60-4.02 were recorded. Different chemical comparison has been shown in table 6.

Table 6: Chemical required for different treatments to produce Spirulina.

T_1 (KM)		T_2 (PSPM)		T_3 (PSPM with Baking soda)	
Chemical Composition	Quantity (gL^{-1})	Chemical Composition	Quantity (gL^{-1})	Chemical Composition	Quantity (gL^{-1})
$NaHCO_3$ (Lab grade)	16.80	$NaHCO_3$ (Lab grade)	16.80	$NaHCO_3$ (Baking soda)	16.80
$NaNO_3$	2.50	K_2HPO_4	0.50	K_2HPO_4	0.50
NaCl	1.00	Urea	0.67	Urea	0.67
Na-EDTA	0.08	PSP	0.50	PSP	0.50
$CaCl_2$	0.04	-	-	-	-
$FeSO_4$	0.01	-	-	-	-
$MgSO_4$	0.20	-	-	-	-
K_2SO_4	1.00	-	-	-	-
K_2HPO_4	0.50	-	-	-	-

Table 7: Approximate chemical cost of different treatments (15.0L medium) to produce Spirulina for 3 months

T_1 (KM)			T_2 (PSPM)			T_3 (PSPM with Baking soda)		
Chemical composition	Quantity (g)	Cost (Tk)	Chemical composition	Quantity (g)	Cost (Tk)	Chemical composition	Quantity (g)	Cost (Tk)
$NaHCO_3$	252.00	352.80	$NaHCO_3$	252.00	352.80	$NaHCO_3$	252.00	37.80
$NaNO_3$	37.50	187.50	K_2HPO_4	7.50	30.00	K_2HPO_4	7.50	30.00
NaCl	15.00	15.00	Urea	10.05	0.15	Urea	10.05	0.15

Na-EDTA	1.20	1.20	PSP	7.50	-	PSP	7.50	-
$CaCl2$	0.60	0.60	-	-	-	-	-	-
$FeSO_4$	0.15	0.15	-	-	-	-	-	-
$MgSO_4$	3.00	3.60	-	-	-	-	-	-
$K2SO_4$	15.00	21.15	-	-	-	-	-	-
$K2HPO_4$	7.50	30.00	-	-	-	-	-	-
Total cost = 612.00			Total cost = 382.95			Total cost = 67.95		
Production								
T_1(KM)			T_2 (PSPM)			T_3 (PSPM with B.S)		
Wet wt. (g)	Dry wt. (g)		Wet wt. (g)	Dry wt. (g)		Wet wt. (g)	Dry wt. (g)	
700.00	76.0		600.00	60.00		525.00	52.50	

Subsequently harvesting was done in every 12 days. After each harvesting, chemical added partially. The growth of cells was varied in treatment 1, treatment 2 and treatment 3 found. The growth rate of S. *platensis* was higher in T1 than T2and T3but there was no significant difference. Higher growth of cells was found due to the favorable water quality parameter and suitable amount of nutrients. In different treatments growth of S. platensis were observed and showed in Table 10.

Table 8: Cell weight of *S. platensis* between T_1 and T_2

Treatments	Initial weight (gL-1)	Final weight (gL-1)
T1	0.147±0.009	1.056 ± 0.02
T2		1.031 ± 0.02
T3		1.001 ± 0.056

Expt.4. Supplementation of Spirulina in the formulated diet for improving the growth of carp species *(Labeo rohita)*

Experimental procedure and design

The feeding trial is being conducted in a static indoor rearing system of Freshwater Station, BFRI, consisting of a series of glass aquariums (30-L each). The same aged and uniform size of *L. rohita* is randomly distributed (1.8 ± 0.05 g) of 10 fish per 30-L glass jar. Aeration is used to maintain an adequate level of dissolved oxygen in each test jar for the study. The initial wt. of fish was taken before the commencement of the feeding trial, and 7-day intervals were used to adjust the daily feed ratio. Water quality parameters such as temperature, pH, dissolved oxygen, and total ammonia are monitored through weekly sampling. At the beginning of the experiment, 5 fish were sacrificed to determine the initial whole-body composition. At the end of the feeding trial, all fish will be weighed, and the survival rate will be determined. Five to seven fish will be removed from each tank to determine whole body carcass composition. Four experimental diets (iso-nitrogenous and iso-energetic) were formulated to contain 30% crude protein and

7% lipid with 2%, 4%, and 6% spirulina levels in each diet. After that, a challenge test done with *Aeromonas hydrophilla* (1-3×10^5cfuml-[1]) injected intraperitoneally at the end of the feeding trial and the fishes will remain under close observation for 15 days and finally survival rate will be determined.

4.1. Nutrient utilization, growth, and body indices

In the present study, One-way ANOVA analysis revealed that four dietary treatments with different doses of spirulina-based diet significantly ($p < 0.05$) affected the growth performance parameters of *L. rohita* fingerling (Table 5). Diet-2, 2% spirulina supplemented diet showed the highest wt. gain, wt. gain (%), SGR, PER, and lowest FCR among the four dietary groups.

Table 9: Growth performance of *Labeo rohita* fed *Spirulina based diet* indoor rearing system after 8 weeks

Diets[1]	Growth Parameter						
	[2]IW(g)	[3]FW (g)	[4]WG (g)	[5]WG (%)	[6]SGR (%/day)	[7]FCR	Survival (%)
T_1	3.22±0.08	8.56^b ±0.26	5.44^b ±0.35	168.72^b ± 1.61	1.65^b ±0.07	1.95^c ±0.04	96
T_2	3.25±0.07	9.16^c ±0.07	6.21^c ±0.17	190.61^c ± 1.78	1.77^c ±0.08	1.61^a ±0.03	98
T_3	3.16±0.01	8.66^b ±0.17	5.40^b ±0.18	171.30^b ± 0.86	1.66^b ±0.04	1.89^b ±0.02	97
T_4	3.19±0.07	8.29^a ±0.11	5.10^a ±0.36	159.35^a ± 1.87	1.59^a ±0.03	2.13^c ±0.07	96
P value	0.353	<0.001	<0.001	<0.001	<0.001	<0.001	0.882

Data are expressed as mean ± SE, n=3 (One-way ANOVA)) [1]Diets: T_1 (Control diet); T_2:2% Spirulina supplemented diet; T_3: 4% Spirulina supplemented diet; T_4: 6% Spirulina supplemented diet; [2] IW, Initial weight; [3]FW, Final Weight; [4]WG, Weight gain; [5]WG (%), Weight gain percentage; [6]SGR, Specific growth rate; [7]FCR, feed conversion ratio.

Table 10: Relative Percentage Survival (RPS) (%) of challenged *Labeo rohita* fed with Spirulina supplemented diets.

Diets[1]	Initial Fish no.	Final fish no.	Survival	Mortality	Relative Percentage Survival (RPS)
T_1 (Control)	15	8	53.33	46.67	--------
T_2 (2g/kg)	15	11	73.34	26.67	42.80 %
T_3 (4g/kg)	15	10	66.67	33.33	28.59 %
T_4(6g/kg)	15	10	66.67	33.33	28.59 %

[1]Diets: T_1 (Control diet); T_2:2% Spirulina supplemented diet; T_3: 4% Spirulina supplemented diet; T_4: 6% Spirulina supplemented diet;

4.3. Whole body composition of *Labeo rohita*

According to a one-way ANOVA analysis, there is no significant effect on the body composition of *L.rohita* among four experimental diets (Table 11).

Table 11: Whole body composition (on % wet weight basis) of *Labeo rohita* fed spirulina supplemented diet reared in an indoor rearing system for 60 days

[1]Diets	Body composition				
	Initial whole-body composition				
	Moisture	**Crude protein**	**Lipid**	**Total ash**	**Total carbohydrate**
	61.36 ±0.57	29.46±0.45	5.33±0.05	3.40±0.02	1.36±0.05
	Final whole-body composition				
T_1	59.35±0.49	28.36±0.05	4.38±0.06	5.15±0.03	1.95±0.04
T_2	60.72±0.36	30.08±0.32	4.51±0.11	4.34±0.13	2.08±0.05
T_3	57.11±0.34	26.61±0.14	4.06 ±0.25	6.98±0.20	2.74±0.08
T_4	56.32±0.21	26.38 ±0.52	4.38±0.23	6.66±0.33	2.95±0.07
***p*-value**	0.23	0.23	0.76	0.58	0.47

Data are expressed as mean ± SE, n=3 (One-way ANOVA); [1]Diets: T_1 (Control diet); T_2:2% Spirulina supplemented diet; T_3: 4% Spirulina supplemented diet; T_4: 6% Spirulina supplemented diet;

Expt. 5: Supplementation of Spirulina in the formulated diet for improving the growth of carp species *(Labeo rohita)*in pond condition

Experimental procedure and design:

Two experimental ponds situated in the pond complex of Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh, have been selected for the feeding trial. The water depth in each pond has been maintained to a maximum of 1.3 meters. So, all the ponds are being dried and renovated to maintain water depth properly. The follow-up feeding trial was conducted in pond conditions based on results from experiment 04. Two experimental diets (iso-nitrogenous and iso-energetic) were formulated using locally available fish feed ingredients such as fish meal, soybean meal, mustard oil cake, corn flour, wheat flour, and rice bran in different combinations to contain 30% crude protein and 7% lipid with 2% spirulina supplemented diet. The bite-sized (2.0-4.0 mm) pellet feeds are made from a semi-auto pellet machine.

Table 12: Growth parameters of Rui, *Labeo rohita* in different treatments in pond condition.

[1]Diets	Growth Parameter				
	[2]IW(g)	**[3]FW (g)**	**[4]WG(g)**	**[5]WG (%)**	**[5]FCR**
T_1	6.82±0.08	22.56^a ±0.26	15.76^a±1.26	238.72^a ± 1.61	2.10^c ±0.04
T_2	6.85±0.07	30.16^b ±0.07	23.31^b±2.36	340.61^b ± 1.78	1.69^a ±0.03
***p*-value**	0.353	<0.001	<0.001	<0.001	<0.001

Data are expressed as mean ± SE, n=3 (One-way ANOVA); [1]Diets: T_1 (Control diet); T_2:2% Spirulina supplemented diet; [2]IW, Initial weight; [3]FW, Final Weight; [4]WG, Weight gain; [5]WG (%), Weight gain percentage; [6]SGR, Specific growth rate; [7]FCR, feed coefficient rate.

Development of Breeding and Culture Technique of Needle Fish and River Catfish

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Objectives:

- To develop larval rearing technique of Kakila fish in captive condition
- To develop breeding and larval rearing technique of Bacha fish in captive condition
- To develop culture technique of Kakila and Bacha fish in captive condition
- To study the histology of the gonadal development of Bacha fish round of the year

Achievements (2023-24)

Exp. 1: Development of nursing and culture technique of Kakila fish

The **s**tudy was conducted to develop nursing and culture technique of kakila. After hatchling production, brood were transferred to another tank or pond immediately. 1st feeding was started 72 hours of hatching while yolk sacs became absorbed. Chick egg meshed was supplied as first feed. Collected zooplankton was supplied to hatchling with chick egg meshed simultaneously. At the age of 6/7 days, fries were graded according to their size and shifted to another tank for avoiding cannibalism. After 15 days, those graded fingerlings were shifted to different hapa in the earthen pond. Then the fingerlings were placed in the earthen pond for 30 days. Then released to grow out pond for culture. Survival rate was found about 50% in this condition. Probably this occurred due to cannibalism and feeding problem. Feeding was followed as per Table1:

Table 1: Feeding chart of larvae/fry

Spawn/Fry age (Days)	Feed	% Body weight	Feeding frequency (times/day)
03-05	Egg Yolk	40%	4
06-19	Brine shrimp flake + Zooplankton	30%	3
20-29	Brine shrimp flake + Live fish fry	30%	3
30-40	Live fish fry + Shrimp + Live feed	20%	3

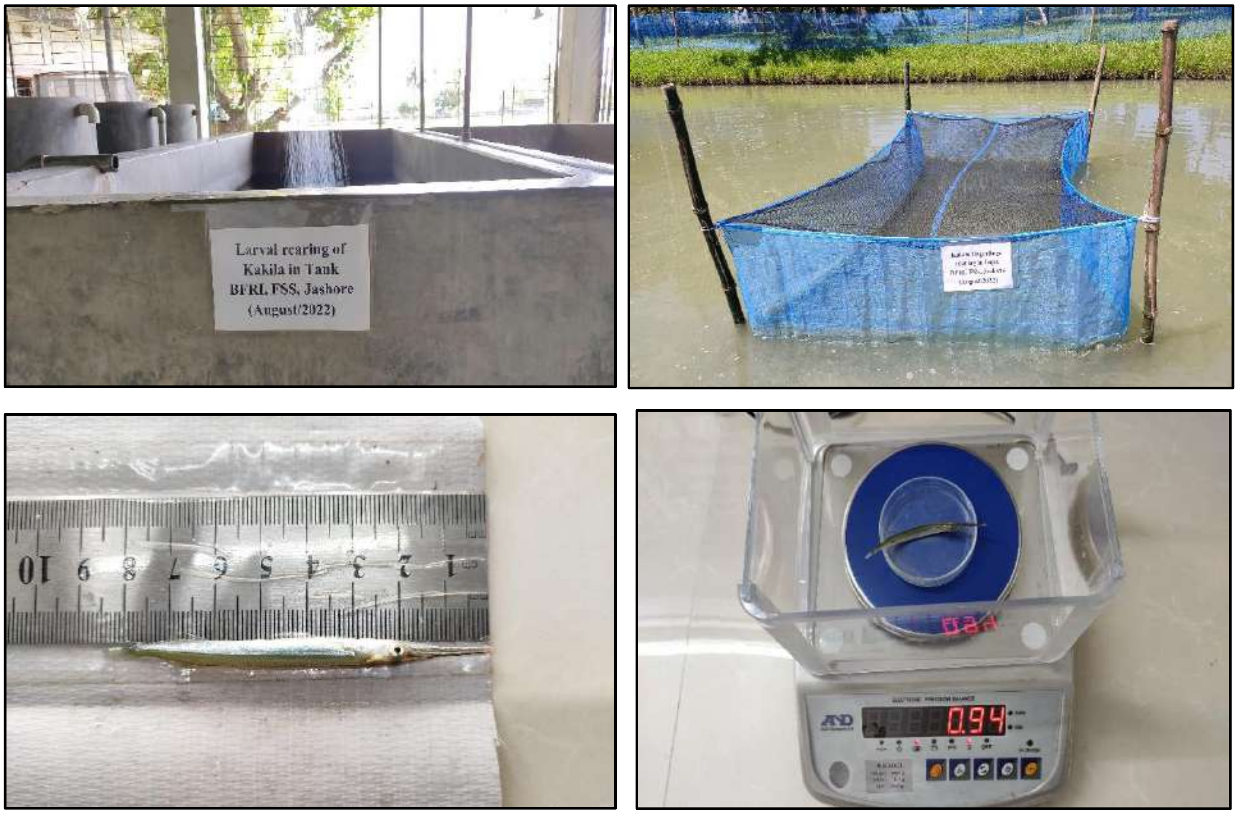


Fig. 01. Nursery technique development of kakila fish

Table 2: Monthly average water quality parameters of Kakila fish pond water of FSS, Jashore

Parameters	Jul/23	Aug/23	Sep/23	Oct/23	Nov/23	Dec/23	Jan/24	Feb/24	Mar/24	Apr/24	May/24	June/24
Temperature (˚c)	30.4±0.14	29.55±0.77	30.4±0.14	29.65±0.56	25.1±0.28	20.1±0.77	20.03±0.14	22.05±0.28	23.9±0.14	31.5±2.12	31±1.41	32.05±0.21
DO (mg/l)	5.05±0.07	4.6±0.14	4.7±0.14	6.21±0.07	5.12±0.14	4.65±0.07	4.55±0.07	4.55±0.07	5.2±0.07	4.85±0.21	4.79±0.07	4.95±0.21
pH	7.87±0.17	7.45±0.07	7.9±0.14	7.27±0.03	8.30±0.07	8.07±0.14	8.18±0.07	8.50±0.07	7.85±0.14	8.0±0	7.95±0.07	7.75±0.07
Total Alkalinity (mg/l)	164.5±0.70	165±0.04	167.5±0.70	151.5±2.12	170.5±0.70	165.5±0.70	165 ± 0.04	170±0.04	163±0.70	166±0	163.5 ± 0.70	165±1.41
Total Hardness (mg/l)	171.5±2.12	174±1.41	171±1.41	168 ± 0.70	171±0.04	166±0.04	167±2.82	171±0.71	168±1.41	169.5±0.70	172.5 ± 0.70	172.5±3.54
NH_3 (mg/l)	0.07±0.03	0.17±0.03	0.15±0.07	0.02±0.07	0.07±0.17	0.05±.07	0.07±0.71	0.04±0.71	0.05±0.07	0.02±.03	0.01±.07	0.07±.03

Water quality parameters were in optimum range during study period

Exp. 2: **Development of breeding and larval rearing technique of Bacha fish**

Methodology

The **s**tudy was conducted to observe growth, gonadal maturation of Bacha (*Eutropiichthys vacha*). For this study, Fishes were stocked in the selected pond with stocking density of 80 dec^{-1}. Fishes were fed with supplementary feed @8% to 3% body weight concurrently daily. The reported result demonstrates the fish's high metabolic activity from May to August.

Table. 03. Showing total length (cm), body weight (gm), GSI (%) of *E. vacha* fish during study period (n=20)

Months	No. of fish examined	Body length (cm)	Body weight (g)	Gonado Somatic Index (GSI) (%)
July/2023	20	19.75± 0.18	60.96± 0.18	0.52
August/2023		20.1± 0.22	61.17±0.13	**0.89**
September/2023		21.33±0.23	62.01±0.15	0.77
October/2023		22.05±0.25	62.32±0.18	0.22
November/2023		22.73±0.29	63.0±0.13	0.37
December/2023		23.0±0.31	63.05±0.19	0.66
January/2024		21.04±0.23	59.53± 0.22	0.42
February/2024		20.64±0.14	56.10± 0.25	0.39
March/2024		20.68±0.13	57.98± 0.15	0.41
April/2024		21.38±0.21	60.38±0.28	0.49
May/2024		21.78±0.17	62.48±0.18	0.54
June/2024		22.90±0.18	63.17±0.25	0.72

After development of brood fish, induced breeding trial will be done with different hormone doses at hatchery by maintaining scientific protocols.

Table. 04: Different hormone doses for Bacha (PG and Synthetic)

Species	Expected time	Type of Hormone	Treatments	Dose of Injection (mg /kg BW)		
				Female		Male
				1st	2nd	
E. vacha	July-August	Synthetic	T_1	0.5	-	1.0
			T_2	1.0	-	
			T_3	1.5	-	
		PG	T_1	3.0	6.0	3.0
			T_2	6.0	9.0	6.0
			T_3	7.0	12.0	6.0

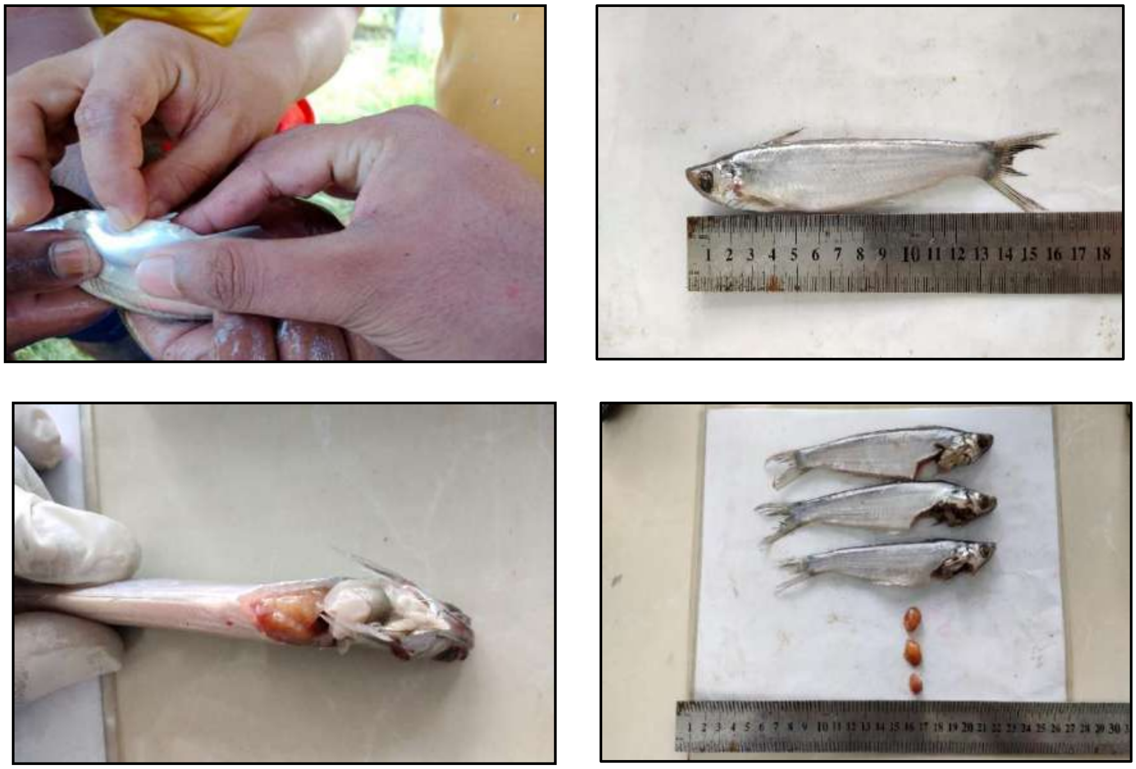


Fig. 02. GSI and fecundity observation

Table 5: Monthly average water quality parameters of Bacha fish pond water of FSS, Jashore

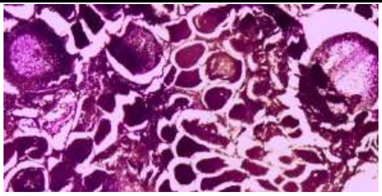
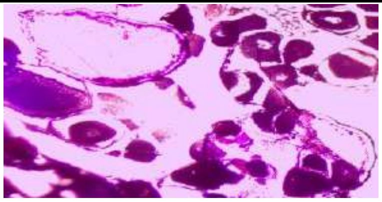
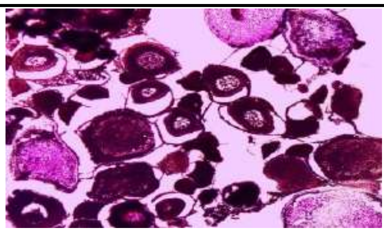
Parameters	**Jul/23**	**Aug/23**	**Sep/23**	**Oct/23**	**Nov/23**	**Dec/23**	**Jan/24**	**Feb/24**	**Mar/24**	**Apr/24**	**May/24**	**Jun/24**
Temperature (^{0}c)	30.4 ±0.28	29.55 ±0.77	30.4± 0.14	29.65 ±0.63	28.2±0.28	21.7± 0.56	21.01± 1.92	23.5± 0.42	28±0.14	33.5± 0.70	31± 1.41	32.1± 0.57
DO (mg/l)	5.05 ±0.07	4.6± 0.14	4.7±0.14	4.65± 0.07	4.7±0.14	4.65± 0.07	4.55± 0.07	4.55± 0.07	4.9± 0.07	4.95±.07	4.75± 0.07	4.8±0.14
pH	7.87 ±0.17	7.45 ±0.07	7.9±0.14	7.72± 0.03	7.85±0.07	7.9±0.14	7.95± 0.07	7.75± 0.07	8.0±0.14	8.0±0	7.77± 0.03	7.85± 0.07
Alkalinity (mg/l)	164.5 ±0.7	165± 0.14	167.5 ±0.70	165±0.07	166.5± 0.70	165.5 ±0.7	166 ± 0.04	171± 0.04	163±0.70	166.5 ±0.70	163.5± 0.70	164±1.41
Hardness (mg/l)	171.5 ±2.12	174± 1.41	171± 1.41	171.5 ±2.12	172.5± 0.70	170.5 ±0.70	169± 2.82	170± 0.71	174±1.41	175.5 ±0.70	171± 1.41	171.5 ±0.70
NH_3(mg/l)	0.07 ±0.03	0.17 ±0.03	0.15± 0.07	0.25± 0.03	0.07±0.17	0.1±0.07	0.07± 0.17	0.04± 0.07	0.05±0.14	0.15± 0.07	0.02±0.07	0.07± 0.17

Water quality parameters were in optimum range during study period.

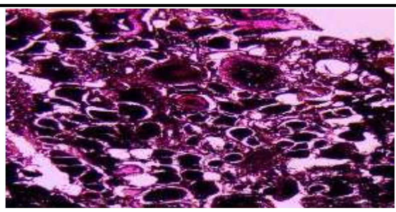
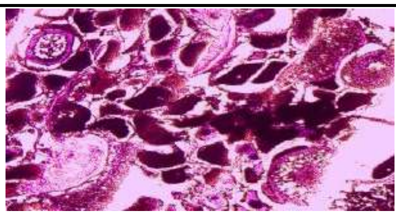
Exp.03: Histology study of kakila and Bacha fish round of the year

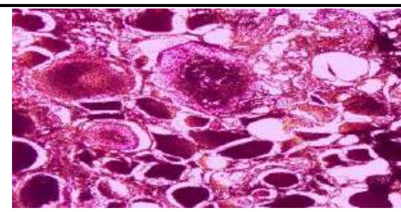
Gonado Somatic Index (GSI) of Kakila and Bacha fish were estimated monthly. Collected Gonad were preserved and send to BFRI Shrimp Research Station, Bagerhat for histological study.

Histological observation of Kakila

Gonad stage	Characteristics	Figure	Months
Immature gonad	1. Only young females possess this stage 2. Undeveloped oocyte (UO) and pre mature oocyte (PMO) are well organized 3. This stage does not present follicles in vitellogenesis		**April& May**
Developing gonad (early perinucleolar stage)	1. At this stage the females are considered entering the reproductive cycle 2. Oogonia, PG oocytes, CA oocytes are predominant 3. As maturation progresses, the quantity of vtg oocytes increases		**Early June**
Developing gonad (late perinucleolar stage)	1. Females are considered entering the reproductive cycle 2. Oogonia, PG oocytes, CA oocytes are predominant 3. Follicles with CA and PG 4. As maturation progresses, the quantity of vtg oocytes increases		**Late June**

Histological observation of Bacha

Gonad stage	Characteristics	Figure	Months
Immature gonad	1. Only young females possess this stage. 2. Oogonia and primary PG follicles are well-organized in the ovigerous lamellae. 3. This stage does not present follicles in vitellogenesis.		**April& May**
Developing gonad (early perinucleolar stage)	1. At this stage the females are considered entering the reproductive cycle. 2. Oogonia, PG oocytes, CA oocytes are predominant 3. As maturation progresses, the quantity of vtg oocytes increases		**Early June**

Developing gonad (late perinucleolar stage)	1. Females are considered entering the reproductive cycle. 2. Oogonia, PG oocytes, CA oocytes are predominant. 3. As maturation progresses, the quantity of vtg oocytes increases		**Late June**

Assessment of existing hatchery management practices and dissemination of BFRI evolved improved germplasm in Jashore region

Researchers

: Mollah N S Mamun Siddiky, SSO
: Nasima Begum, SO
: Md. Rakibul Islam, SO

Objectives:

- To assess the present status of fish seed production and hatchery management practices in Jashore
- To identify the major problems and constraints in fish hatchery and nursery management
- To evaluate the role of value chain actors and their functions for producing quality fish seed
- To improve the quality of existing brood stock of tengra , pabda, gulsha, and BFRI Suborno Rui in Jashore region through dissemination of BFRI evolved improved germplasm.

Achievements (2023-24):

Experiment/study: Rearing of BFRI improved germplasm (Suborno Rui) brood in FSS pond complex and distribution to the selected hatcheries in Jashore region.

The study was conducted to observe the development of BFRI improve germplasm Suborno rui (*Labeo rohita*). For this study, ponds were selected in BFRI, FSS campus, Jashore. Ponds were prepared by drying followed by liming (1 kg dec^{-1}), fertilization with organic fertilizer (5-6 kg dec^{-1}) as well as inorganic fertilizer (100g decimal^{-1} urea & 75g dec^{-1} TSP). Improved germplasm of Suborno rui fingerlings were collected from BFRI Freshwater Station, Mymensingh and were stocked in the ponds of BFRI, FSS, Jashore. The stocking density was 8-10 dec^{-1}. Fishes were fed with formulated feed and supplementary feed @10% to 3% body weight concurrently daily. Length-weight and water quality parameters (temperature, DO, pH, alkalinity, ammonia) data were monitored (Figure 1, 2 ,3 and table 1) and growth parameters were calculated. These improved Suborno rui fingerlings will be reared around 2-3 years to attain brood size.

Table 1: Water quality parameters of experimental ponds

Parameters/ Date	Temp (°c)	DO (mg/l)	pH	NH_3(mg/l)	TA (mg/l)	TH(mg/l)
23-Aug	28.22±0.18	4.8±0.22	7.5±0.11	0.08±0.03	165.5±2.12	170.5±2.5
23-Sep	29.25±0.86	4.55±0.06	7.61±0.16	0.1±0.07	165.5±0.7	169.5±0.6
23-Oct	27.81±0.61	4.59±0.09	7.55±0.04	0.02±0.001	166±0.70	171.5±1. 7

23-Nov	28.5 ±0.11	4.3±0.18	7.64±0.15	0.08±0.03	165.5±2.12	170.5±2.7
23-Dec	25.25±0.29	4.35±0.05	7.63±0.15	0.1±0.07	165.5±0.7	169.5±3.8
24-Jan	**19.41±1.30**	4.88±0.03	7.45±0.02	0.02±0.004	166±0.70	171.5±0.7
24-Feb	22.2±0.65	4.95±0.09	7.73±0.06	0.03±0.002	167.5±1.41	170.5±2.9
24-Mar	24.2±0.14	4.77 ±0.03	7.62±0.24	0.02±0.001	167.5±2.12	171±1.41
24-Apr	**31.0±0.9**	4.89 ±0.05	7.33±0.21	0.01± 0.004	167.8±2.81	169±2.32
24-May	30.5±0.11	4.84 ±0.03	7.82±0.31	0.03± 0.002	166.15±3.1	170±2.58
24-Jun	29.7±0.17	4.99 ±0.06	7.51±0.11	0.03± 0.003	166.44±2.5	171±3.21

From the above mentioned table 1 the highest temperature was in April and the lowest temperature was in January. Due to climate change the temperature was higher compare to previous year. All water quality parameters were in suitable range for fish culture.

Figure 1 : Growth monitoring of Suborno rui

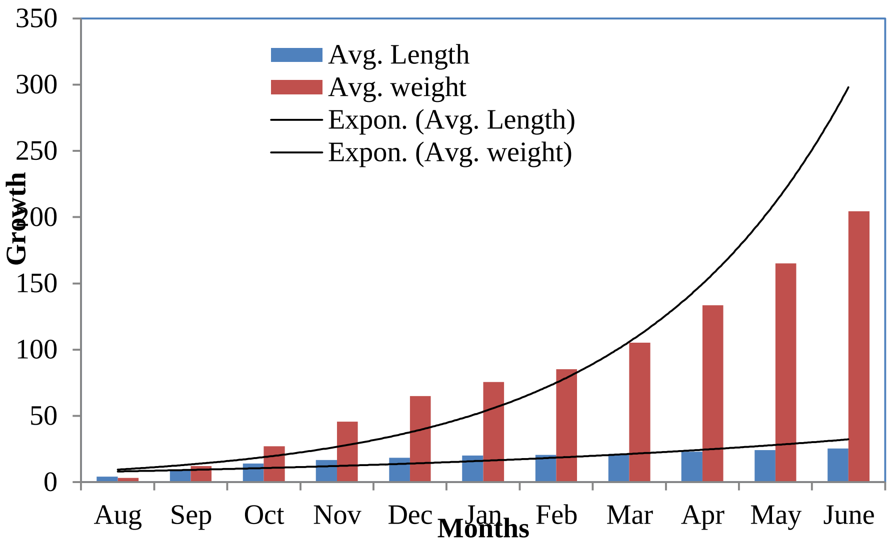


Figure 2: Month wise growth of Suborno rui

The initial weight of Suborno rui was 3.2 ±0.33 g and weight till June was 255 ± 3.4 g. From the growth graph it is observed that the growth of Suborno rui was uniform and it was exponential.

Figure 3: Pictorial view of Suborno Rui

After rearing of these improved rui fishes around 2-3 years, those improved brood rui will be distributed to the govt. registered renowned private hatcheries in Jashore to produce mass seed production in Jashore region.

12.2. Name of the experiment: Comparative growth study of *Mystus tengara* among BFRI evolved improved germplasm, wild source and hatchery produced.

The study were conducted to observe the development of *Mystus tengara* of BFRI improve germplasm wild source and hatchery produced. For this study, ponds were selected in BFRI, FSS campus, Jashore. Ponds were prepared by drying followed by liming (1 kg dec^{-1}), fertilization with organic fertilizer (5-6 kg dec^{-1}) as well as inorganic fertilizer (100g $decimal^{-1}$ urea & 75g dec^{-1} TSP). Improved germplasm of tengra fry were collected from BFRI Freshwater Station, Mymensingh, hatcheries from Jashore region and wild source (Modhumoti, Chitra and Gorai river). Then they were stocked in the ponds of BFRI, FSS, Jashore. The stocking density were 500 ind/dec^{-1}. Fishes were fed with supplemented floating fish feed @10% to 3% body weight concurrently daily. Length-weight and water quality parameters (temperature, DO, pH, alkalinity, ammonia) data were monitored (table 2,3) and growth parameters were calculated monthly.

We have collected *M. tengara* from three sources *i.e.* BFRI evolved, hatcheries from Jashore region and wild source (Bhairab river of Meherpur and Betna River of Satkhira).

Table 2: Water Quality Parameters of experimental ponds

Parameters	Temp (°c)	DO (mg/l)	pH	NH_3(mg/l)	TA (mg/l)	TH(mg/l)
Feb/24	22.2±0.65	4.95±0.09	7.73±0.06	0.03±0.002	167.5±1.41	170.5±2.9
March/24	24.2±0.14	4.77 ±0.03	7.62±0.24	0.02±0.001	167.5±2.12	171±1.41
April/24	31.0±0.9	4.89 ±0.05	7.33±0.21	0.01± 0.004	167.8±2.81	169±2.32
May/24	30.5±0.11	4.84 ±0.03	7.82±0.31	0.03± 0.002	166.15±3.1	170±2.58
June/24	29.7±0.17	4.99 ±0.06	7.51±0.11	0.03± 0.003	166.44±2.5	171±3.21

From the above mentioned table 4 the highest temperature was in April and the lowest temperature was in February. Due to climate change the temperature was higher compare to previous year. All water quality parameters were in suitable range for fish culture.

Figure 4: Growth monitoring of Tengra

Table 3: growth parameters of *M. tengara*

Months	Avg. Length			Avg. weight		
	T_1(BFRI)	T_2(Hatchery)	T_3(Wild)	T_1(BFRI)	T_2(Hatchery)	T_3(Wild)
March	3.82±0.77	3.86±0.11	3.80±0.52	0.58±0.94	0.66±0.83	0.62±0.78
April	5.72±1.40	5.88±0.72	5.95±0.78	5.07±0.10	4.72±0.58	4.88±0.52
May	8.26±0.79	8.16±0.36	8.17±0.24	8.32±1.21	8.18±0.77	7.86±0.24
June	9.32±0.36	9.18±0.18	8.86±0.40	12.42±0.87	12.13±0.2	11.83±1.17

The initial weight of Tengra of three treatments were 0.58 to 0.66 g and the final weight till June were 11.83 to 12.42g. The highest growth performance was found in T_1.

Figure 5: Pictorial view of *M. tengara*

After completion of six months of culture period, those improved broods will be distributed to the govt. registered renowned private hatcheries in Jashore to produce mass seed production in Jashore region.

Culture suitability of *Barilius barila*, *Labeo angra* and *Labeo dero* under Polyculture in Farmers Pond of Northern Region of Bangladesh

Researcher(s) Dr. Azhar Ali, PSO
Mst. Sonia Sharmin, SSO
Maliha Hossain Mou, SSO
Srebash Kumar Saha, SO

Objectives of the project:

- To evaluate the production potentials of *Barilius barila*, *Labeo angra* and *Labeo dero* under polyculture system in seasonal water bodies of farmer's field;
- To assess the water quality parameters of cultured water bodies;
- To assess the BCR of culture technologies and
- To disseminate suitable culture techniques of *Barilius barila*, *Labeo angra* and *Labeo dero* in different aqua-ecological zones in the northern part of Bangladesh.

Achievements in 2023-24):

Development of polyculture technique of *Labeo dero* with other carp species in farmer's ponds (2023-24)

For this study, a total of 06 (six) seasonal ponds were selected in 06 (six) different Upazila of Rangpur division (Table 1) with the concerning of relevant Upazilla Fishery Officer (UFO/ SUFO). The six (06) ponds were divided into three groups. Each group treated as one treatment e.g. treatment-I (T_1), treatment-II (T_2), treatment-III (T_3) and each pond were considered as one replication. The list of location wise selected ponds and experimental design are presented in Tables 1 and 2 respectively.

Table 1. Location wise list of selected farmer's ponds

Sl.No.	Location (Upazila, district)	No. of farmer's pond	Pond area (decimal)
1	BFRI, Saidpur, Nilphamari	01	15
2	Saidpur, Nilphamari	01	14
3	Sadar, Nilphamari	01	12
4	Chirirbandar, Dinajpur	01	13
5	Khanshama, Dinajpur	01	14
6	Taraganj, Rangpur	01	12

Pond preparation

The ponds were prepared by dewatering, liming and fertilization. The spawn was stocked in the prepared ponds as per experimental design (Table 2). Before beginning the experiment, the native and unwanted fishes were removed from the pond by repetitive netting then ponds were completely dried to eradicate unwanted crustaceans/fishes/eggs that remained in cysts. Pond embankments were prepared. Netting and fencing were done for complete restriction of unwanted predators like animals/birds. Pond bottoms were treated with lime at the rate of 1 kg/decimal and left for 3 days. Then the ponds were filled with groundwater and fertilized with Urea and TSP 1.15 and 1.5 ppm respectively. After sufficient phytoplankton production fingerlings of Kursha and other fish species were stocked according to the stocking densities presented in Table 2 and the Initial length and weight of fingerlings were measured before releasing into the pond are showed in Table 3 respectively.

Table 2. Polyculture of *Labeo dero* (Pattern-III) with other carps in the farmer's ponds

Treatments	Species combination	Stock. Density (indi. dec.$^{-1}$)	Fingerlings size
T_1	Kursha+Rui+Catla+Silver carp+Sarpunti+ GIFT	250+6+4+5+10+5	
T_2	Kursha+Rui+Catla+Silver carp+Sarpunti+ GIFT	300+6+4+5+10+5	Over wintered
T_3	Kursha+Rui+Catla+Silver carp+Sarpunti+ GIFT	350+6+4+5+10+5	

Stocking of fingerling and feeding managements

- The ponds were stocked as per experimental design (Table 2) on May 2024.
- The fish are being supplied with commercial feed @8-5% body weight containing (30-35%) protein.

Table 3: Stocking of Kursha in the farmer's pond with different stocking densities

Parameters	**Treatments**		
	T_1	**T_2**	**T_3**
Stock. Dens. (indi.dec^{-1})	250	300	350
Culture Period	150	150	150
Initial Length (cm) of Kursha	5.4±0.4	5.3±0.1	5.5±0.2
Initial weight (g) of Kursha	1.50±0.1	1.48±0.1	1.53±0.1

After 45 days of culture, the growth performances of Kursha and physico-chemical parameters of the experimental ponds are presented in Tables 4 and 5 respectively.

Table 4: Growth performances of *Labeo dero* under polyculture system in the farmer's pond (up to June,2024)

Parameters	Treatments		
	T_1	T_2	T_3
Stock. Dens. (indi.dec^{-1})	250	300	350
Culture Period	150	150	150
Initial Length (cm)	5.4±0.4	5.3±0.1	5.5±0.2
Initial weight (g)	1.50±0.1	1.50±0.1	1.51±0.1
3rd Sampling weight (g)	8.1±1.20	7.4±1.0	6.3±1.10
Weight gain (g)	6.6±1.2	5.9±1.04	4.79±0.98
% weigh gain	14.66±1.5	13.11±1.2	10.67±1.3
ADGw (g day^{-1})	0.15±0.2	0.13±0.1	0.111±0.1
SGRw(g day^{-1})	3.74±0.6	3.54±0.2	3.18±0.3

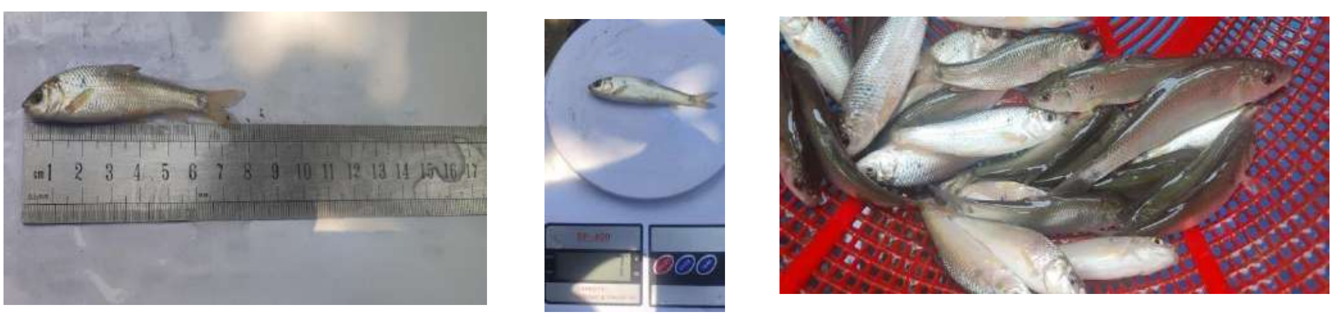


Fig.: 1a Fig.:1b Fig.:1c

Figure 1. Measuring length (1a), weight (1b) and sampling (1c) of *Labeo dero* fingerling.

Table 5. Physico-chemical parameters of the experimental ponds

Parameters	T_1	T_2	T_3
Water Temperature(°C)	30.2±3.2	29.8±2.4	28.9±2.3
DO (mgL^{-1})	6.62±0.23	6.84±0.40	6.32±.34

DO Saturation (%)	107.36±4.42	106.4±4.47	75.6±7.45
pH	7.93±.12	7.46±.09	7.18±.08
mVpH	-72.1±9.27	-56.2±1.2	-62.65±3.4
Conductivity (μScm^{-1})	136.71±18.7	155.12±.6.32	125.2±5.3
Absolute Conductivity (μScm^{-1})	114±8.96	123±6.87	142±4.5
Oxidation Reduction Potential (mV ORP)	130.82±34.78	145.8±22.65	148.45±12.78
Alkalinity (mgL^{-1})	92.4±7.65	102.5±6.3	98.4±7.2
Salinity (mgL^{-1})	0.05±0.01	0.03±0.0	0.02±0.0
Total Dissolved Solids (mgL^{-1})	53.42±8.44	63.72±6.23	72.52±3.2
Hardness (mgL^{-1})	111.42±4.89	112.22±27	103±33

Above study is being conducted for 5 month of culture period and final harvest will be done 2024. Stocking densities of 250, 300 and 350 ind./dec designate as T_1, T_2 and T_3 respectively. The initial weight of Kursha was 1.5g and up-to June 2024 highest weight gain was observed in T_1 was 6.6g, whereas 5.9g and 4.7g found in T_2 and T_3 respectively. Specific growth rate was highest in T_1 3.74 (g day^{-1}) compare to other treatments. T_1 already having good growth performance compare to other. The reason may be lowest stocking densities having highest feeding access. Water temperature, pH, DO, Alkalinity TDS and hardness was suitable in each sampling period. This sampling will continue until final harvest on October 2024. FCR, BCR and survival rate of total fish production will be analyzed.

Study on the Present Status of Aquatic Biodiversity of Teesta and Its Adjacent Rivers

Researcher(s) : Maliha Hossain Mou, SSO & PI
: Dr. Azhar Ali, PSO
: Mst. Sonia Sharmin, SSO
: Srebash Kumar Saha, SO

Objectives:

- To assess aquatic biodiversity of Teesta and its adjacent rivers.
- To determine spatio-temporal variability of biodiversity status of Teesta and its adjacent rivers.
- Complete cataloging of fish species of Teesta and its adjacent rivers.

Achievements in the past (2023-24)

Site Selection

The study was carried out in six sampling sites of Teesta and its adjacent river which are located across four districts. These are-1. Teesta Barrage, Hatibanda, Lalmonirhat 2. Belka, Sundarganj, Gaibanda; 3. Tepa Madhupur, Kaunia, Rangpur; 4. Kolkonda, Gangachara, Rangpur; 5. Dalia, Joldhaka, Nilphamari and 6. Betgari, Gangachara, Rangpur.

Water quality parameter observation

During this research period water quality parameters of Teesta and its adjacent rivers was recorded monthly. All data was collected by using Hanna digital multiparameter. Data showed in the following Table 1,

Table 1: Physico-chemical parameters of water of Teesta and its adjacent rivers

Parameters (Unit)	Teesta (Mean ± SE)	Buri Teesta (Mean ± SE)	Ghaghat (Mean ± SE)
Water Temperature(°C)	26.84±2.67	28.02±2.31	27.58±3.65
DO (mgL^{-1})	5.86±0.23	5.14±0.40	6.04±.34
DO Saturation (%)	102.36±4.42	106.4±4.47	75.6±7.45
pH	7.73±.12	7.64±.09	7.18±.08
Conductivity (μScm^{-1})	126.71±18.7	145.12±.6.32	165.2±5.3
Absolute Conductivity (μScm^{-1})	124±8.96	143±6.87	162±4.5
Oxidation Reduction Potential (mV ORP)	201.82±34.78	206.65±22.65	148.45±12.78
Resistivity (KΩcm)	0.0088± 0.003	0.0068±0.002	0.0059±0.001
Alkalinity (mgL^{-1})	56.4±7.65	52.5±6.3	53.94±7.2
Salinity (mgL^{-1})	0.07±0.01	0.07±0.0	0.08±0.0
Total Dissolved Solids (mgL^{-1})	63.42±9.44	73.72±6.23	82.52±3.2
Hardness (mgL^{-1})	104.42±4.89	106.22±27	113±33

Fig.: 1a

Fig.:1b

Fig.:1c

Fig.: 1d

Fig.:1e

Fig.:1f

Figure 1. Sampling of different sampling sites (1a) Water quality parameters meeasurement at Teesta rivers (1b) Ghaghat river (1c) Plankton collection (1d) Benthos collection (1e) water quality parameters at Kaunia Teesta point (1f) Depth measuring at Buri Teesta rivers.

Phytoplankton and Zooplankton availability in Teesta and Its Adjacent River

Phytoplankton and Zooplankton was collected from sampling site by using plankton net, preserved in 7 to 10% buffered formalin solution and taken into FSS, Saidpur Laboratory. Up to this research period 23 species of phytoplankton and 11 species of were zooplankton were identified. Among the phytoplankton, there were 9 species of bacillaryophyta, 6 species of charophyta, 4 species of chlorophyta, 2 species of cyanophyta and 2 species of dinophyta. Among the zooplankton, there were 4 species of Copepoda, 3 species of Cladocera, 2 species of Rotifera and Crustaceans larvae. Qualitative and quantitative abundance of phytoplankton and zooplankton are presented in Table 2, 3 and 4 respectively.

Table 2: List of Phytoplankton identified up to research period of Teesta and its adjacent rivers.

Bacillariophyta	Cyanophyta	Charophyta	Chlorophyta	Dinophyta
Cyclotella	*Spirunila*	*Spirogyra*	*Chlorella*	*Dinophysis*
Fragilaria	*Oscillatoria*	*Closterium*	*Ulothrix*	*Ceratium*
Navicula		*Cosmarium*	*Pediastrum*	
Melosira		*Micrasterias*	*Scenedesmus*	
Asterionella			*Volvox*	
Biddulphia			microspora	
Surirella				
Tabellaria				
Synedra				

Fig.: 2a

Fig.:2b

Fig.:2c

Figure 2. Phytoplanktons such as (2a) Ulothrix (2b) Navicula (2c) Pediastrum etc. of Teesta and adjacent rivers

Table 3: List of Zooplankton identified up to research period of Teesta and its adjacent rivers.

Copepoda	Rotifera	Cladocera	Crustacean larvae
Diaptomus	Brachionus	Dyaphanosoma	Nauplius
Bosmina	*Notholka*	Moina	*Skistodiaptomus*
Cyclops			

Table 4: Counting of Phytoplankton and Zooplankton of Teesta and its adjacent rivers

River Name	Phytoplankton (Cells/L)	Zooplankton (Cells/L)
Teesta	10.23×10^3	1.19×10^3
Buri Teesta	10.97×10^3	1.34×10^3
Ghaghat	9.13×10^3	0.99×10^3

Fig.: 3a

Fig.:3b

Fig.:3c

Figure 3. Zooplanktons such as (2a) Diaptomus (1b), (3b) Cyclops and (3c) Dhapnia etc. of Teesta and adjacent rivers

12.5 Available Benthic invertebrate in the Teesta and its adjacent rivers

Benthic community was collected from sampling sites by using Ekman Dredge and taken into FSS, Saidpur Laboratory. Collected specimen showed in the following Table 4 as follows,

Table 5: List of identified Benthos in FSS, Saidpur Laboratory

Class	Family	Local Name	Scientific Name
Bivalvia	Unionidae	Unio	*Unio sp.*
	Sphaeriidae	Musculium	*Musculium sp.*
Gastropoda	Limnaedae	Patla shamuk	*Limnaea sp.*
	Unionidae	Jhinuk	*Lamellidens marginalis*
Insecta	Viviparidae	Choto shamuk	*Viviparous bengalensis*
	Paludomidae	Choto gulshamuk	*Paludomus conica*
Branchiura	Argulidae	Fish lics	*Branchiura sp.*

12.6 Fish species cataloging of Teesta and adjacent rivers

Almost 09 fish species sample were collected and carried to the FSS laboratory for morphometric and meristic character analysis. Fish species is being under cataloging process and it will continue throughout next year. Fish species were catalog based on following criteria;

1) Name
2) Classification
3) Figure
4) Morphometric and meristic character
5) Habitat/ distribution
6) Red list status in Bangladesh

Table 6 showed the already catalog fish species.

Table 6: List of Fish species under catalog (June, 2024)

SI	Species name	Scientific name	Common name	Habitat	Red List Status
1	Boirali	*Barilius barila*	Barred baril	Teesta	LC
2	Jarua	*Chigunious chagunio*	Chaguni	Atrai	EN
3	Kala bata	*Crossocheilus latius*	Gangetic Latia Stone roller	Teesta	EN
4	Balachta	*Somileptes gongota*	Moose-faced Loach	Jamunessori (Atrai)	EN
5	Kajuli	*Ailia coila*	Gangetic ailia	Teesta	LC
6	Gutum	*Lepidocephalus guntea*	Guntea loach	Atrai	VU
7	Vacha	*Eutropiichthys vacha*	schilbid catfish	Chikli (Teesta)	LC
8	Narkeli chela	*Salmostoma bacaila*	Large razorbelly minnow	Chikli (Teesta)	LC
9	Natua/Puiya	*Acanthocobitis botia*	Mottled loach	Burikhora (Teesta)	LC

N.B: CR=critically Endangered; VU=Vulnerable; EN= Endangered; LC= Least Concern

1) *Barilius Barila* (Hamilton)
Local Name: Boirali, barali, koksha
English Name: Barred baril

Fin formula: D 9(2/7); P_1 13; P_2 9; A 13-14 (3/10-11).

Characteristics

- Body slender and jaws equal; maxilla reaching below the anterior third of eye. Adults with rows of pores on head.
- Head 5.0-5.5; height 5.2-5.4 in total length. Eye 3.5-4.0 in head; snout 0.7-1.0, interorbital 0.7-1.0
- Dorsal fin almost entirely advances in anal fin, pectoral nearly as long as head, lower lobe of caudal longer. Fins are yellowish, stained with black externally.
- Characteristic muscular pad present in front bases of pectoral. Body colored by vertical blue band with pinkish fins and dark olivaceous back.

2) *Salmostoma bacaila*
Local Name: Narkeli chela
English Name: Large razorbelly minnow

Fin formula: D. 10(2/8); P_1. 12-13; $P_{2.}$ 9; A. 14-15(2/13).

Characteristics

- Body elongates, head compressed with flat sides. Mouth narrow and subterminal, snout overhanging, two pairs of barbels longer than orbit.
- Eyes situated high up on head. Scales small, lateral line complete with 44-47 scales.
- Body silvery with pinkish tinge, fins reddish with light outer edges, dorsal fin apex black.
- Pelvics and anal tinged with red. Head 3.4-3.6 in standard, 4.4-4.7 in total length.
- Height 3.2-3.7 in standard, 4.2-4.8 in total length. Eye 40-5.6 in head, snout 1.6-2.4, interorbital 1.3-2.0

3) *Eutropiichthys vacha*
Local Name: Vacha, Garua Bacha
English Name: Batchwa, Vacha, Bacha
Fin formula: D I/7, P_1 I/13; P_2 1/5; A 3/44; C 22

Characteristics

- Body is elongated and compressed.
- Body silvery but darker along the back grey and white ventrally.
- Mouth is wide and extended to posterior margin of eye.
- Barbel 4 pairs, one pair nasal, one pair mandibular and two pair maxillaries.
- Dorsal, pectoral and caudal fins are white with black edges. Pelvic and anal fin are white and adipose fin greyish in color.
- Dorsal fin short, spine weak, smooth anterior and serrated on posterior border.

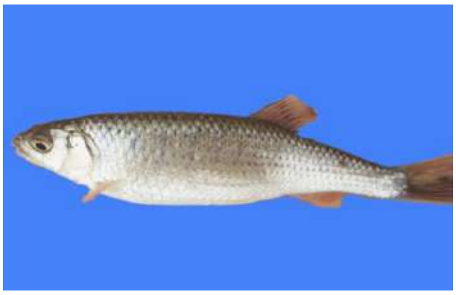
4) *Acanthocobitis botia*
Local name: Natua/Puiya
English/Common Name: Mottled Loach

Fin formula: D.13 (2/11); P_1. 10; $P_{2.}$ 8 (1/7); A. 6 (I/5).

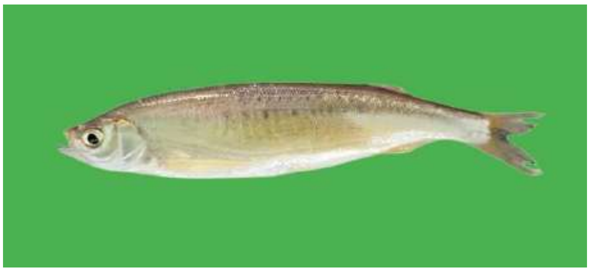
Characteristics

- Body elongates, cylindrical, anteriorly, compressed posteriorly.
- Mouth crescentic, surrounded by fleshy lips, lower lip notched at middle farming two rounded lobes.

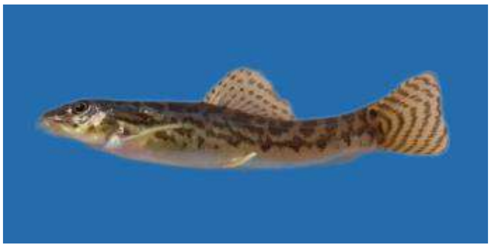
- Eyes situated high, near dorsal surface, 4 rostal and 2 maxillary barbells.
- Dorsal fin is much nearer to snout than to caudal base.
- Pectorals nearly as long as head, reaching below origin of dorsal.
- Edge of dorsal straight 10-14 irregular dark bands descend from back and end in dark spots below lateral line.



4a. Boirali



4b. Narkeli chela



4c. Natua

4dVacha

Figure 4. Fish species such as (4a) Boirali (1b), (4b) Narkeli chela (4c) natua and (4d) Vacha etc. of Teesta and adjacent rivers

This was a chronological study of sampling of different biodiversity components such as phytoplankton, zooplankton, fish, benthos and analyzing it's in the laboratory. In this fiscal year 2023-24 physico-chemical parameters of water of Teesta, Buri Teesta and Ghaghat river were shown in Table 1 and key findings is that all data was suitable for open water fish habitat. But in the moonson period the turbidity rate was seen higher in Teesta compare to Buri Teesta and Ghaghat river. 23 species of Phytoplankton, 11 species of Zooplankton and 7 species of benthos were identified mostly found in Buri Teesta River and 09 endangered fish spices have already been cataloged. This cataloging process will continue in the next year.

Development of Breeding Techniques and Mass Seed Production of Some Important Stream Fishes in Northern Region of Bangladesh

Researcher(s) : Dr. Azhar Ali, PSO & PI
: Maliha Hossain Mou, SSO
: Mst. Sonia Sharmin, SSO
: Srebash Kumar Saha, SO

Objectives:

To achieve this goal the proposed research has been designed with the following objectives:

- To collect, identify and domesticate of important stream fishes.
- To study reproductive biology and brood development of those fish species.
- To develop breeding protocol and mass seed production of those fish species.
- To establish nursery techniques of those successful breed fishes.

Achievement in the past (2023-24)

Collection of fingerling/sub-adult of the stream fishes from the Atraii, Kakra and Teesta River:

A total of

- 3000 fries of *Crossocheilus latius;*
- 200 fingerlings of *Botia lohachata;*
- 50 sub-adults of *Labeo boga; and*
- 10 sub-adults of *Raiamas bola* were collected from the Teesta, Atraii, Korotoa, Chikli, Kanchan and Burikhora river of Northern region of Bangladesh. Collected fishes were studied the reproductive biology and also domestication in ponds at the Freshwater Sub-Station, Saidpur, Nilphamari.

12.2 Expt. No 1: Domestication and brood development of *C. latius* with carp in captive condition

These experiments were conducted at the BFRI, FSS, Saidpur for a period of 12 months of each year during July 2023 to June 2024. The experiment was concerned with the observation of the growth, gonadal maturation and also yield performance of Candidates fishes under polyculture system in the captive condition. For domestication of *Crossocheilus latius* species in captive condition, firstly tried to adapt with artificial feed in brood rearing pond. Sub-adult fish species were kept in pond and regular feeding behavior were observed.

Pond preparation and experimental design

Three ponds were divided into three groups. Each pond was considered as one treatment e.g. treatment-I (T1), treatment-II (T2), treatment-III (T3). The ponds were 10-12 decimal in size. The water depth was maintained at 1.5 meter. The ponds were prepared by dewatering, liming (01 kg/decimal) and fertilization (Urea 100 g and TSP 50-100 g /decimal). The experimental design is presented Table 1.

Table 01. Brood rearing of *Crossocheilus latius* with carps in captive condition

Treatments	Stocking densities (Ind./dec.)	Stock. dens. (Other carps)			
	C. latius	Catla	Silver	Rohu	Rajpunti
T_1	50	25%	20%	30%	25%
T_2	100				
T_3	150				

Sources of fingerlings: Natural sources fingerling of candidate fishes and hatchery produced fingerlings of carps were stocked.

Stocking size: The average 1.5-2.0g weight of fingerlings of *C. latius* fish with same (15-20g) size fingerlings of carps were stocked.

Supplementary feeding: Commercial fish feed (containing 28-30% protein) was supplied @ 8-5% of their total biomass every day in the pond during morning hours commencing from the first day of stocking. Feed supply will be adjusted by estimating the standing crop by random sampling of the stock. Food conversion ratios will be estimated by the following formula:

$$FCR = \frac{\text{Total supplementary feed given (kg)}}{\text{Total body weight gain (kg)}}$$

Sampling and data collection

Fishes were sampled once in every fortnightly for periodic check on growth. Total length in cm and weight in g were recorded for growth study. The growth rate was estimated by using the following formulas:

$$\text{Growth (\%)} = \frac{W_2 - W_1}{W_1} X\, 100$$

Where, W_2 = Final weight in g, W_1 = Initial weight in g

Specific Growth Rate (SGR) were determined by using the following formula:

$$\text{SGR (\% day}^{-1}) = \frac{Ln W_2 - Ln\ W_1}{T_2 - T_1} X\ 100$$

Water quality Parameter

Water Quality data such as water depth (cm), water temperature (°C), dissolved oxygen (mg l^{-1}), water pH, Soil pH, and ammonia (mg l^{-1}), Conductivity (µScm^{-1}), Alkalinity (mgL^{-1}), Total Dissolved Solids(mgL^{-1}) and Hardness (mgL^{-1}) of the experimental ponds were collected fortnightly.

Growth Performance of *C. latius* with carps in captive condition

After the culture tenure 90 days from January 2024 to June 2024, the growth performances of *C. latius* and water quality parameter of the experimental ponds are presented in Tables 2 and 3 respectively.

Table 02: Growth Performance of *C. latius* in brood development pond (30 June, 2024)

Parameters	Treatments		
	T_1	T_2	T_3
Initial weight (g)	1.4±0.2	1.2±0.3	1.3±0.2
6^{th} sampling weight (g)	5.6±0.4	4.8±0.2	4.2±0.4
Weight gain (g)	4.2±0.3	3.5±0.4	3.0±0.3
SGRw (% day^{-1})	1.54±0.04	1.49±0.05	1.34±0.06
ADGw (g day^{-1})	0.05±0.002	0.04±0.003	0.03±0.002

Table 03. Physiochemical parameters in three treatments of brood development pond

Water quality parameters	T_1	T_2	T_3
Water Temperature (°C)	26±1.0	25.67±.50	27.2±1.2
Water pH	7.50±.02	7.8±0.6	7.9±0.25
Soil pH	6.9±0.3	6.7±0.3	7.1±0.3
DO (mg L^{-1})	6.2±0.10	5.9±0.2	5.3±0.24
NH_3 (mg L^{-1})	0.05±.03	0.07±.02	0.09±.03
Alkalinity (mgL^{-1})	92.4±7.65	102.5±6.3	98.4±7.2
TDS ((mgL^{-1})	69.52±5.2	93.23±6.3	72.52±3.2

12.3 Expt. No. 2 Studies of reproductive parameters of *C. latius*

Studies of Sex ratio and GSI values of *Crossocheilus latius*

The sex ratio and gonadosomatic index (GSI) of *Crossocheilus latius* was studied to know the spawning season of this fish. A total of 226 fishes were dissected during November 2023 to June 2024 and studied monthly. The results are presented in Table 4.

Table 04. Sex ratio and GSI values of *Crossocheilus latius* during November 2023 to June 2024

Month	Individual	♂	♀	♂:♀	GSI% (♀)
July, 23	21	09	12	1.0:1.44	8.28±0.32
August, 23	24	08	18	1.0:1.87	6.41±0.42
September, 23	25	10	15	1.0:1.60	4.22±0.21
October, 23	22	09	13	1.0:1.44	2.46±0.27
November, 23	27	11	16	1.0:1.54	1.49±0.21
December, 23	26	12	14	1.0:1.17	0.69±0.10
January, 24	31	14	17	1.0:1.21	0.61±0.21

February, 24	26	12	14	10:1.17	0.72±0.21
March, 24	31	13	18	1.0:1.46	2.84±0.10
April, 24	27	11	16	1.0:1.54	4.23±0.21
May, 24	23	10	13	1.0:1.30	7.46±0.32
June, 24	29	12	17	1.0:1.70	9.26±0.12

Studies of absolute and relative fecundity of *Crossocheilus latius*

The fecundity was estimated by gravimetric method. The method was used as described by Blay (1981). The fecundity was calculated using the following formula. The results are presented in Table 5.

$$F = \frac{N \times \text{Gonad weight}}{\text{Sample weight}}$$

Where, F is the fecundity and N is the number of eggs in the sample.

Table 05. Mean values of relative fecundity of *C. latius*

Class interval(cm)	Parameters			Fecundity (Per 100g bwt)
	TL (cm)	BW (g)	GW (g)	
3.1–4.0	9.05±1.04	4.5±0.85	0.42±0.05	1491±239
Range	8.20-10.09	3.65-5.35	0.37-0.47	1252-1730
4.1–5.6	9.79±1.75	5.95±1.03	0.45±0.03	1786±127
Range	8.04-11.54	4.9-6.9	0.42-0.49	1611-1913

1a **1b** **1c**

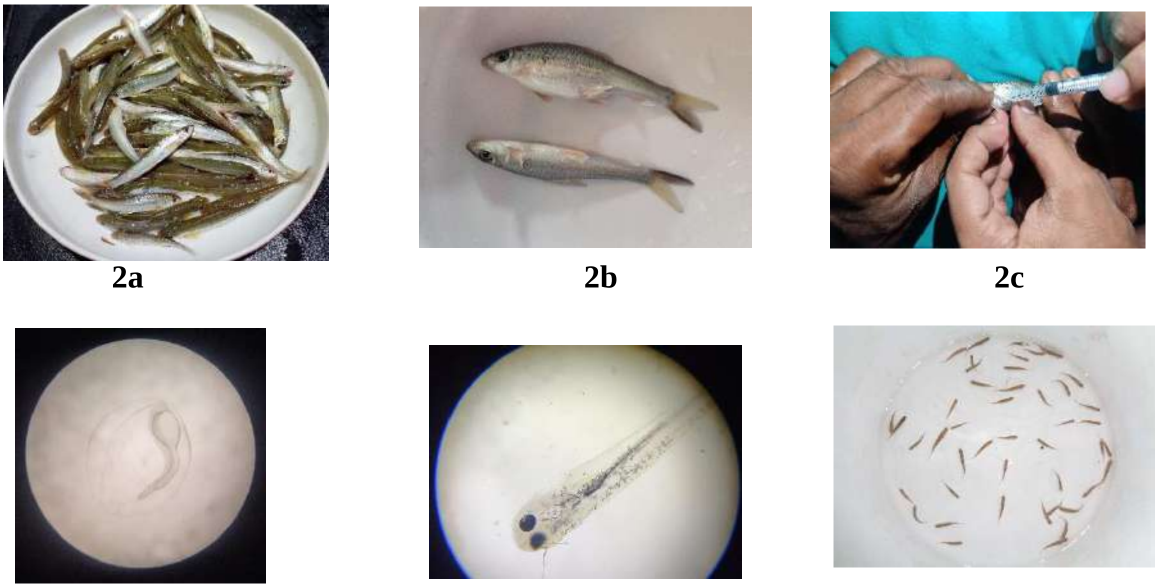
Figure 1: GSI & Fecundity study 1a) Mature egg of C *latius* 1b) *C latius* fish 1c) Egg measurement

12.4 Expt. No. 3 Primary success of induced breeding of *C. latius* using synthetic hormone

The experiment was conducted at the hatchery complex of BFRI, FSS, Saidpur to determine the reproductive response of *C. latius* using different types of hormones. The results are presented in Tables 06, and 07.

Table 06. Spawning response of C. *latius* to PG under natural method

Trial	Ovuhom (ml kg^{-1})		Latency period (hrs)	Incub. Temp. (^0C)	% of egg release	% of fertilization	% of hatching	Remarks
	M	F						
T_1	0.1	0.1	10-12	26.0-28.0	30-50	40	30	Comparatively better amount of fertilization and hatching observed in **T_3**.
T_2	0.1	0.2	10-12	26.0-28.0	60-70	70	60	
T_3	0.1	0.3	10-12	26.0-28.0	75-80	75	70	
T_4	0.2	0.4	10-12	26.0-28.0	50-60	50	45	



2a **2b** **2c**

2d **2e** **2f**

Figure 2. Brood fish of C. *latius* (1a) Brood male and female (1b) Injecting hormone of Ovuhom (1d) Embryonic stage (1e) microscopic view of larvae and (1d) 30 days old Fry of C. *latius*.

As this study having aim to artificial breeding success of some endangered fish species under red list categories from northern part of Bangladesh, we set some objective for this project and target some stream fishes to domesticate and breeding in captive condition. In this 2023-24 fiscal year almost 3500 fish species have been collected from different open waterbody. Among them *Crossocheilus latius* was most. This species is tried to domesticate in captive condition and regular gonad maturation was observed. The highest GSI of this fish was found in the month of June and highest fecundity was measured 3416/10g body weight. Several trials were conducted for artificial breeding success of *Crossocheilus latius* and using the doses of 0.1 and 0.3 ((ml kg^{-1}) b.w. in male and female respectively with synthetic hormone ovuhom got succeed. Now the hatchling is being observed for its survival and nursery development.

Development of Induced Breeding and Culture Techniques of Gangetic Endangered Fish Species

Researcher(s):

: Dr. David Rintu Das, Principal Scientific Officer
: Md. Shariful Islam, Senior Scientific Officer
Md. Moniruzzaman, Scientific Officer
Mahmudul Hasan Mithun, Scientific Officer
Shahanaj Parvin, Scientific Officer

Objectives of the Project :

- ✓ Identification of reproductive biology of Khorsula (*Rhinomugil corsula*) and Gang tengra (*Gagata youssoufi*) fish.
- ✓ Development of induced breeding techniques of Kajuli, Khorsula and Gang tengra fish.
- ✓ Culture feasibility of Batashi and Pialy with other species in pond condition.

Achievement for the year 2023-2024

Expt-1: Identification of reproductive biology of Khorsula (*Rhinomugil corsula*) and Gang tengra (*Gagata youssoufi*) fish.

GSI of Khorsula *(Rhinomugil corsula)*

For the calculation of GSI, every month about 10 fish were examined which body length were 18.12± 2.15 cm and body weight were 110.10 ± 1.95 g. In case of male and female *R. corsula*, it was found that the value of GSI gradually increased from April and then it increased sharply from May and became higher during the month of June and July. Highest GSI value was of female and male recorded as 14.55 ± 2.50 % and 3.19 ± 1.50 %, respectively in the month of July (Figure 1) which it indicated that July is the peak breeding season of *R. corsula*.

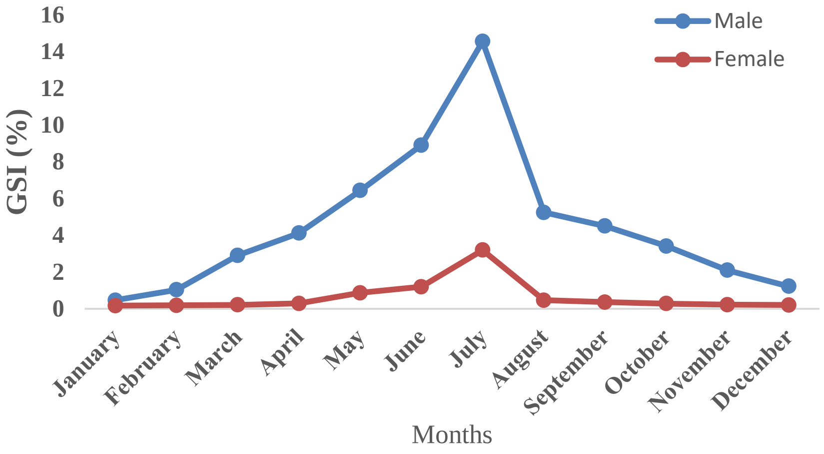


Figure 1. Monthly mean values of gonado-somatic index (GSI) of male and female *R. corsula*

Table 1. Fecundity of *Rhinomugil corsula*

Month	No. of fish examined	Body weight (g) (Mean±SD)	Ovary weight (g) (Mean±SD)	Fecundity (Mean±SD)	Ova diameter (mm) (Mean±SD)
April	10	113.44±1.23	5.99±0.11	10150±350	0.35±0.03
May	10	120.22±2.11	6.10±0.17	12435±765	0.38±0.05
June	10	125.43±2.21	9.78±0.38	28830±1670	0.57±0.06
July	10	136.45±2.39	11.10±0.41	42890±2920	0.65±0.07
August	10	125.31±2.45	1.87±0.09	2215±879	0.27±0.05

GSI of Gang tengra *(Gagata youssoufi)*

For the calculation of GSI, every month about 10 fish were examined which body length were 7.32± 2.66 cm and body weight were 7.10 ± 1.95 g. In case of male and female *G. youssoufi*, it was found that the value of GSI gradually increased from April and then it increased sharply from May and became higher during the month of June, July, and August. Highest GSI value was of female and male recorded as 10.25 ± 2.11 % and 2.89 ± 1.32 %, respectively in the month of July (Figure 2) which it indicated that July is the peak breeding season of *G. youssoufi*.

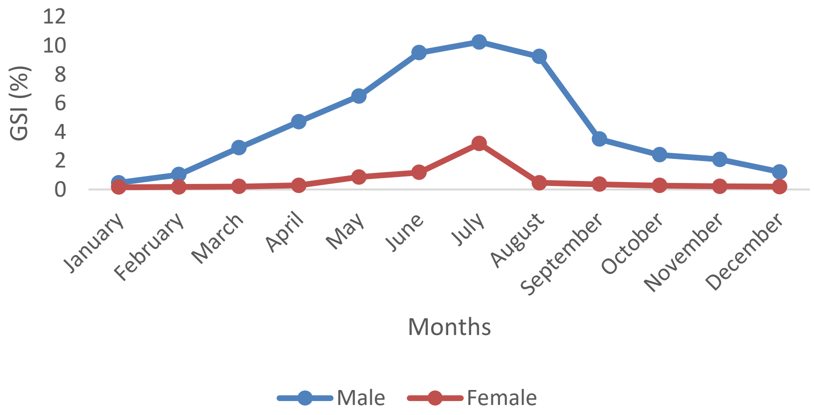


Figure 2. Monthly mean values of gonado-somatic index (GSI) of male and female *Gagata youssoufi*

Table 2: Fecundity and Ova diameter range of *Gagata youssoufi*

Month	No. of fish examined	Body weight (g) (Mean±SD)	Ovary weight (g) (Mean±SD)	Fecundity (Mean±SD)	Ova diameter (mm) (Mean±SD)
April	10	5.12±1.23	1.19±0.11	1830±350	0.35±0.03
May	10	6.91±1.11	1.65±0.17	2670±377	0.38±0.05
June	10	6.99±1.29	1.99±0.33	3160±420	0.57±0.06
July	10	8.45±1.39	2.09±0.46	5100±433	0.65±0.07
August	10	6.26±1.45	1.87±0.09	2636±321	0.27±0.05

GSI of *Ailia coila*

For the calculation of GSI, every month about 10 fish were examined which body length were 9.12± 2.15 cm and body weight were 5.17 ± 2.05 g. In case of female *A. coila*, it was found that the value of GSI gradually increased from March to June and then it increased sharply in the month of July and then gradually slowed down. Highest GSI value was recorded as 6.78 ± 1.50 % in the month of July (Fig. 3) which indicated that July is the peak breeding season of *A. coila*.

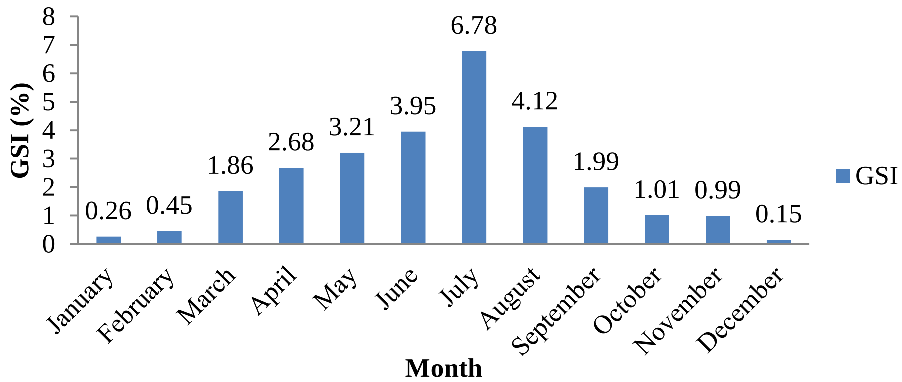


Figure 3: Monthly mean values of Gonado-somatic index (GSI) of female *A. coila*.

Table 3: Fecundity and Ova diameter range of *Ailia coila*

Month	No. of fish examined	Fecundity range	Ova diameter (mm) (Mean±SD)
June	10	2500-2700	0.29±0.03
July	10	3000-3500	0.40±0.05
August	10	1830-2110	0.19±0.07

Expt-2: Development of induced breeding techniques of Kajuli, Khorsula and Gang tengra fish.

Collection of brood fishes

The sexually matured, strong, and diseased free broods were collected from the River Jamuna, River Atrai & Roktodaho Beel of Sirajgonj, Naogoan, and Bogura districts of Bangladesh and were stocked in the ponds at the rate of 400-500 brood/decimal averaging 3-4 g each for domestication for breeding purposes.

Pond preparation and rearing of brood Fish

Before collecting the brood fishes, the pond was prepared by eradicating predatory and unwanted fishes through dewatering followed by drying. Aquatic vegetation was removed manually and harmful aquatic insects were removed by using rotenone and phostoxin. Liming was done at the rate of 1 kg/decimal and after 7 days of liming, inorganic fertilizers such as Urea and TSP were used at the rate of 200g and 100g per decimal, respectively for enhancing the natural production of phytoplankton and zooplankton in the brood pond. Seven days after fertilization, fishes were released into the brood pond with intensive care. During the rearing period, water quality parameters were maintained within the optimum level for the proper growth and maturation of the fishes. Measured values of water quality parameters are shown in the following (Table 4):

Table 4: Recorded water quality parameters during the rearing and breeding period of the brood fishes

Water quality parameters	Values	
	Mean ± SD	Range
Water Temperature (°C)	29.91 ± 1.55	28.36 - 31.46
pH	7.82 ± 0.11	7.71 - 7.93
DO (mg/l)	5.53 ± 0.38	5.15 - 5.91
Transparency (cm)	29.12 ± 1.22	27.9 - 30.34
Free CO_2 (mg/ l)	0.30 ± 0.06	0.24 - 0.36
Total ammonia (mg/l)	0.11 ± 0.05	0.06 - 0.16

Feeding

The fish were fed with a commercial special diet (Agatha Super Floating Premium, 0.8 mm, Code-1102) which was given twice a day at the rate of 8-10% body weight per daybased on gain in average body weight of fish.The proximate composition of fish feed, according to manufacturer is presented in (Table 5).

Table 5. Proximate composition of the fish feed

Component	% composition
Moisture	11
Protein	40
Metabolic Energy (ME):	3300 Kcal/Kg
Lysine	2
Fibre	6
Calcium	1.5-3.5
Phosphorous	0.8-1.8
Vitamin E	300 mg/kg

Induced breeding trial of Kajuli *(A. coila)* by applying PG doses

For the development of induced breeding techniques of Kajuli fish, we conducted two trial during the months of June and July of 2023. There were three treatments in each trial with three replications each. During the month of June, we injected 2, 4 and 6 mg PG/kg BW of female and 1, 2 and 3 mg PG/kg BW of male but none of the fish was ovulated. Another trial was conducted during the month of July where 7, 8 and 9 mg PG/kg BW of female and 3.5, 4 and 4.5 mg PG/kg BW of male were injected but none of the fish was also responded that time.

Table 6: Trial for induced breeding of Kajuli *(A. coila)* by applying PG doses

Trial	Treatment	Weight of brood fish (g)		PG dose (mg/kg BW)		Ovulation
		Female	Male	Female	Male	
Trial 1 (June)	T1	5.22±0.18	4.53±0.24	2	1	No ovulation
	T2	5.33±0.29	4.34±0.21	4	2	
	T3	5.18±0.24	4.93±0.23	6	3	
Trial 2 (July)	T1	6.20±0.16	5.83±0.21	7	3.5	No ovulation
	T2	6.53±0.20	5.39±0.29	8	4	
	T3	6.78±0.21	5.97±0.33	9	4.5	

Induced breeding trial of Khorsula (*Rhinomugil corsula*) by applying PG doses

For the development of induced breeding techniques of Khorsula fish, we conducted two trial during the months of June and July of 2023. There were three treatments in each trial with three replications each. During the month of June, we injected 14, 16 and 18 mg PG/kg BW of female and 7, 8 and 9 mg PG/kg BW of male but none of the fish was ovulated. Another trial was conducted during the month of July where 20, 22 and 24 mg PG/kg BW of female and 10, 11 and 12 mg PG/kg BW of male were injected and this time female ovulation occurred but none of the male fish didn't released sperm. For that why no eggs were fertilized.

Table 7: Trial for induced breeding of Khorsula (*Rhinomugil corsula*) by applying PG doses

Trial	Treatment	Weight of brood fish (g)		PG dose (mg/kg BW)		Ovulation
		Female	Male	Female	Male	
Trial 1 (June)	T1	85.21±1.88	75.23±1.24	14	7	No ovulation
	T2	83.35±1.89	72.37±1.28	16	8	
	T3	87.12±1.94	74.13±1.20	18	9	
Trial 2 (July)	T1	96.20±2.16	78.83±2.21	20	10	No ovulation
	T2	92.53±2.20	77.39±2.29	22	11	No ovulation
	T3	97.78±2.21	78.97±2.33	24	12	Female ovulated but male didn't responded

Expt. 3: Culture feasibility of Batashi and Pialy with other species in pond condition.

The trial was set up in a Completely Randomized Design (CRD) with three treatments (stocking density) having three replicates each.

Table 8. Stocking ratio of Batashi and Pialy with other species

Species	Treatment 1 (nos./dec)	Treatment 2 (nos./dec)	Treatment 3 (nos./dec)
Batashi	1000	1000	1000
Pialy	1000	1000	1000
Gulsha	200	300	400
Rui	10	12	14
Catla	8	10	12
Total	**2218**	**2322**	**2426**

Feeding

Commercial floating feed of 0.3 mm size was given twice a day. Feeding adjustment was done every two weeks (25% down to 8% of BW). The amount of feeds used per treatment was recorded daily.

Sampling

The total culture period will be 180 days where sampling of fish (n = 25) for length (cm) and weight (g) gain will be done fortnightly, using measuring scale and electrical balance. Water quality (temperature, dissolved oxygen, pH, alkalinity, ammonia), were measured using relevant equipment.

Statistical Analysis

All the data collected during experiment were recorded and preserved on a computer spreadsheet. Growth and yield parameters of fish were analyzed statistically by one-way ANOVA and DMRT (Duncan Multiple Range Test) using the statistical software (Statistix 10).

Water quality parameters

Table 9. Mean and range values of water quality parameters measured during the experimental period of 120 days

Water quality parameters	**T1** Mean ± SD	**T2** Mean ± SD	**T3** Mean ± SD
Water Temperature (°C)	28.40 ± 1.30a (27.10-29.70)	28.40 ± 1.30a (27.10-29.70)	28.40 ± 1.30a (27.10-29.70)
pH	7.50±0.20a (7.30-7.70)	7.40 ± 0.20ab (7.40-7.60)	7.10 ± 0.40b (6.70-7.50)
DO (mg l^{-1})	5.40 ± 0.50a (4.90-5.90)	5.30 ± 0.50a (4.80-5.80)	5.10 ± 0.60a (4.50-5.70)
Transparency (cm)	33.10 ± 2.10a (31.00-35.20)	33.10 ± 2.40a (30.70-35.50)	33.30 ± 2.30a (31.00-35.60)
Total ammonia (mg l^{-1})	0.10 ± 0.02a (0.08-0.12)	0.14 ± 0.04a (0.10-0.18)	0.17 ± 0.06a (0.11-0.23)

Growth and production performance

Table 10. The growth and production performance of batashi and pialy in polyculture are given below during the experimental period of 180 days

Parameters	**Species**	**Treatment**		
		T1	**T2**	**T3**
Initial Length (cm)	*Batashi*	2.56±0.17	2.54±0.08	2.52±0.10
	Pialy	2.66±0.19	2.44±0.78	2.82±0.15
	Gulsha	3.97±0.21	4.20±0.30	4.07±0.15
	Rui	8.67±1.51	9.44±1.25	9.99±1.21
	Catla	8.23±1.58	8.67±1.29	8.10±1.33
Final Length (cm)	*Batashi*	9.99±0.11a	8.85±0.07b	8.13±0.30c
	Pialy	9.93±0.31a	8.75±0.57b	7.99±0.16c
	Gulsha	15.80±.09a	12.64±0.25b	11.80±0.19bc
	Rui	27.97±0.34a	25.56±0.48b	23.97±0.37c
	Catla	26.20±0.81a	23.95±0.28b	21.69±0.43c
Initial Weight (g)	*Batashi*	1.01±0.12	1.00±0.11	0.99±0.07
	Pialy	1.01±0.12	1.10±0.11	1.02±0.06
	Gulsha	1.83±0.06	1.73±0.06	1.60±0.10
	Rui	20.82±0.60	20.66±1.45	19.07±0.80
	Catla	19.56±0.50	19.97±0.58	18.02±0.29
Final Weight (g)	*Batashi*	10.69±0.14a	8.44±0.11b	7.20±0.07c
	Pialy	11.28±0.24a	9.22±0.20b	8.15±0.17c
	Gulsha	15.09±0.05a	10.27±0.25b	8.32±0.22c
	Rui	580.07±5.35a	511.21±1.00b	459.24±1.71c
	Catla	560.21±6.01a	490.87±5.50b	430.55±2.91c
SGR (% day^{-1})	*Batashi*	1.70±0.10a	1.68±0.10b	1.66±0.05ab

	Pialy	1.77±0.05a	1.76±0.10ab	1.74±0.12b
	Gulsha	1.87±0.05a	1.58±0.03b	1.52±0.03c
	Rui	1.85±0.02a	1.80±0.04b	1.78±0.02c
	Catla	1.86±0.04a	1.83±0.02ab	1.81±0.05b
ADWG (g)	*Batashi*	0.06±0.00a	0.05±0.00b	0.05±0.00b
	Pialy	0.06±0.00a	0.06±0.00a	0.06±0.00a
	Gulsha	0.11±0.00a	0.09±0.00b	0.08±0.00c
	Rui	3.11±0.03a	2.73±0.01b	2.61±0.01c
	Catla	3.00±0.01a	2.75±0.02b	2.61±0.03c
Survival (%)	*Batashi*	87.30±4.60a	85.71±4.70ab	84.95±2.40b
	Pialy	86.78±4.22a	83.65±3.25b	81.47±3.48c
	Gulsha	89.40±4.31a	83.60±3.11b	75.21±2.41c
	Rui	95.41±2.31a	89.89±1.31b	80.71±2.11c
	Catla	92.71±2.11a	83.71±2.11b	79.11±2.01c
Gross Production (Kg/dec)	*Batashi*	9.33±1.51a	7.23±1.21b	6.12±1.41c
	Pialy	9.79±1.11a	7.71±1.01b	6.64±1.21c
	Gulsha	2.70±1.01a	2.57±1.31ab	2.50±1.22b
	Rui	5.53±1.22a	5.51±1.25a	5.19±1.27ab
	Catla	4.15±1.12a	4.10±1.15ab	4.09±1.21ab
Total Production (Kg/dec)		31.50±1.19a	27.12±1.30b	24.54±1.26c
FCR		2.09±0.06c	2.37±0.02b	2.63±0.06a

Results of the present study demonstrated that, significant differences was observed among the treatments in weight gain, SGR, survival rates and gross production. A significantly higher ($p<0.05$) total production of 31.50±1.19 kg/ dec in 180 days was obtained in T1 treatment compared to T2 and T1.

Species Availability and Develop a Suitable Technology of Fermented Dried Fish Product (Shidol) in Floodplain Region of Bangladesh

Researcher(s):

: Md. Moniruzzaman, Scientific Officer

: Dr. David Rintu Das, Principal Scientific Officer

Shishir Kumar Dey, Scientific Officer

Mahmudul Hasan Mithun, Scientific Officer

Shahanaj Parvin, Scientific Officer

Objectives of the Project :

- Refinement of suitable technology for the production good quality Shidol.
- Evaluation of the sensory test of Shidol
- Field validation of BFRI made shidol in some selected areas of Bangladesh.

Achievement for the year 2023-2024

Expt 01: Refinement of suitable technology for the production good quality Shidol.

i) **Survey of the available SIS species and ingredients used in *Shidol* preparation:**

We visited several local fish markets to know the availability of different SIS found in the floodplain region for the preparation of *Shidol*. Some locally available fish species were given below:

- Puti
- Taki
- kholisha
- Mola
- Chanda
- Chela
- Batashi
- Dhela

ii) Collection of raw materials

Raw materials (SIS) were collected from local fish market.

iii) Dressing, Cutting, Gutting

After collection, raw materials (SIS) were gutted immediately. Generally, women workers involve in dressing, cutting and gutting.

iv) Salting

After gutting fish salting were done to protect fish from fly, insect, or their larval infestation. Sometime salts were used to get extra weight of fish. Generally, 125 g salt was used for 1 kg of fish.

Figure 1. Salting of gutted fish.

v) The Smoking Kiln

The smoking kiln made with steel as a rectangular box of 105×75×45 cm3 size. Horizontally, the chamber divided into two equal parts by placing a horizontal perforated iron net-frame and the bottom portion used as base for burning saw dust wooden logs/chips as smoke source. 50-55^{0}C temperature (external of Kiln) was maintained manually by controlling the outlet of the smoking chamber. During smoking operation, fishes were turned over in the middle period.

Figure 2. Smoking of salted fish in Smoking Kiln.

vi) Sun drying in ring tunnel

After Smoking fishes were sun dried (4-6 days) in ring tunnel fish dryer and net covered drying busket. Each fish turned over 8-10 times/day.

Figure 3. Sun drying of smoked fishes.

vii) **Processing of *Shidol***

After grinding, arum green stems pulp mixed with fish powder to make paste. The arum green and fish powder ratio was 2:1 and other ingredients like garlic, ginger and salt was added as required amounts. Once all mixed, the paste was rubbed with turmeric powder and mustard oil and made round ball by hand. Turmeric powder and mustard oil mixture used to protect the cake from blowfly, beetle or other insect attack. After that it was kept into sealed earthen pot for anaerobic fermentation for 3-4 days. Carbohydrates of arum green steams serve as media for fermentation bacteria to grow and draw their metabolic energy. This fermentation process added desirable flavor and taste to the final product. After fermentation, the paste again mixed well and made round shape by hand. Then the round shaped shidol were dried under sunlight for another 4-6 days into ring tunnel fish dryer to protect it fly, insects, birds and rodents.

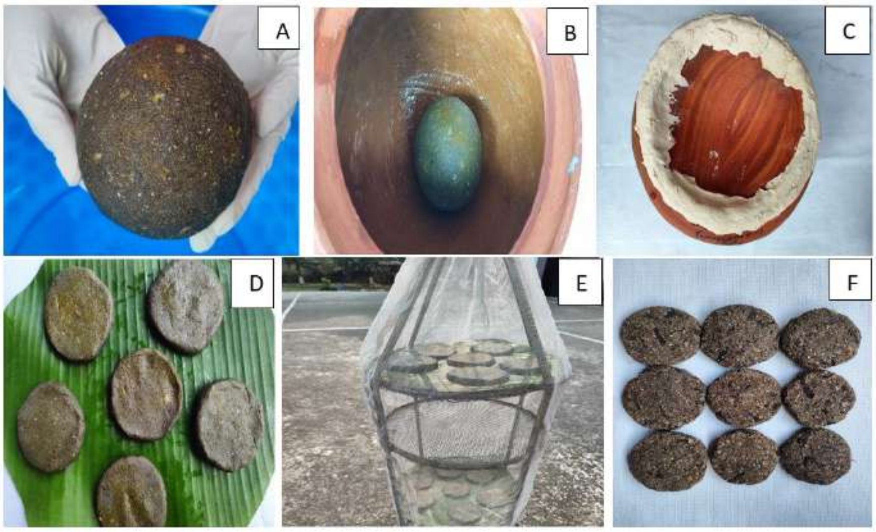


Figure 4. (A) Shidol paste after mixing all ingredients, (B, C) kept in airtight earthen pot for anaerobic fermentation, (D) round shape of shidol (E) drying shidol in ring tunnel fish dryer (F) final product of shidol after drying.

In these experiment three different types of *Shidol* samples (Sample-1, Sample-2 and Sample-3) was prepared by using different amount of ingredients and their special characters showed in Table- 1.

Table 1: Materials used in processing of *Shidol*

Sample name	Origin/ District	Materials	Special Characters
Sample 1	Bangladesh Fisheries Research Institute.	Aram green (57%), Dry SIS (28%), Salt (1.99%), Salt (1.99%), Garlic (7.2%), Ginger (3.6%), Turmeric powder (0.7%), Mustard oil (7.2%)	Salting, Smoking, sun drying
Traditional	Rangpur, Nilphamari, Kurigram, Dinajpur, Gaibanda, Lalmonirhat, Panchagarh	Aram green (70%), Dry SIS (17.54%), Salt (3.50%), Garlic (7.01%), Ginger (0.70%), Turmeric powder (0.35%), Mustard oil (0.70%)	Sun drying

Shidol samples were stored in different preservation unit and their preparation cost was shown in table 2.

Table 2: Preservation unit and production cost of *Shidol*

Sample name	Preservation unit	Sample preparation cost/Market price (Tk.)
Sample-1	Atmospheric temperature	34.28TK/100g
Traditional/Local	Atmospheric temperature	45tk/100g

Vacuum packaging

Processed Shidol were then sealed by vacuum packaging machine (DZ-280/2SD) in sealer bags to extend the shelf life and reduce the oxidative reaction and lowest bacterial count in the product at relatively low cost and stored at atmospheric temperature (15-34^{0}c). Vacuum packaging represents a static form of hypobaric storage which is widely used in the food industry due to its effectiveness in reducing oxidative reactions in the product at relatively lower costs. In vacuum packaging, the product is contained in a package made of a material having low oxygen permeability and is sealed air tight after evacuating the air. All samples will be tested at BCSIR lab, Rajshahi. Proximate composition (% dry weight) of *Shidol* samples were shown in table 3.

Figure 5. (A) Shidol vacuum packaging by vacuum sealer machine, (B) vacuum packaged shidol.

Table 3: Proximate composition (% dry weight) of *Shidol* samples

Parameters	BFRI made Shidol			Traditional Shidol		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Moisture (%)	18.73	17.56	19.73	25.52	24.42	26.72

Crude lipid (%)	18.92	18.15	20.19	14.05	13.25	14.78
Crude Protein (%)	33.48	30.21	37.15	31.80	29.55	34.58
Ash (%)	14.42	13.98	14.92	14.28	14.10	14.82
Fiber (%)	5.70	5.43	6.20	6.37	5.90	6.87
Carbohydrate (%)	7.64	2.20	11.65	8.88	5.02	11.67

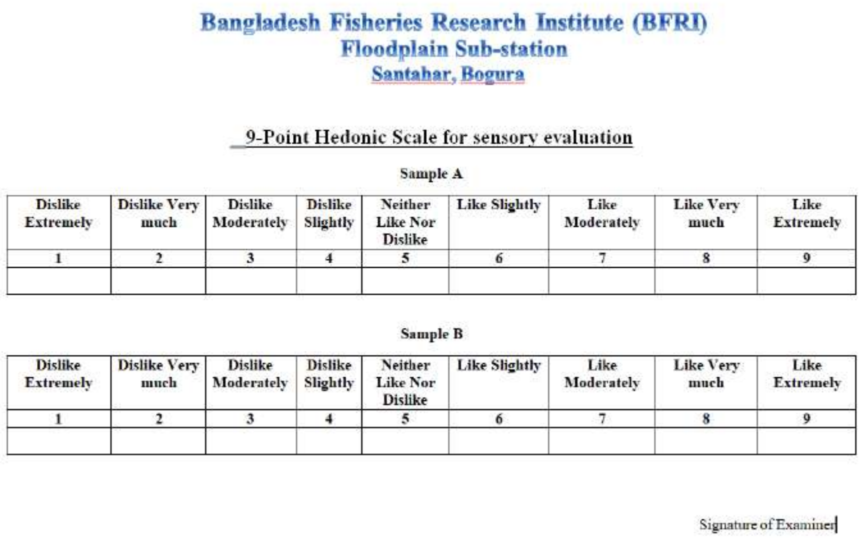
Table 4: Results of Biochemical and microbiological test after 90 days of preservation period of Shidol

Days	pH (27°C)		Total Viable Count (log CFU/g)		Total Yeast Mold (log CFU/g)		Total Coliform Count (log CFU/g)		TVBN (mg/100gm)		Peroxide value (meq O_2 /kg	
	A	B	A	B	A	B	A	B	A	B	A	B
0	6.19	7.31	2.8	8.8	1.1	3.56	0	0.1663	20.1	176.21	2.1	7.1
15	6.25	7.45	1.3	7.4	2.1	5.34	0	0.2499	26.25	198.6	4.2	10.2
30	6.4	7.76	1.8	6.4	2.3	6.5	0	0.2499	33.15	225.69	7.2	15.2
45	6.26	7.26	1.4	5.5	2.8	6.7	0	0.4978	37.25	249.62	7.4	20.4
60	6.3	7.71	1.29	5.7	3.1	7.4	0	0.6367	41.21	268.3	8.6	27.6
75	6.45	7.54	1.14	5.66	3.12	7.59	0	0.6745	42.11	271.21	8.98	29.35
90	6.75	7.66	1.06	5.41	3.15	7.98	0	0.6841	42.68	273.29	9.01	31.58

A= BFRI made shidol and B= traditionally made shidol

Expt 02: Evaluation of the sensory test of Shidol

The samples for the sensory evaluation were tested by 9-point hedonic scale method. All the will be randomly presented to the 10 panelist. During 90 days of sensory analysis, BFRI made Shidol had better taste than traditional Shidol.


Bangladesh Fisheries Research Institute (BFRI)
Floodplain Sub-station
Santahar, Bogura

9-Point Hedonic Scale for sensory evaluation

Sample A

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
1	2	3	4	5	6	7	8	9

Sample B

Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
1	2	3	4	5	6	7	8	9

Signature of Examiner

Figure 6: 9-point hedonic scale for sensory analysis.

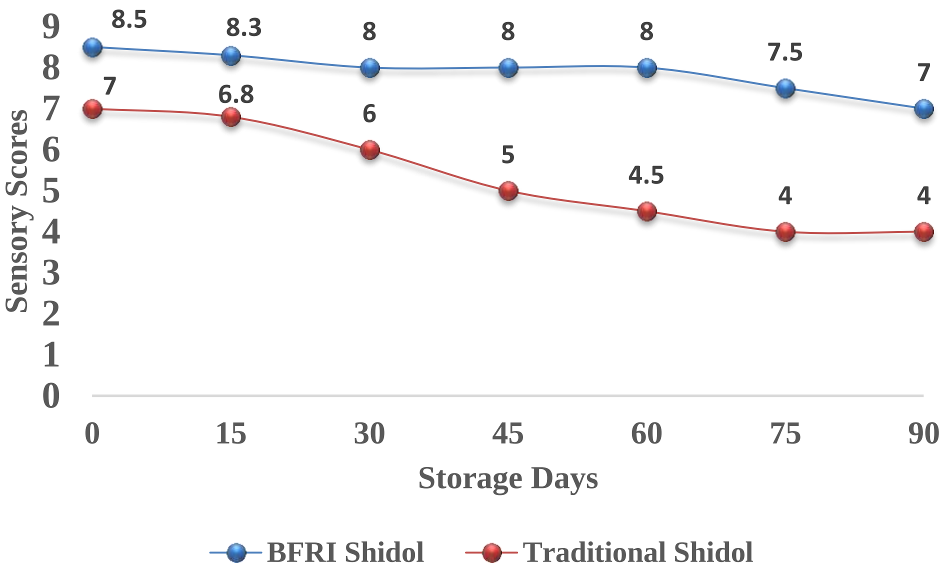


Figure 7. Sensory analysis of BFRI Shidol and traditional Shidol.

Expt 02: Field validation of BFRI made shidol in some selected areas of Bangladesh.

For the field validation of BFRI made shidol, initially we selected two person (1 male and 1 female) for making them entrepreneurs. We provided them practical knowledge about how to make good quality shidol and we will provide them all logistic support to produce shidol by using BFRI technology. We will also help them for creating marketing channel so that the can easily sell their shidol.

Figure 8: Selected entrepreneurs for making BFRI shidol (above) and provide practical knowledge about how to make good quality shidol.

Investigation on Fish Biodiversity and Ecological condition of Jamuna and Atrai River in the Context of Climate Change

Researchers

: Md. Mehedi Hasan Pramanik, SSO
: Dr. David Rintu Das, PSO
Md. Shariful Islam, SSO
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Objective of the project:

i) To assess the fish biodiversity of Jamuna and Atrai River
ii) To study the effects of climatic factors and their associated events on the Jamuna and Atrai River ecology and biodiversity of fish
iii) Identification of spawning and collection sites of carp fish eggs and larvae in River Jamuna.

(Objectives of this study comply with the sustainable development goal 14: Conserve and sustainably use the oceans, seas and marine resources for sustainable development)

Achievement (July2023-June 2024)

Study Areas

Five sampling points of Jamuna River

a. Kurigram Sadar Upazila, Kurigram
b. Gaibandha Sadar Upazila, Gaibandha
c. Sariakandi Upazila, Bogura
d. Sirajganj Sadar Upazila, Sirajganj
e. Shivalaya Upazila, Manikganj

Four sampling points of Atrai River

f. Dhamoirhat Upazilla, Naogaon;
g. Mohadebpur Upazilla, Naogaon;
h. AtraiUpazilla, Naogan;
i. Chatmohor Upazilla, Pabna.

Sampling spots will be determined by site selection visits (considering the convenience of transport, travel, accommodation arrangements etc.).

Study 1: To assess the Fish biodiversity of Jamuna and Atrai River

i) Fish specimen collection and identification

Samples of different fish species were collected from the fisherman's catch landed at different fish landing centers of the selected sampling stations and from fish bazar as well. A digital camera was used to capture the photos of different fish species. The collected fish samples were identified by analyzing their morphometric and meristic characteristics following (Rahman 1989 and 2005, Talwar and Jhingran 1991). The valid scientific names of the identified fish species were ensured by checking from catalogue of life (Roskov *et. al*, 2000), reputed Books and responsible online Sites. (Table: 1)

Table 1: List of fish found at various points of Jamuna and Atrai River

River Name	Sampling Point	Available Fish Species (Local Name)
Jamuna	➢ Gosaibari Ghat, Dhunot, Bogura ➢ Closer Point, Sirajgang ➢ Sariakandi Feri Ghat, Sariakhandi, Bogura ➢ Balasi Ghat, Gaibandha	Tatkini, Chapila, Ritha, Phasa, Kalibaus, Gang Magur, Kaiyakanta, Guchi Baim, , Jebra Sipchela, Chela, Kajoli, Batasi, Bourani, Kholisa, Chanda, Punti, Darika, Mola, Guchi Baim, Punti, Pabda,Taki, Banchpatari, Tara Baim, Gulsa Tengra, Bhagha air, Boal, Air, Rui, Catla, Ghaura Bacha, Shol, Gojar, Bele, Darika, Guchi Baim, Hilsa, Pangus, Jebra
Atrai	➢ Dhakbanglo Ghat, Mohadebpur Upazilla, Naogaon; ➢ Shimultolai BGB Camp Ghat, Dhamoirhat, Upazilla, Naogaon; ➢ Mach Bazar Ghat, Atrai Upazilla, Naogan	Rui, Air, Mola, Chanda, Bhagha air, Rui, Catla, Pabda, Taki, Boal, Shing, Magur, Bhagha air, Baim

ii) Determination of IUCN conservation status

IUCN conservation status is being determined after all designated sampling spots have been sampled. Data collection is ongoing

iii) Diversity indices analysis
Data collection is ongoing.

Study 2: To study the effects of climatic factors and their associated events on the Jamuna and Atrai River ecology and biodiversity of fish

i) Physico-chemical parameters
Water quality parameters (Temperature, Dissolved Oxygen, pH, and Transparency) were collected monthly basis from different sampling spots of Jamuna River.
The water temperature at Gaibandha Point in Jamuna River during the period from January to May averaged 30.2°C and ranged from 23 to 34 °C. Sirajganj point averaged 30.4°C and 24 to 34°C. On the other hand, the temperature at Atrai Point of Atrai River averaged 31.15°C and the range was 24° to 35°C.
In terms of transparency (cm), the values at Gaibandha and Sirajganj points of Jamuna River were respectively: 21.10 and 22.10 cm; and the range were 15-26 and 14-27 cm. It was 25.09 at Atrai Point in Atrai River and the range was 18-28 cm.

The values of pH, DO (mg/1), Temperature (°C), and Transparency (cm) were favorable for the river. In other words, the water quality of the Atrai and Jamuna rivers was tolerable. (Table: 2)

Table 2: Water quality parameters of sampling sites (Jamuna and Atrai River)

	January to May 2024		
Water quality parameters	**Jamuna River (Gaibandha)**	**Jamuna River (Sirajganj)**	**Atrai River (Mohadebpur)**
Water Temperature (°C)	30.2 ± 1.00 (23-34)	30.4 ± 0.90 (24-34)	31.15 ± 1.10 (24-35)
pH	7.50±0.20 (7.30-7.70)	7.40 ± 0.20 (7.40-7.60)	7.10 ± 0.40 (6.70-7.50)
DO (mg l^{-1})	6.10 ± 0.30 (4.9-7.9)	6.80 ± 0.20 (4.8-7.8)	5.13 ± 0.30 (4.5-5.7)
Transparency (cm)	21.10 ± 0.70 (15-26)	22.10 ± 0.90 (14-27)	25.09± 1.30 (18-28)

ii) Plankton sample collection and **identification:**
Replicate plankton samples, each of 50 L were collected from various spots around each sampling station by means of a bucket and filtered through bolting silk plankton net of 50µ. The filtrate were transferred to another bottle and preserved immediately in 1:100 Lugol's solution. Qualitative and quantitative analysis of planktons were done following drop count method (APHA 1995). Identification of plankton was made following Ward and Whipple (1959) and Presecot (1962).
A total of 4 group of phytoplankton and 3 group of zooplankton were identified of which Chlorophyceae in phytoplankton population and Rotifera in zooplankton population were dominant. (Table:3 and 4)

Table 3: List of different plankton groups of Jamuna River.

Plankton Type	Plankton Groups	Location			
		Gosaibari Dhunot, Bogura	**Closer Point, Sirajganj**	**Sariakandi Feri Ghat, Bogura**	**Balasi Ghat, Gaibandha**
Phytoplankton	Bacillariophyceae	*Asterionella sp. Coscinodiscus sp. Navicula sp. Nitzsehia sp. Synedra sp.*	*Coscinodiscus sp., Nitzsehia sp., Synedra sp.*	*Coscinodiscus sp. Synedra sp.,*	*Coscinodiscus sp. ,Navicula sp. Nitzsehia sp.*
	Cyanophyceae	*Spirulina sp Anabaena sp. Aphanocapsa sp. Coelosphaerium sp. Microcystis sp. Spirulina sp.*	*Coelosphaerium sp. sp., Microcystis sp. sp., Anabaena sp., Polycystis sp.*	*Coelosphaerium sp.,Polycystis sp., Spirulina sp.*	*Anabaena sp., Oscillatoria sp., Aphanocapsa sp., Microcystis sp.*
	Euglenophyceae	*Euglena sp. Phacus sp.*	*Phacus sp.,*	*Euglena sp.*	*Euglena sp.*
	Chlorophyceae	*Pediastrum sp. Spirogyra sp. Closterium sp. Volvox sp.*	*Pediastrum sp. Spirogyra sp., Pandorina sp.*	*Pediastrum sp. Spirogyra sp., Volvox sp.*	*Pediastrum sp. Spirogyra sp. Volvox sp., Ankistrodesmus sp.*
Zooplankton	Copepoda	*Cyclops sp. Nauplius sp.*	*Cyclops sp.*		
	Rotifera	*Lecane sp., Brachionus sp. Keratella sp.*	*Brachionus sp., Keratella sp.*	*Keratella sp. Lecane sp., Nauplius sp.*	*Lecane sp., Nauplius sp.*
	Branchiopoda	*Bosmina sp. Moina sp.*	*Moina sp.*	*Bosmina sp.*	*Bosmina sp. Moina sp.*

Table 4: List of different plankton groups of Atrai River.

Plankton Type	**Plankton Groups**	**Genus**
Phytoplankton	Bacillariophyceae	*Navicula* sp., *Synedra* sp., *Coscinodiscus* sp., *Asterionella* sp.
	Cyanophyceae	*Spirulina* sp., *Microcystis* sp., *Nostoc* sp., *Anabaena* sp., *Aphanocapsa* sp.
	Coscinodiscophyceae	*Coscinodiscus* sp.,
	Euglenophyceae	*Euglena* sp,. *Phacus* sp.,
	Chlorophyceae	*Spirogyra* sp., *Pandorina* sp. *Pediastrum* sp., *Scenedesmus* sp.
Zooplankton	Copepoda	*Cyclops* sp., *Nauplius* sp.
	Rotifera	*Brachionus* sp.
	Branchiopoda	*Bosmina* sp. *Moina* sp.,

iii) CPUE (Catch Per Unit Effort): CPUE of fish species were estimated (Kg/100m net/hour). Fish sampling were done in every selected sampling station to collect data, catch composition of different gear used and their catch per unit effort (CPUE). CPUE were estimated by the formula-Kg/100m net/hour. Data collection is ongoing.

iv) Gear Study

We observed that seine, lift, drag, cast, gill, fixed net and fish trap used widely during the sampling period in those study areas. Different types of fishing gear were presented in table 5.

Table 5: List of different Gear of Jamuna and Atrai River

Location	**Net name and type**
Jamuna River	**Net**
	Seine net (Ber, Kochal, Ghurni ber Jal), Lift net (Vesha Jal/Dharma Jal), Cast net (Jhaki/Khepla Jal), Drag net (Moi, Thela Jal), Gill net (Fash/Current, Okal Jal Jal) and Fixed net (Panti , Mosari/Vadai/Behundi Jal),
	Fish Trap
	China Duari, Darki, Polo
Atrai River	**Net**
	Seine net (Ber/Vadai/Moshari Jal), Cast net (Jhaki/Khepla Jal), Drag net (Moi Jal), Gill net (Puntijal, Koi Jal and Fash Jal) and Fixed net (Behundi Jal)
	Fish Trap
	Khalsane, Bana, Polo

v) Length-Weight Data: The length and weight of Jamuna river fish during the mentioned period is being recorded by collecting and measuring samples from commercial catches including landing sites.

vi) Correlate between climate and Field data

After sampling all the designated sampling spots, the collected climate data will be correlated.

Study 3: Identification of spawning and collection sites of carp fish eggs and larvae in River Jamuna.

i) Spawning and collection site data collection through the FGD survey and direct observation

Collection of relevant data on carp spawning sites (egg hatching area, larval migration area) from DOF officials, fishermen, aratdar, and key informants through FGD/direct observation is in progress.

Spawning site (Egg releasing spot, Larvae roaming area) of Carp Fish data were collected by FGD/direct observation from the concerned DoF official, fisherman, artdar and key informants.

Primarily at 2 locations on the Jamuna River, information was found on the sale of spawn collected from the river

- Gosaibari Ghat, Dhunot, Bogura
- Balasi Ghat, Gaibandha

Data collection is ongoing

Collection of detailed information on these two places is in progress.

Assessment of Effectiveness of Existing Hilsa Sanctuaries for Sustainable Production in Bangladesh

Researchers: Mezbabul Alam, SO
Md. Shariful Islam, SSO
Rumana Yasmin, SSO

Objectives

1. Reassess the previously identified spawning and nursery grounds and
2. Identification of new spawning and nursery grounds of Hilsa

Achievements (July 2023-June 2024)

Study-1 : Reassess the previously identified spawning and nursery grounds

Research was conducted on the following spawning and nursery grounds:

- From Veduria in Bhola District to Char Rustam in Patuakhali District (about 100 km area of Tentulia River) (3rd Hilsa Sanctuary, Tetulia River, Figure-1)

Figure 1: Sampling sites in Tetulia River

Physico-chemical parameters

Mean values and ranges of Physico-chemical parameters over the study period are presented in Table 1. Physico-chemical parameters such as air and water temperature (°C), transparency (cm), DO (mg/l), CO_2 (mg/l), pH, total hardness (mg/l), and total alkalinity (mg/l) were determined. The air and water temperature of the study areas were found to vary from 22 to

31^{o}C and 22.6 to 30 ^{o}C, respectively. Dissolved oxygen and free CO_2 ranged between 5 to 7 mg/l and 7.2 to 13 mg/l, respectively. The study areas pH and transparency salinity varied from 7.5 to 8, 8.1 to 29 cm respectively. No saline intrusion was observed in the study areas during the period. The results of the Physico-chemical parameters indicated that the parameters were within the suitable ranges for fishes in study areas.

Table 1: Physico-chemical parameters of 3rd Hilsa Sanctuary, Tetulia River

Parameters	Tetulia River	Standard value
Air temperature (°C)	28.06 ± 2.10 (22-31)	20-30 (EQS,1997)
Water temperature (°C)	26.41 ± 2.19 (22.6-30)	20-30 (EQS,1997)
DO (mg/l)	6.13 ± 0.52 (5-7)	4-6 (EQS,1997)
CO_2 (mg/l)	10.225 ± 1.98 (7.2-13)	6 ppm or less (EQS,1997)
pH	7.63 ± 0.15 (7.5-8)	6.5-8.5 (EQS,1997)
Transparency (cm)	16.55 ± 7.93 (8.1-29)	40 or less(Rahman,1992)
Salinity (ppt)	0.00 ± 0.00	0-10 (Rahman,1992)
Alkalinity (mg/l)	71.33 ± 15.96 (54-99)	>100 (Rahman,1992)
Hardness (mg/l)	97.58 ± 53.90 (62-270)	40-400 ppm (Boyd,1998)

Plankton identification

Following the drop count method, qualitative and quantitative analysis of planktons were done (APHA 1995). Plankton identification was made following Ward and Whipple (1959) and Prescott (1962). Ten plankton groups were identified in the qualitative study of plankton; among them seven were phytoplankton and three were zooplankton groups (Table 2). Among the seven phytoplankton groups, 24 genera were identified. Bacillariophyceae, Zygnematophyceae and Chlorophyceae were the most dominant groups of phytoplankton. But in the case of three zooplankton groups, almost six different genera were observed, including the same proportion.

Table 2: Qualitative assessment of Plankton at 3rd Hilsa Sanctuary, Tetulia River

Group	Genus	Genus

		No.
Chlorophyceae	*Pediastrum, Volvox, Scenedesmus, Acanthocystis,Microspora*	05
Ulvophyceae	*Ulothrix*	01
Zygnematophyceae	*Spirogyra, Nitzschia, Netrium, Staurastrum(end), Gonatozygon*	05
Bacillariophyceae	*Navicula, Gomphonema, Asterionella, Diatoma, Frustulia, Stephanodiscus, Cyclotella*	07
Cyanophyceae	*Spirulina, Rivularia,Oscillatoria*	03
Dinophyceae	*Ceratium*	01
Euglenophyceae	*Euglena*	01
Copepoda	*Cyclops, Nauplius*	02
Rotifera	*Brachionus, Keratella*	02
Cladocera	*Daphnia, Bosmina*	02

The quantitative study of phytoplankton observed a higher number on the lower side of the Tetulia River than on the upper side (Table 3).

Table 3: Quantitative assessment of plankton in 3rd Hilsa Sanctuary, Tetulia River

Sampling sites	Total Plankton (cells/L)	Total Phytoplankton (cells/L)	Total Zooplankton (cells/L)	Phytoplankton (%)	Zooplankton (%)
Bheduria	32×10^2	25×10^2	7×10^2	78.12	21.88
Char Kalmi	34×10^2	27×10^2	7×10^2	79.41	20.59
Kalaiya	35×10^2	26×10^2	9×10^2	74.28	25.72

CPUE of Hilsa

Hilsa CPUE in Tetulia River were observed higher amount in September and October and gradually lower in other months. (Figure-2)

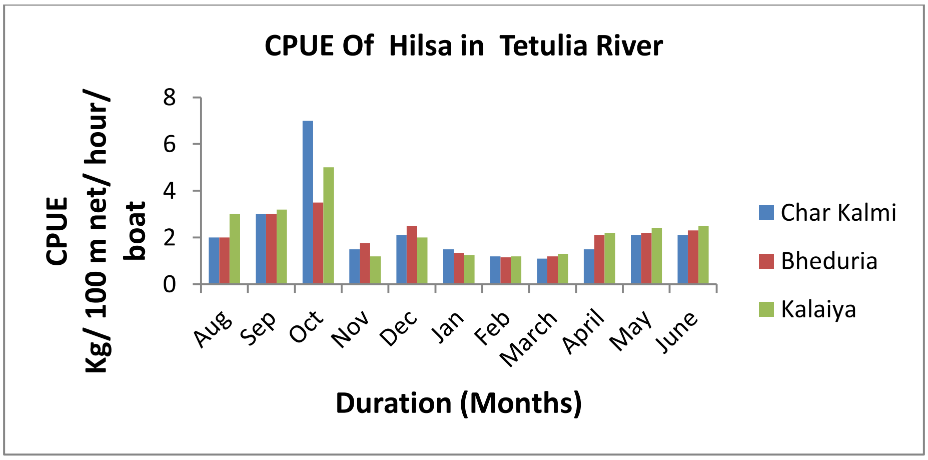

Figure-2: CPUE of Hilsa in Tetulia River

CPUE of Jatka

Jatka CPUE in Tetulia River were varied from a range of 4-5 kg/100 m net/ hour/ boat (figure-3)

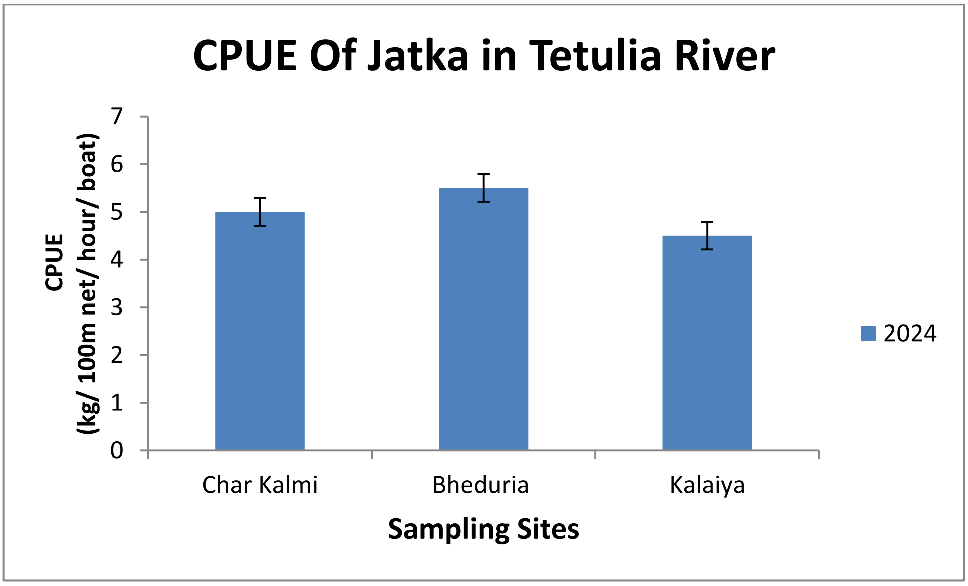

Figure-3: CPUE of Jatka in Tetulia River

Research was also conducted on the following spawning and nursery grounds:

- 40 km area of Andharamanik River in Kalapara Upazila of Patuakhali district (4th Hilsa Sanctuary, Andarmanik River , figure-4)

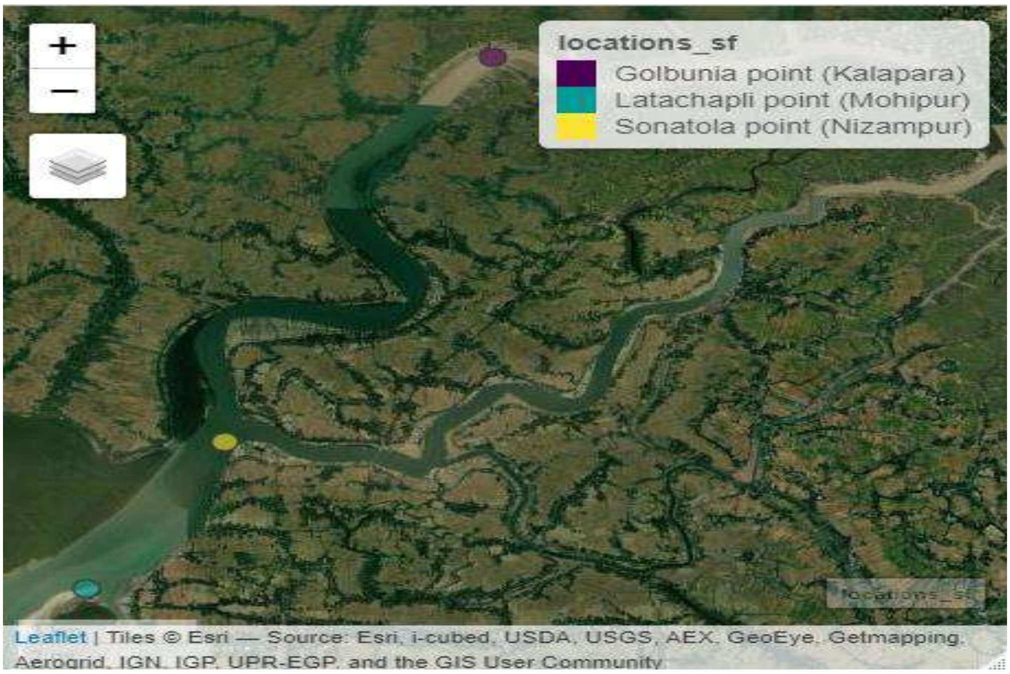

Figure 4: Samplig sites in Andarmanik River

Physico-chemical parameters

Mean values and ranges of Physico-chemical parameters over the study period are presented in Table 4. Physico-chemical parameters such as air and water temperature (°C), transparency (cm), DO (mg/l), CO_2 (mg/l), pH, total hardness (mg/l), and total alkalinity (mg/l) were determined. The air and water temperature of the study areas were found to vary from 23 to 32°C and 22.8 to 31 °C, respectively. Dissolved oxygen and free CO_2 ranged between 5 to 6 mg/l and 7.2 to 13 mg/l, respectively. The study areas pH and transparency salinity varied from 7.5 to 8.1, 11 to 29 cm respectively. The saline intrusion was observed in the study areas during the period ranging from 15-20 .The results of the Physico-chemical parameters indicated that the parameters were within the suitable ranges for fishes in study areas except saline intrusion in dry season.

Table 4: Physico-chemical parameters of 4th Hilsa Sanctuary, Andarmanik River)

Parameters	Andarmanik River	Standard value
Air temperature (°C)	29.06 ± 2.10 (23-32)	20-30 (EQS,1997)
Water temperature (°C)	26.41 ± 2.19 (22.8-31)	20-30 (EQS,1997)
DO (mg/l)	5.13 ± 0.52 (5-6)	4-6 (EQS,1997)
CO_2 (mg/l)	10.50 ± 1.85 (7.2-13)	6 ppm or less (EQS,1997)
pH	7.75 ± 0.15 (7.5-8.1)	6.5-8.5 (EQS,1997)
Transparency (cm)	18.55 ± 10.93 (11-29)	40 or less(Rahman,1992)
Salinity (ppt)	0.00 ± 0.00	0-10 (Rahman,1992)
Alkalinity (mg/l)	71.33 ± 15.96 (54-99)	>100 (Rahman,1992)
Hardness (mg/l)	97.58 ± 53.90 (62-270)	40-400 ppm (Boyd,1998)
Saline intrusion	12 ± 3.5 (15-20)	0-10 (Rahman,1992)

Plankton identification

Following the drop count method, qualitative and quantitative analysis of planktons were done (APHA 1995). Plankton identification was made following Ward and Whipple (1959) and Prescott (1962). Five plankton groups were identified in the qualitative study of plankton; among them three were phytoplankton and two were zooplankton groups (Table 5). Among the three phytoplankton groups, 16 genera were identified. Bacillariophyceae, Zygnematophyceae and Chlorophyceae were the most dominant groups of phytoplankton.

But in the case of three zooplankton groups, almost six different genera were observed, including the same proportion.

Table 5: Qualitative assessment of Plankton at 4th Hilsa Sanctuary, Andarmanik River)

Group	Genus	Genus No.
Chlorophyceae	*Pediastrum, Volvox, Scenedesmus, Acanthocystis,Microspora*	05
Bacillariophyceae	*Navicula, Gomphonema, Asterionella, Diatoma, Frustulia, Stephanodiscus, Cyclotella, Coscinidiscus*	08
Cyanophyceae	*Spirulina, Rivularia,Oscillatoria*	03
Copepoda	*Cyclops, Nauplius*	02
Rotifera	*Brachionus, Keratella*	02

The quantitative study of phytoplankton observed a higher number on the lower side of the Andarmanik River than on the upper side (Table 4).

Table 6: Quantitative assessment of plankton in 4th Hilsa Sanctuary, Andarmanik River)

Sampling sites	Total Plankton (cells/L)	Total Phytoplankton (cells/L)	Total Zooplankton (cells/L)	Phytoplankton (%)	Zooplankton (%)
Mohipur	28×10^2	22×10^2	6×10^2	78.12	21.88
Hajipur	26×10^2	22×10^2	4×10^2	79.41	20.59
Kalapara	31×10^2	24×10^2	7×10^2	74.28	25.72

Study-2: Identification of New Spawning and Nursery grounds of Hilsa

Sampling was conducted from Noria to Maowa Ghat (25 km) of Padma river for the extension of 5th Hilsa sanctuary (figure-5).

Figure-5: Sampling sites in lower Padma River

Physico-chemical parameters:

Mean values and ranges of Physico-chemical parameters over the study period are presented in Table 7. Physico-chemical parameters such as air and water temperature (°C), transparency (cm.), DO (mg/l), CO_2 (mg/l), pH, total hardness (mg/l), and total alkalinity (mg/l) were determined. The air and water temperature (°C) of the study areas were found to vary from 24 to 32^oC and 23 to 31^oC, respectively. Dissolved oxygen and free CO_2 ranged between 4.5 to 8 mg/l and 7 to 11.8 mg/l, respectively. The study areas pH, transparency and salinity varied from 7.5 to 8.5, 16.5 to 33 cm and 0 ppt, respectively. Salinity intrusion was not observed in the Padma river during the study period. Alkalinity and hardness ranged from 49 to 121 mg/l and 60 to 129 mg/l during the study period. The analytical results of the Physico-chemical parameters indicated that the parameters were within suitable ranges for fishes in the study areas

Table 7: Physico-chemical parameters of Padma river

Parameters	Padma river	Standard value
Air temperature (^oC)	29 ± 2 (24-32)	20-30 (EQS,1997)
Water temperature (^oC)	227.17 ± 1.94 (23-31)	20-30 (EQS,1997)
DO (mg/l)	5.73 ± 0.84 (4.5-8)	4-6 (EQS,1997)
CO_2 (mg/l)	9.74 ± 1.45 (7-11.8)	6 ppm or less (EQS,1997)
pH	8.11 ± 0.31 (7.5-8.5)	6.5-8.5 (EQS,1997)
Transparency (cm)	27.94± 3..76 (33-16.5)	40 or less(Rahman,1992)
Alkalinity (mg/l)	65.67± 18.66 (49-121)	>100 (Rahman,1992)
Hardness (mg/l)	79.88 ±16.53 (60-129)	40-400 ppm (Boyd,1998)
Salinity (ppt)	0	0-10 (Rahman,1992)

Plankton identification

Following the drop count method, qualitative and quantitative planktons were analyzed (APHA 1995). Plankton identification was made following Ward and Whipple (1959) and Prescott (1962). In the qualitative study among nine plankton groups six phytoplankton and three zooplankton groups (Table 8) were identified. Among the six phytoplankton groups, 21 genera were identified where Bacillariophyceae, Zygnematophyceae and Chlorophyceae were the most dominant groups. But in the case of zooplankton groups, five different genera were observed where Cladocera and Rotifera were the dominant group (Table 8).

Table 8: Qualitative assessment of Plankton of Padma River

Group	Genus	Genus No.
Chlorophyceae	*Pediastrum, Volvox, Scenedesmus, Acanthocystis*	04
Ulvophyceae	*Ulothrix*	01
Zygnematophyceae	*Spirogyra, Nitzschia, Netrium, Staurastrum(end), Gonatozygon*	05
Bacillariophyceae	*Navicula, Gomphonema, Asterionella, Diatoma, Frustulia, Stephanodiscus, Cyclotella*	07
Cyanophyceae	*Spirulina, Rivularia, Oscillatoria*	03
Dinophyceae	*Ceratium*	01
Copepoda	*Nauplius*	01
Rotifera	*Keratella, Brachionus*	02
Cladocera	*Bosmina, Moina*	02

The quantitative study of phytoplankton observed a higher amount in Noria (38×10^2 cells/ l) of Padma river than in Lauhajang (36×10^2 cells/ l) and Mawa ghat (32×10^2 cells/ l) (Table 09).

Table 09: Quantitative assessment of plankton in Padma River

Sampling sites	Total Plankton (cells/l)	Total Phytoplankton (cells/l)	Total Zooplankton (cells/l)	Phytoplankton (%)	Zooplankton (%)
Noria	38×10^2	30×10^2	8×10^2	78.94	21.06
Lauhajang	36×10^2	27×10^2	9×10^2	75	25
Mawa	32×10^2	25×10^2	7×10^2	78.12	21.88

CPUE of Jatka

Jatka CPUE in Padma River was varied from a range of 0.3-4.5kg/100 m net/ hour/ boat. It was higher in the lower Padma region than the upper region. (figure-6)

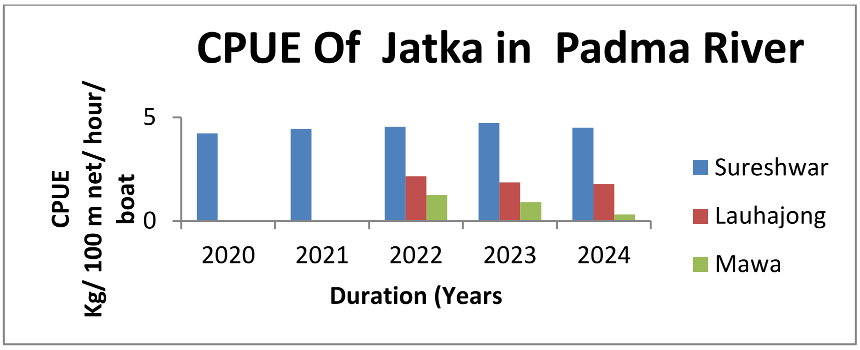

Figure-6: CPUE of Jatka in Padma River

- Sampling was conducted in the following locations from Ramgoti to Tankirkhal of existing 1st Hilsa sanctuary for the expansion of existing sanctuaries in Lower Meghna estuary (figure-7)

- Ramgoti, Laxmipur
- Tankirkhal, Noakhali

Figure 7: sampling sites in Lower Meghna River (Hatiya Channel)

Physico-chemical parameters:

Mean values and ranges of Physico-chemical parameters over the study period are presented in Table 10. Physico-chemical parameters such as air and water temperature (°C), transparency (cm.), DO (mg/l), CO_2 (mg/l), pH, total hardness (mg/l), and total alkalinity (mg/l) were determined. The air and water temperature (°C) of the study areas were found to vary from 25 to 31^{o}C and 23 to 29^{o}C, respectively. Dissolved oxygen and free CO_2 ranged between 5 to 7 mg/l and 7.2 to 13 mg/l, respectively. The study areas pH, transparency and salinity varied from 7.5 to 7.82, 17 to 29 cm and 10-17 ppt, respectively. Salinity intrusion was observed in the areas during the periods. Alkalinity and hardness ranged from 54 to 120 mg/l and 62 to 235 mg/l during the study period. The analytical results of the Physico-chemical parameters indicated that the parameters were within suitable ranges for fishes in the study areas.

Table 10: Physico-chemical parameters of 1st Hilsa sanctuary (Ramgoti to Tankirkhal)

Parameters	Average values (Ramgoti to Tankirkhal)	Standard value
Air temperature (°C)	27.83 ± 1.94 (25-31)	20-30 (EQS,1997)
Water temperature (°C)	26.21 ± 2.04 (23-29)	20-30 (EQS,1997)
DO (mg/l)	6.12± 0.60 (5-7)	4-6 (EQS,1997)
CO_2 (mg/l)	10.38 ± 1.99 (7.2-13)	6 ppm or less (EQS,1997)
pH	7.62 ± 0.14 (7.5-7.82)	6.5-8.5 (EQS,1997)
Transparency (cm)	22.67 ± 4.47 (17-29)	40 or less(Rahman,1992)
Alkalinity (mg/l)	73.67± 20.62 (54-120)	>100 (Rahman,1992)
Hardness (mg/l)	97.91 ± 48.24 (62-235)	40-400 ppm (Boyd,1998)
Salinity (ppt)	15.5 ± 0.70 (10-17)	0-10 (Rahman,1992)

Plankton identification

Following the drop count method, qualitative and quantitative planktons were analyzed (APHA 1995). Plankton identification was made following Ward and Whipple (1959) and Prescott (1962). In the qualitative study among ten plankton groups seven phytoplankton and three zooplankton groups (Table 11) were identified. Among the seven phytoplankton groups, 19 genera were identified where Bacillariophyceae, Zygnematophyceae and Chlorophyceae were the most dominant groups But in the case of three zooplankton groups, almost six different genera were observed, including the same proportion. **(**(Table 8)**)**

Table 11: Qualitative assessment of Plankton of 1st Hilsa sanctuary (Ramgoti to Tankirkhal)

Group	Genus	Genus No.
Chlorophyceae	Pediastrum, Volvox, Scenedesmus, Microspora	04
Ulvophyceae	Ulothrix	01
Zygnematophyceae	Spirogyra, Nitzschia, Netrium, Gonatozygon	04
Bacillariophyceae	Navicula, Gomphonema, Asterionella, *Diatoma*, Frustulia, Cyclotella	06
Cyanophyceae	Spirulina, Rivularia,	02
Dinophyceae	Ceratium	01
Euglenophyceae	Euglena	01
Copepoda	Cyclops, Nauplius	02
Rotifera	Brachionus, Keratella	02
Cladocera	*Daphnia*, *Bosmina*	02

The quantitative study of phytoplankton observed a higher amount in Ramgoti (35×10^2 cells/l) of Meghna river than in Tankirkhal (33×10^2 cells/ l) (Table 9).

Table 12: Quantitative assessment of Plankton of 1st Hilsa sanctuary (Ramgoti to Tankirkhal)

Sampling sites	Total Plankton (cells/l)	Total Phytoplankton (cells/l)	Total Zooplankton (cells/l)	Phytoplankton (%)	Zooplankton (%)
Ramgoti	35×10^2	28×10^2	7×10^2	80	20
Tankirkhal	33×10^2	26×10^2	7×10^2	78.78	21.21

CPUE of Jatka

Jatka CPUE at 1st Hilsa sanctuary of Meghna River was varied from a range of 4-5 kg/100 m net/ hour/ boat. It was silghtly lower than the previous years because of high saline intrusion during the period of January to March. (Figure-8)

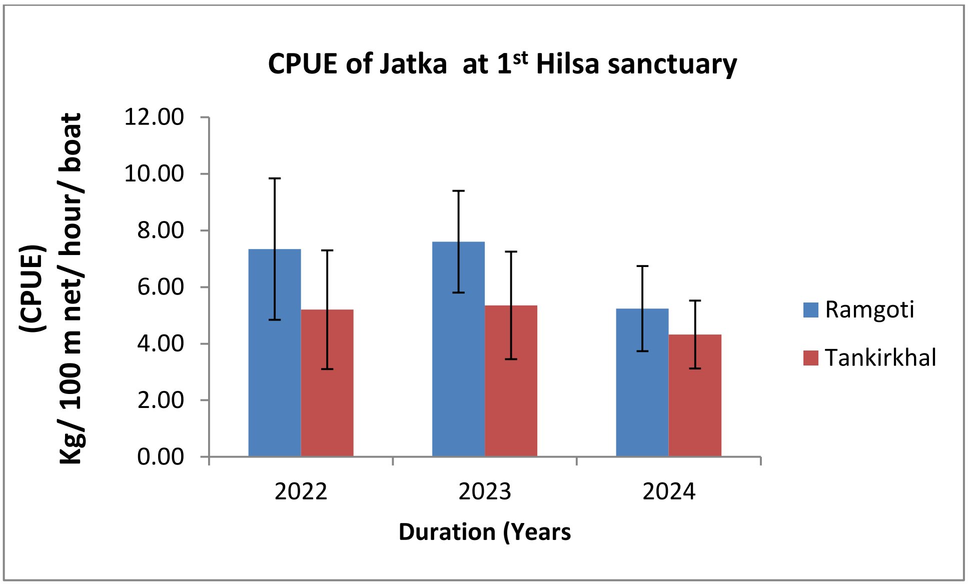

Figure-8: CPUE of Jatka at 1st Hilsa sanctuary of Meghna River

Domestication and Conservation of Commercially Important Threatened Riverine Finfish

Researchers:

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: Md. Mozzammel Hoque, SSO
: Mezbabul Alam, SO

Objectives:

- To collect the selected riverine fishes from wild sources
- To study the food, feeding habits, and reproductive parameters of the collected fishes
- To develop the brood management techniques of the fishes in captive conditions
- To develop the induced breeding techniques of the selected fishes in captive conditions
- To develop the nursery rearing techniques of the selected fishes in captive conditions
- To assess the growth and yield performance of selected fishes in captive conditions

Achievements

Experiment 1: Collection of fry/fingerling/sub-adult/adult fish

A total of 500 sub-adults of *Rita rita* and 600 sub-adults of *Silonia silondia* were collected from the wild sources to domesticate in ponds at the BFRI, RS, Chandpur.

Experiment 2: Studies of the food, feeding habit and reproductive parameters of the selected fishes

A total of 100 *S. silondia were* examined and details of information on the dissected *S. silondia* fishes are present in Table 1. No empty gut was observed in all the dissected fishes. Out of 100 *S. silondia*'s stomachs 13 stomachs (13%) were quarter filled (<25% filled), 51 stomachs (51%) were half filled (25-50% filled), and 24 stomachs (24%) were three quarter filled (50-75% filled) whereas, 12 stomachs (10%) were full filled (75-100% filled). The *S. silondia* fish consumes a wide range of food. Fish, crab body particles, crab chelate leg, shrimp, shrimp chelate leg, rotifers, debris, and worms were found frequently in the examined gut.

Table 1: Information on the dissected *S. silondia*

Total length (cm)	Weight (g)	Gut length (cm)	Gut weight (g)
18.08±1.22	30±6.78	20.7±1.48	3.84±0.51

A total of 100 *R.rita were* examined and details of information on the dissected *R. rita* fishes are present in Table 2. No empty gut was observed in all the dissected fishes. Out of 100 *R.rita's* stomachs12 stomachs (12%) were quarter filled (<25% filled), 40 stomachs (40%) were half filled (25-50% filled), and 27 stomachs (27%) were three quarter filled (50-75% filled) whereas, 21stomachs (21%) were full filled (75-100% filled). A total of eight major groups of prey items which includes mollusk, worm, insect, plant, copepod, teleost, detritus, and debris were made up of the stomach contents.

Table 2: Information on the dissected *R. rita*

Total length (cm)	Weight (g)	Gut length (cm)	Gut weight (g)
33.64±6.42	502.91±16.78	48.57±1.38	34.84±12.51

A total of 100 *P. pangasius were* examined and details of information on the dissected *P. pangasius* fishes are present in Table 3. No empty gut was observed in all the dissected fishes. Out of 100 *P. pangasius's* stomachs 27 stomachs (27%) were quarter filled (<25% filled), 40 stomachs (40%) were half filled (25-50% filled), and 18 stomachs (18%) were three quarter filled (50-75% filled) whereas, 15 stomachs (15%) were full filled (75-100% filled). The *P. pangasius* fish consumes a wide range of food. Snail, mussel, crab body particles, crab chelate leg, shrimp, shrimp chelate leg, rotifers, debris, and worms were found frequently in the examined gut.

Table 3: Information on the dissected *P. pangasius*

Total length (cm)	Weight (g)	Gut length (cm)	Gut weight (g)
34±4.32	352.91±10.78	52.57±3.38	32.84±15.51

Reproductive biology:

To know the reproductive biology of selected fish , data were collected on total length, weight, , sex ratio and Gonadosomatic index (GSI) which are presented in Table-4.

Table-4: Reproductive parameter of *S. silondia, R. rita and P. pangasius*

Species	Total length (cm)	Weight (g)	Sex Ratio	Gonad length (cm)	Gonad Weight (g)	GSI
S. silondia	32.66±3.54	270.66±81.74	M :F=1:1.15	33.50±1.64	30.07±14.94	10.65±2.32
R. rita	33.64±6.42	502.91±16.78	M :F=1:1	6.5±2.22	4.13±1.41	0.71±0.51
P. pangasius	34±4.32	352.91±10.78	M :F=1:1.2	9.5±0.41	22.13±0.41	6.61±1.71

Experiment 3: Brood management techniques of the selected fishes in captive condition

This experiment is being conducted in earthen ponds under natural conditions at BFRI, RS, Chandpur for 12 months, from December, 2023 to November 2024. The experiment was concerned with observing the growth, gonadal maturation and yield performance of *L. boggut*, *S. silondia* and *G. gagata* fishes under a polyculture system in the captive condition. Three ponds were selected for each candidate species. Three ponds will be divided into six parts. Each pond is considered as one treatment, e.g., Treatment-1 (T_1), Treatment-2 (T_2), Treatment-3 (T_3) and each part is considered as one replication. The ponds are 20 decimal in size. The water depth is maintained at 1-1.5 meters. The ponds were prepared by dewatering, liming (1 kg decimal^{-1}) and fertilization (Urea 100 g and TSP 50-100 g decimal^{-1}). The experimental design is presented in Table 5.

Table 5: Experimental design for brood rearing of of P. pangasius, S. silondia and R. rita in captive condition

Treatment	Stock. dens. (indi. ha^{-1})		
	S. silondia	*R. rita*	*P. pangasius*
T_1	2,000	2,000	2,000
T_2	2,500	2,500	2,500
T_3	3,000	3,000	3,000

Sources of fingerlings: Wild sources fingerlings of selected fishes were stocked.

Supplementary feeding: Commercial fish feed (containing 28-30% protein) is being fed @ 8-5% of their total biomass daily. Feed supply is being adjusted by estimating the standing crop by random stock sampling.

Sampling and data collection: Fishes are sampled once every fortnightly for a periodic check on growth. The growth study is recorded as total length in cm and weight in g.

Water quality data on water depth (cm), water temperature (^{O}C), secchi disc transparency (cm), dissolved oxygen (mg l^{-1}), water pH, free CO_2 (mg l^{-1}) and ammonia (mg l^{-1}) is recorded fortnightly.

Water quality parameters were congenial for brood rearing. Water quality parameters of different treatments are mentioned in Table 6.

Table 6: Water quality parameters in three treatments

Parameters	T_1	T_2	T_3
Water Temperature (^{o}C)	32±1.71	31.5±2.06	31.5±1.71
DO (mg l^{-1})	6.25±0.35	5.8±0.28	6±0.14
CO_2	12.1±1.56	11.75±1.77	12.9±2.40
pH	7.25±0.35	7.25±0.35	7.25±0.35
NH_3	0	0	0
Transparency (cm)	30±1.51	35±1.80	28±1.43

Weight of the selected fishes during stocking and average body weight of the selected fishes in pond condition after 180 days of culture are shown in table 7.

Table 7: Initial weight and average body weight of the fishes in pond condition after 180 days of culture

Treatment	Initial weight (g)		ABW (g) after 180 Days	
	S. silondia	*R. rita*	*S. silondia*	*R. rita*
T1	19.52	20.87	48±4.41	56.25±6.35
T2	20.22	20.23	46.88±5.22	55.80±4.28
T3	18.75	19.55	46.25±6.06	54.40±8.14

Climate Change and Anthropogenic Actors Affecting Fisheries Resources and Livelihoods in Riverine Ecosystem of Bangladesh

Researchers

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Objectives

1. To determine the effects of climate change on biophysico-chemical parameters of water and water quality index
2. To determine the effects of climate change on riverine fish species diversity
3. To determine the effects of climate change on livelihood of riverine fisher's along the Meghna river Basin in Bangladesh
4. To know the present status of the impact of climate change on riverine ecology, fish diversity and livelihood of the fisher's with probable future impacts

Achievements

Location of the study areas

The study was conducted to assess the impact of climate change on the river ecology, diversity of fish fauna and the livelihoods of fisher's folk in three fishing communities viz. Upper (S_1), and Lower (S_2) Meghna river basin and Andharmanik river basin (S_3) from Bangladesh Fisheries Research Institute, Riverine Station, Chandpur. Most of the fishermen of the selected communities were professional and they were engaged in fishing round the year. In the selected areas, all types of fishing remain prohibited under the "Fish Protection and Conservation Act 1950" during March-April while juvenile Hilsa (jatka) are abundant in the upper and lower stretches of Padma and Meghna rivers as well as during the peak spawning time of Hilsa during the October-November.

Data collection

In order to address the project objectives, data was collected through direct field observations and from the secondary sources. The secondary data mainly comprised of meteorological data and it was collected from the Water Development Board, Meteorological Department and from published literatures. However, in this report, the results obtained from direct filed observations are depicted. To study the impact of climate change, almost 30 fishers were randomly selected for interviews. The questionnaire mainly focused on fishers' livelihood characteristics, level of dependency on fisheries, and exposure to climatic hazards.

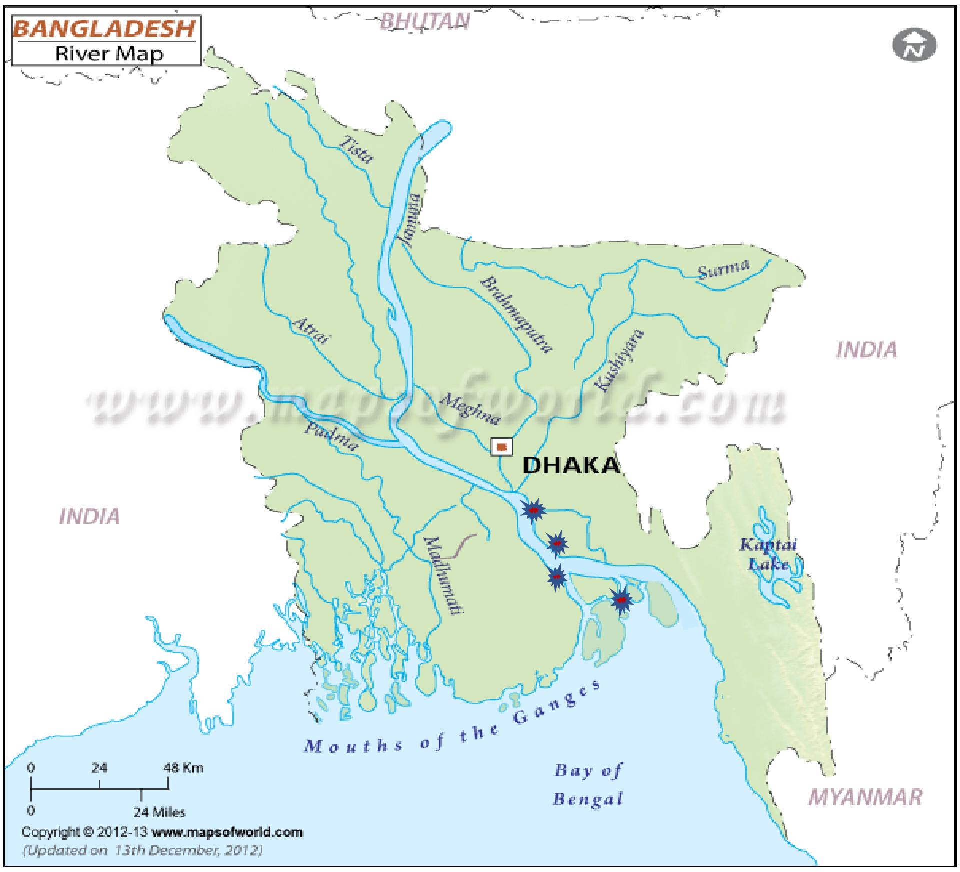


Figure 1. Sampling locations on the map of Bangladesh.

Results

List of Anthropogenic causes of climate change in Bangladesh

In Bangladesh, a country particularly vulnerable to the impacts of climate change, several anthropogenic factors contribute to climate change. Here's a list tailored to Bangladesh:

1. Deforestation: Bangladesh has experienced significant deforestation due to agricultural expansion, urbanization, and logging. This reduces the number of trees available to absorb carbon dioxide from the atmosphere, contributing to increased greenhouse gas concentrations.

2. Agricultural Practices: Traditional agricultural practices in Bangladesh, such as rice cultivation and livestock farming, contribute to greenhouse gas emissions. Methane emissions from flooded rice paddies and enteric fermentation in livestock are significant sources of emissions.

3. Industrial Growth: Bangladesh's rapid industrialization, particularly in sectors such as textiles and manufacturing, has led to increased emissions of greenhouse gases and other pollutants.

4. Burning of Biomass: The burning of biomass for cooking and heating purposes, especially in rural areas where access to clean energy sources is limited, releases carbon dioxide and other greenhouse gases into the atmosphere.

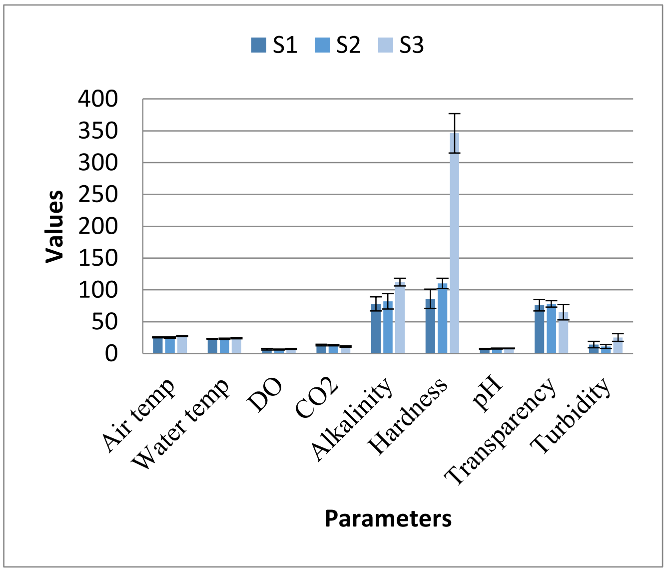
5. Urbanization and Infrastructure Development: Urbanization and the construction of infrastructure such as roads, buildings, and dams can lead to land use changes and associated emissions, including the release of stored carbon from soil and vegetation.

6. Energy Production: The reliance on fossil fuels for electricity generation and other energy needs contributes to greenhouse gas emissions. Bangladesh is investing in coal-fired power plants to meet its growing energy demand, which further exacerbates climate change.
7. Waste Management: Inadequate waste management practices, including open dumping and burning of waste, release methane and carbon dioxide into the atmosphere.
8. Transportation: Like many developing countries, Bangladesh faces challenges related to transportation emissions, particularly from vehicles powered by fossil fuels.
9. Riverbank Erosion and Coastal Development: Changes in river courses and coastal erosion, exacerbated by anthropogenic activities such as riverbank encroachment and unsustainable coastal development, contribute to habitat loss and increased vulnerability to climate change impacts.

Addressing these anthropogenic factors requires a combination of mitigation and adaptation strategies, including transitioning to renewable energy sources, promoting sustainable land use and forest management practices, improving waste management systems, enhancing resilience to climate impacts, and integrating climate considerations into development planning and policies.

Physico-chemical parameters of water in the study areas

Water quality parameters of three stations exhibited considerable fluctuations. The maximum air temperature (27.3±0.7ºC) and water temperature (24.2±1.1ºC) were found at Station 3 (S_3). The maximum and minimum value of water transparency were found 78+5 cm and 65+12 cm at S_2 and S_3, respectively. The average highest dissolved oxygen content 7.3±0.7 and 6.8+0.8, (mg/L), respectively was found at (S_2 and S_3). On the contrary, the average CO_2 were beyond 15 mg/L in all stations. The maximum alkalinity 112+6 mg/L and hardness 346+31 mg/L were also found at S_3 (Figure 2). The average pH was found just slightly above the neutral limit in the studied sampling sites. The results of the studied physico-chemical parameters reveled that water quality parameters were found within the acceptable limits for the growth of fish in all sampling sites.



Parameters	**Standard**
Air temp	20-30 (EQS,1997)
Water temp	20-30 (EQS,1997)
DO	4-6 (EQS,1997)
CO_2	6 ppm (EQS,1997)
Alkalinity	>100 (Rahman, 1992)
Hardness	200-500 (DOE, 2003)
pH	6.5-8.5(EQS,1997)
Transparency	40 or less (Rahman, 1992)

Figure 2. Water quality parameter of sampling stations

Species diversity

A biodiversity index is used to describe the diversity of a sample or community by a single number. The concept of the "species diversity" involves two components: the number of species or richness and the distribution of individuals among species. The value of a diversity index increases both when the number of species and evenness increases. For a given number of species, the value of a diversity index is maximized when all species are equally abundant. Shannon-Wiener diversity index considers both the number of species and proportion of each species while evenness and dominance indices represent the number of species present in an ecosystem as well as the relative abundance of each species.

The results of diversity index revealed that Simpson's index was found highest in station S_2 (0.65) and Shannon-Wiener index were found highest in S3 (1.1). The higher value of Shannon-wiener diversity index was reflected by higher number of species. Furthermore, the highest Evenness index was recorded in stations S_2 (0.18) and the lowest in S_3 (0.17), respectively. Margalef richness value is used as an indicator to compare the species numbers in different sites. Margalef richness value was found highest in S_2 (2.42) and minimum at Sr (1.879). The results of others diversity indices have been furnished in Table l.

Table 1: Diversity indices of species.

Diversity indices	**S_1**	**S_2**	**S_3**
Simpson_1-	0.3338	0.6554	0.485
Shannon_H	0.6817	0.12506	1.099
Evenness_e^H/S	0.172	0.1809	0.171
Brillouin	0.6808	0.7406	1.091
Menhinick	0.3381	0.341	0.2404
Margalef	1.879	2.427	2.139
Equitability_J	0.2393	0.2465	0.3878
Fisher_alpha	2.569	2.93	2.199
Berger-Parker	0.7999	0.7853	0.6974

The study recorded 39 fish species and 2 decapods in the Meghna river basin that belonged to 10 orders. Hilsa (*Tenualosa ilisha*) was the main commercial species of the Meghna and Tetulia rivers. Among the observed fish species, a total of 38.0% of fish belonged to Siluriformes, followed by Cypriniformes, (16.3%), Perciformes (19.0%), Osteoglossiformes (6.0%), Tetraodontiformes (3.3%), Channiformes (3.3%), Synbranchiformes (3.0%), Pleuronectiformes (3.0%) and Decapoda (6.0%) (Table 2 and Figure 3).

Table 2: Availability of hilsa and other commercially important fishes in the study area.

Orders	**Scientific name**	**Local name**
Cypriniformes	*Esomus danrica*	Darkina
	Puntius chola	Chela puti
	Puntius ticto	Tit puti
	Labeo rohita	Rui
	Amblypharyngodon mola	Mola
Siluriformes	*Eutropiichthys vacha*	Bacha
	Ompok pabda	Pabda
	Wallago attu	Boal

	Silonia silondia	Shilong
	Pangasius pangasius	Pangas
	Ailia coila	Kajuli
	Rita rita	Rita
	Sperata aor	Air
	Mystus vitatus	Tengra
	Mystus tengara	Gang Tengra
	Clupisoma garua	Gaura
	Pseudeutropius atherinoides	Batashi
Tetraodontifomes	*Tetraodon cutcutia*	Potka
Channiformes	*Channa punctatus*	Taki
Clupiformes	*Tenualosa ilisha*	Ilish
	Corica soborna	Kachki
Synbranchiformes	*Monopterus cuchia*	Cuchia
Osteoglossiformes	*Notopterus notopterus*	Foli
	Chitala chitala	Chital
Perciformes	*Colisa fasciatus*	Kholisa
	Anabas testudineus	Koi
	Chanda beculis	Chanda
	Glossogobius giuris	Bele
	Nandus nandus	Bheda
	Otolithoides pama	Poa
Pleuronectiformes	*Cynoglossus arel*	Kukurjib
Decapoda		Shrimp
	Scylla serrata	Kakra

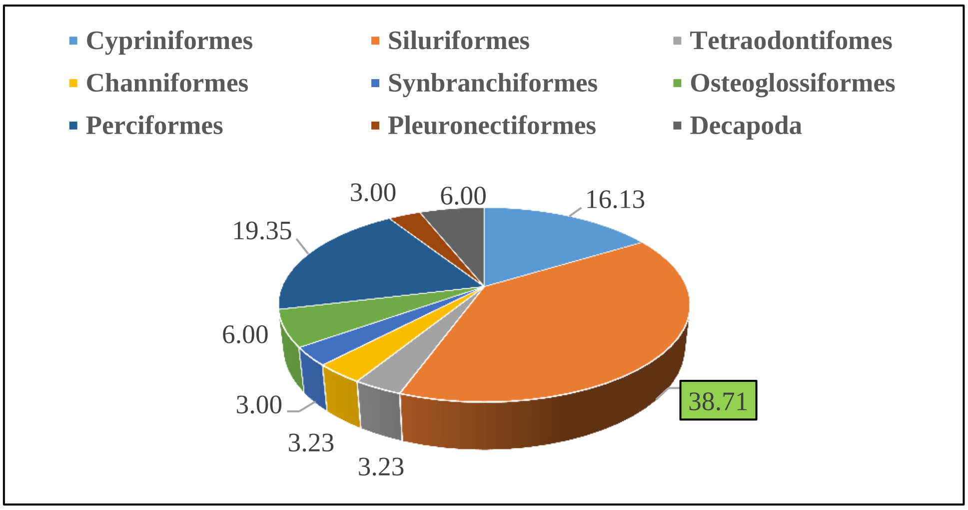


Figure 3. Species composition of the study area

Social profiles of the fisherman

The demographic status of the fishing communities was quite a different from the other professional communities. In fisher's village the average number of individuals per household is 5. Among the 30 fishers interviewed, 25 were exclusively involved in fishing, 5 were involved both in fishing and other agricultural farming and small business for the maintenance of their daily life. Among all the fishermen 85% were married, 11% unmarried and 4% were divorced (Figure 4).

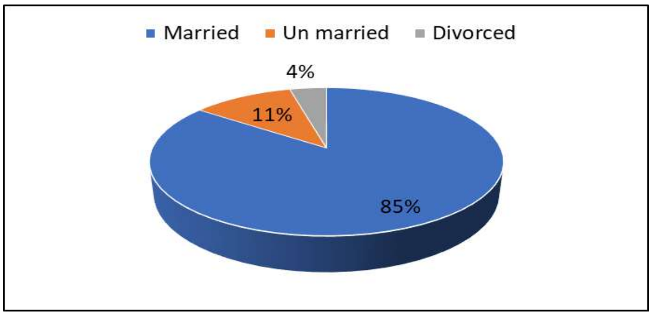


Figure 4. Marital status of the fisher's folk.

Family size and types

Family size is a significant socioeconomic indicator since it has an impact on household income, food consumption, and socioeconomic well-being. According to the survey results, 71% of fisher's have 6-8 family members, 24% have less than 5 family members, 3% have more than 8 family members and 2% have more than 10 family members, respectively. Family structure ranged from joint (consists of grandfather and mother, husband, wife, children, uncles, aunties, nephews etc.) to single nuclear types (consists of husband, wife and children) family (Figure 5).

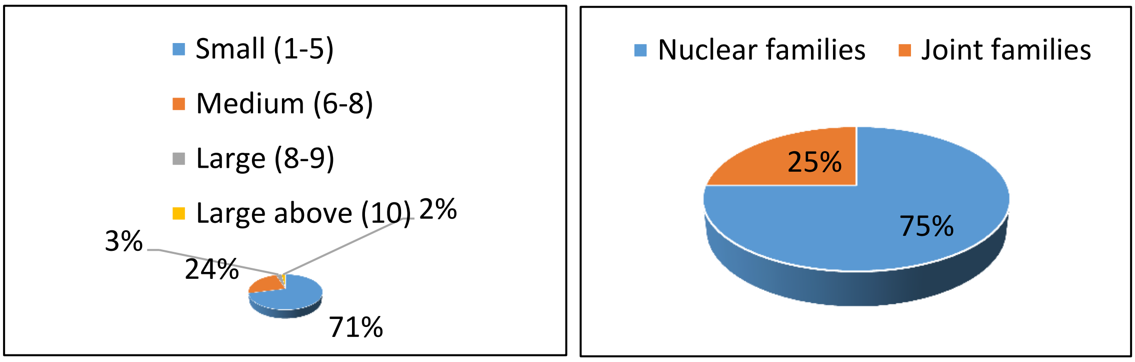


Figure 5. Family size and types of the fisher's folk.

Age structure

The interviewed fishermen's ages ranged from far under 20 to well over 60. Only around 3% of the fishermen were younger than 20. Two-fifths were between the ages of 21 and 40, another 40% were between the ages of 41 and 50, l0% were between the ages of 51 and 60, and the remaining 7% of fisherman were older than 60. The results revealed that people in the age range of 40 to 50 actively participate in fishing more than those in any other age group (Figure 6).

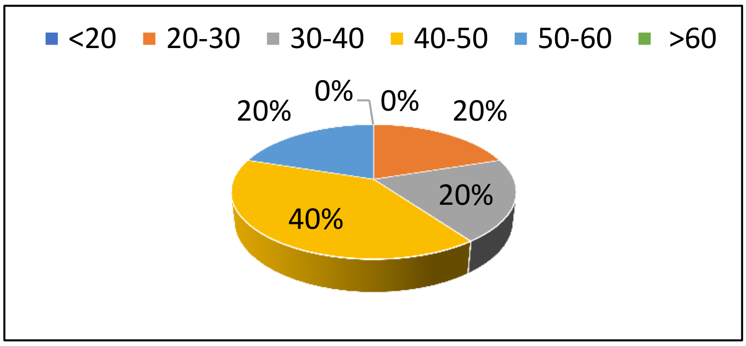
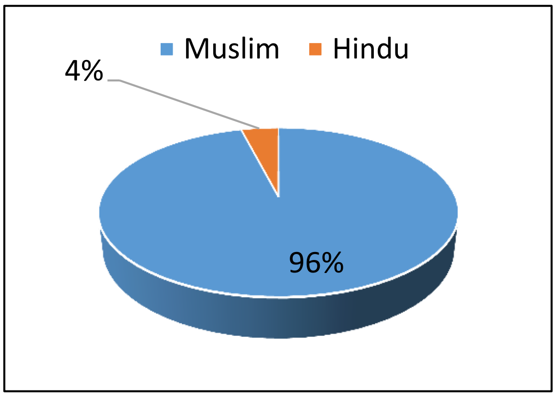


Figure 6. Age structure of the fisher's folk.

Sex composition and religion

The male members of the fisherman's family were preponderant. There were 62% males and 38% females in the fishermen community. Religiously, around 96% were Muslims and 4% were Hindus within the fishing community (Figure 7). The small-scale fishing and trade community was heavily influenced by religious and social stratification.



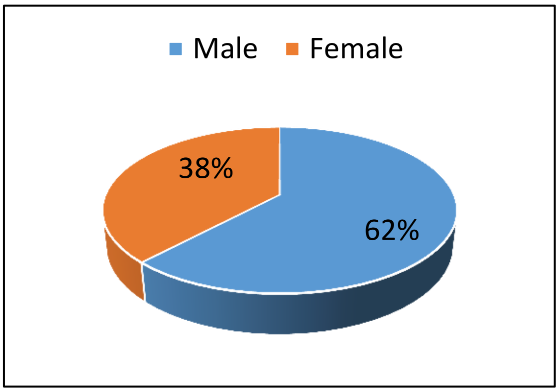


Figure 7. Sex composition and religion of the fisher's folk.

Educational and literacy status

The study revealed that fishermen did not have enough education. Sixty-two percent of small-scale fishermen were either illiterate or only had a primary school education. There were four distinct categories of fishermen depending on their average degree of education. The illiteracy rate was just 15% overall, but 85% among fishermen's kids. About 31% of fisher's kids were in elementary school, 5% in primary school, 2% in high school, and 0.01% in college. It seems the fisherman, despite their lack of access to formal education, were coming to terms with the need of formal education (Figure 8).

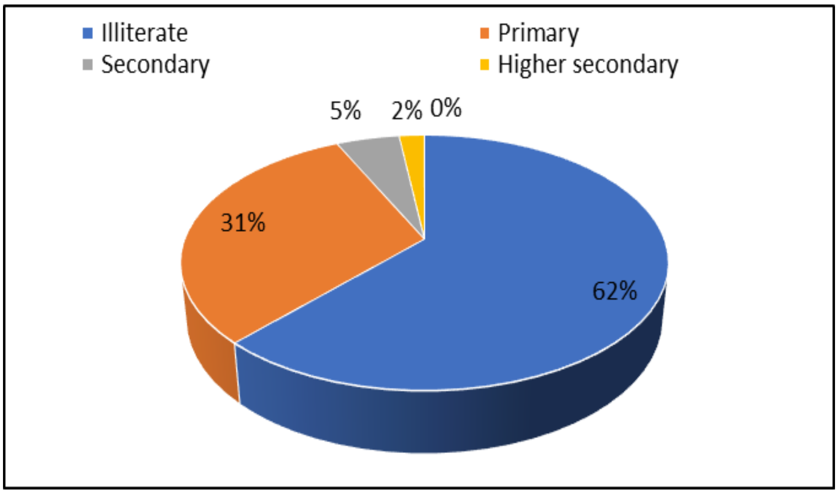


Figure 8. Literacy status of the fisher's folk.

Health facilities

The fishing communities' access to healthcare is inadequate. According to the present investigation, 30% of fisher households experienced major issues and migrate to the upazila health complex, 35%

rely on kabiraj, and 35% (Figure 9) of fisher households depend on untrained/ unlicensed village doctors in drug houses whose owners lack knowledge of current medical science.

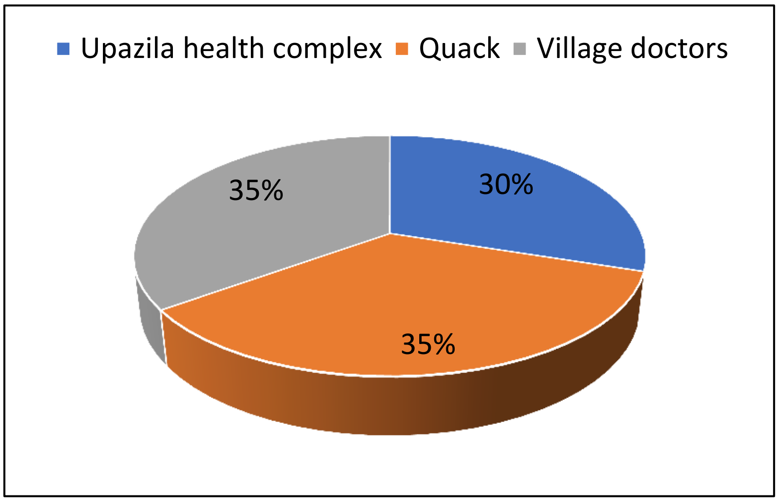


Figure 9. Health facilities of the fisher's folk.

Drinking water facilities

Access to safe to drinking water is yet ensured in the studies areas. Some fisher's community still use pond water, river water, well water for drinking, cooking and bathing. It was observed that about 91% people use tube well, 7% people use well, 1% people use river and 1% people use pond water as a source of drinking water (Figure 10).

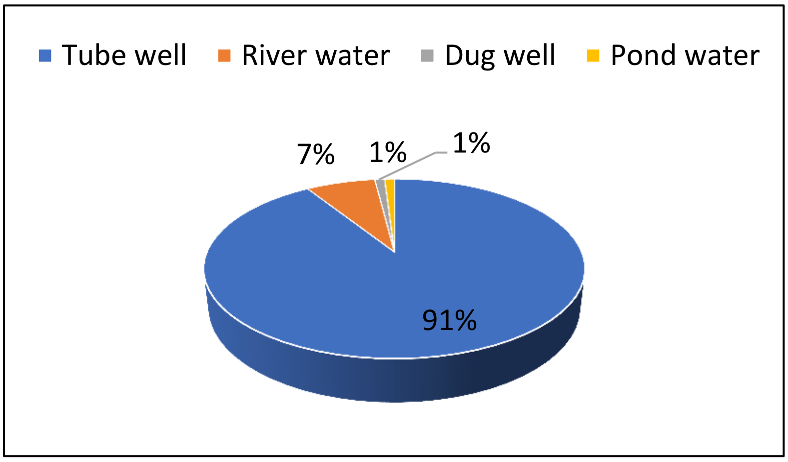


Figure 10. Drinking water facility of the fisher's folk.

Housing and infrastructure

In the studied areas, the community houses were of three main types viz. katcha, semi pacca and pacca houses. Kacha-houses were made of bamboo spill and tin with mud flooring and semi pacca-was made of wood and tin with cement floor whereas pacca houses were made of bricks and tin with cement floor. Under the present study, it was found that among the fisher's community, 88% of housing structures were kacha, 10% were semi pacca and only 2% were pacca houses (Figure 11). Road and transportation system were found under developed.

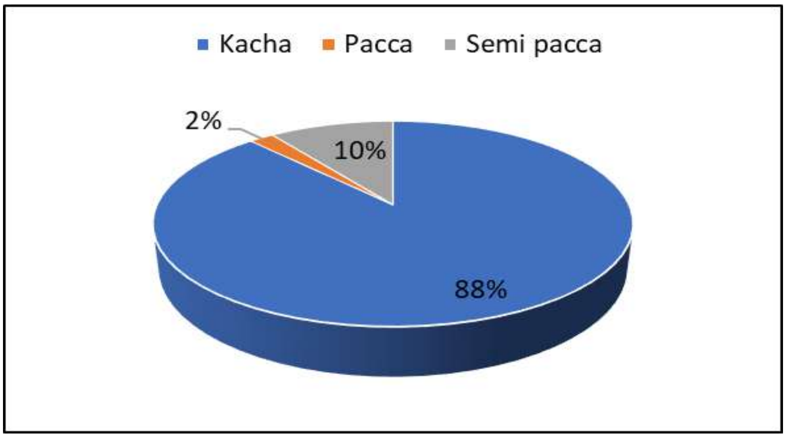


Figure 11. Housing and Infrastructure facilities of the fisher's folk.

Fishing assets

Respondents to the study informed that the annual cost of maintaining and repairing their boats were between 20,000 and 50,000 Tk. (BDT). About 82% of the fishermen had their own boat and equipment. The remaining 18% contribute to the Mohajons' boat in some way, either as manual laborers or by sharing in the catch.

Development of value added Hilsa Products and Optimization of Storage Condition

Researchers

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Objectives of the study

i. To determine the overall quality and shelf-life of ready-to-cook (RTC) hilsa fish ball and other products under vacuum and modified atmosphere packaging at refrigerated (4˚C) storage condition
ii. To know the effects of vacuum packaging on overall quality and shelf life of hilsa fish under ice and frozen storage condition
iii. To determine the overall quality and shelf-life of ready-to-eat (RTE) hilsa fish crackers under vacuum and modified atmosphere packaging at room temperature.

Achievements

Experiment-1: The overall quality and shelf-life of ready-to-cook (RTC) hilsa fish ball and other products under vacuum and modified atmosphere packaging at refrigerated (4˚C) storage condition

Sample collection

Hilsa fish (*Tenualosa ilisha*) weighing 0.8±0.15 kg collected from the Chandpur fish landing center were brought to the Hilsa Lab of Bangladesh Fisheries Research Institute, Riverine Station, Chandpur under iced condition. The fish were then kept in freezing condition for further processing.

Preparation of hilsa fish ball

Preparation of boneless hilsa flesh: After thawing overnight, the frozen hilsa were cleaned and seasoned with a bit of salt and turmeric powder. To remove the bones, the fish were pressure cooked on high for 10 minutes, then simmered on low for about an hour. This softened the small pin bones and made it easy to extract the larger straight bones. The boneless hilsa flesh was then used to make fish balls.

Recipe of hilsa ball: The following ingredients were used to prepare hilsa fish ball:

a) Boneless hilsa flesh- 500 g
b) Gingerpaste-15 g
c) Garlic paste- 8 g
d) Onion- 50 g

e) Green chili- 4 pcs
f) Corn flour- 15 g
g) Pepper powder-1 g
h) Soy sauce- 500 g
i) Dried bread crumbs-1cup (½ cup for mixing, ½ cup for breading)
j) Salt- as per taste
k) Lemon juice- 2 tsp
l) Egg- 2

First onion, garlic, ginger and green chilies were sautéed in vegetable oil which is then mixed with cooked boneless flesh in a bowl. Then all ingredients including corn flour, pepper powder, soy sauce, dried bread crumbs, salt and lemon juice were added and mashed well. Fish ball was then prepared with hands and dipped in the batter (prepared with eggs) individually and placed on the plate of breadcrumbs. Rolled the balls evenly and kept it in the refrigerator for half an hour. Hilsa fish ball are now ready for packaging.

Figure: **Preparation of hilsa fish ball**

Biochemical and Microbiological Analysis

pH

A Ready-to-Cook (RTC) product, Hilsa Fish Ball, was created and its pH level and microbial growth were monitored at refrigeration temperatures. The initial pH of the Hilsa Fish Balls was 5.85 and remained between 5.86 and 6.28 throughout storage, well within the acceptable range of 6.8 to 7.0 in all packaging conditions (Metin *et al.*, 2001) (Table 1). Implementation of Modified Atmosphere Packaging (MAP) for packaging is currently in progress, with subsequent analysis to follow promptly.

Table 1: pH value of Hilsa fish ball at chilled storage

Treatments	Storage period				
	0d	**5d**	**10d**	**15d**	**20d**
Not Sealed Pack (Control)	5.85±0.08	5.86±0.13	6.17±0.08	6.18±0.01	6.28±0.09
MAP-1 (50% CO_2 + 50% N_2)	MAP installation for packaging is currently underway, and analysis will follow promptly.				
MAP-2 (40% CO_2 + 30% N_2 +30% O_2)					

Aerobic Plate Count (log CFU/g)

The growth and presence of bacteria in food products are crucial indicators of shelf life and quality. Bacterial counts ranging from 2 to 6 logs CFU/g are deemed acceptable for freshly caught freshwater fish (Gelman *et al.*, 2001). The aerobic plate count (APC) of pre-fried hilsa fish balls was measured at 4.08 log CFU/g on agar media, indicating an acceptable initial quality of the fish balls (Table 2). MAP installation for packaging is currently in progress, with subsequent analysis to follow promptly.

Table 2: Aerobic Plate Count (log CFU/g) of Hilsa fish cutlet at chilled storage

Treatments	Storage period				
	0d	**5d**	**10d**	**15d**	**20d**
Not Sealed Pack (Control)	4.03±0.17	5.81±0.08	6.89±0.22	7.52±0.05	8.27±0.14
MAP-1 (50% CO_2 + 50% N_2)	MAP installation for packaging is currently underway, and analysis will follow promptly.				
MAP-2 (40% CO_2 + 30% N_2 +30% O_2)					

Preparation of hilsa fish Cutlet

Preparation of boneless hilsa flesh: After thawing overnight, the frozen hilsa underwent cleaning and seasoning with a pinch of salt and turmeric powder. To debone the fish, it was subjected to high-pressure cooking for 10 minutes followed by simmering on low heat for approximately an hour. This method effectively softened the small pin bones, facilitating the extraction of the larger straight bones. The boneless hilsa flesh was subsequently utilized in preparing fish cutlets.

Recipe of hilsa fish cutlet: The following ingredients were used to prepare hilsa fish cutlet:

Boneless hilsa flesh- 500 g, Gingerpaste-15 g, Garlic paste- 8 g, Onion- 50 g, Green chili- 4 pcs, Corn flour- 15 g, Pepper powder-1 g, Soy sauce- 500 g, Dried bread crumbs-1cup (½ cup for mixing, ½ cup for breading), Salt- as per taste, Lemon juice- 2 tsp, Egg- 2

1. Begin by sautéing onion, garlic, ginger, and green chilies in vegetable oil until they are fragrant and golden brown.
2. In a large mixing bowl, combine the sautéed mixture with the cooked boneless hilsa flesh.
3. To the bowl, add the following ingredients: corn flour for binding, pepper powder for seasoning, soy sauce for flavor, dried breadcrumbs for texture, salt for taste, and lemon juice for a hint of acidity. Thoroughly mix all the ingredients until well combined and mashed.
4. Prepare the batter by whisking eggs until they are well beaten and smooth.
5. With clean hands, shape the hilsa mixture into individual fish cutlets, forming them into desired shapes.
6. Dip each cutlet into the prepared egg batter, ensuring they are fully coated.
7. Once coated with egg, transfer the cutlets onto a plate containing breadcrumbs. Gently press each cutlet into the breadcrumbs to coat them evenly on all sides.
8. Place the breadcrumb-coated cutlets in the refrigerator and let them chill for at least half an hour. Chilling helps the cutlets firm up and hold their shape.
9. After chilling, the hilsa fish cutlets are now ready for packaging, either for immediate consumption or for storing in the freezer for later use.

Biochemical and Microbiological Analysis

pH

A Ready-to-Cook (RTC) product, Hilsa cutlet, was created and its pH level and microbial growth were monitored at refrigeration temperatures. The initial pH of the Hilsa cutlet was 5.83 and remained between 5.86 and 6.29 throughout storage, well within the acceptable range of 6.8 to 7.0 in all packaging conditions (Metin *et al.,* 2001) (Table 1). Implementation of Modified

Atmosphere Packaging (MAP) for packaging is currently in progress, with subsequent analysis to follow promptly.

Table 3: pH value of Hilsa fish cutlet at chilled storage

Treatments	Storage period				
	0d	**5d**	**10d**	**15d**	**20d**
Not Sealed Pack (Control)	5.83±0.09	5.86±0.18	6.10±0.04	6.16±0.04	6.29±0.07
MAP-1 (50% CO_2 + 50% N_2)	MAP installation for packaging is currently underway, and analysis will follow promptly.				
MAP-2 (40% CO_2 + 30% N_2 +30% O_2)					

Aerobic Plate Count (log CFU/g)

Monitoring the growth and presence of bacteria in food products is critical for assessing their shelf life and overall quality. Gelman et al. (2001) established that bacterial counts ranging from 2 to 6 logs CFU/g are considered acceptable for freshly caught freshwater fish. In the case of hilsa fish cutlets, the aerobic plate count (APC) was meticulously measured at 4.03 log CFU/g on agar media, suggesting an initial quality level within acceptable limits (refer to Table 4). Currently, efforts are underway to install modified atmosphere packaging (MAP) for the packaging process. Subsequent comprehensive analysis is scheduled to be conducted promptly, enabling a thorough assessment of the product's microbial status and ensuring its compliance with quality standards.

Table 4: Aerobic Plate Count (log CFU/g) of Hilsa fish cutlet at chilled storage

Treatments	Storage period				
	0d	**5d**	**10d**	**15d**	**20d**
Not Sealed Pack (Control)	4.03±0.17	5.81±0.08	6.89±0.22	7.52±0.05	8.27±0.14
MAP-1 (50% CO_2 + 50% N_2)	MAP installation for packaging is currently underway, and analysis will follow promptly.				
MAP-2 (40% CO_2 + 30% N_2 +30% O_2)					

Impact of Lunar Periodicity, Saline Intrusion, Rainfall and Water Discharge on Hilsa Fisheries in a Changing Climate in Bangladesh

Researchers:

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Objectives

1. To determine the effects of lunar periodicity and tidal fluctuations on hilsa breeding and production
2. To assess the extent and intensity of saline intrusion on hilsa navigation route in Bangladesh and its impact on hilsa abundance and distribution
3. To determine the impacts of rainfall and water discharge on hilsa production
4. To estimate the impact of physico-chemical parameters on hilsa production
5. To update hilsa management interventions and policy guidelines

Achievements in the past

In order to address the aforesaid objectives of the project, the data was collected from direct field observation, as well as some secondary data were accessed from some web sources that provide 24 hours real-time data on different meteorological factors. The data on tidal fluctuations (tidal co-efficient, tidal heights) was collected from “Tide Forecast.com” and Tides4Fishing” website. Lunar periodicity data were accessed from the “Phases of the Moon” app and classical “Time and Date.com”. The data on hilsa demographics (length, weight, percentage of male, female, spent and oozing hilsa) were collected from direct field investigations from the selected sampling locations (Figure 1).

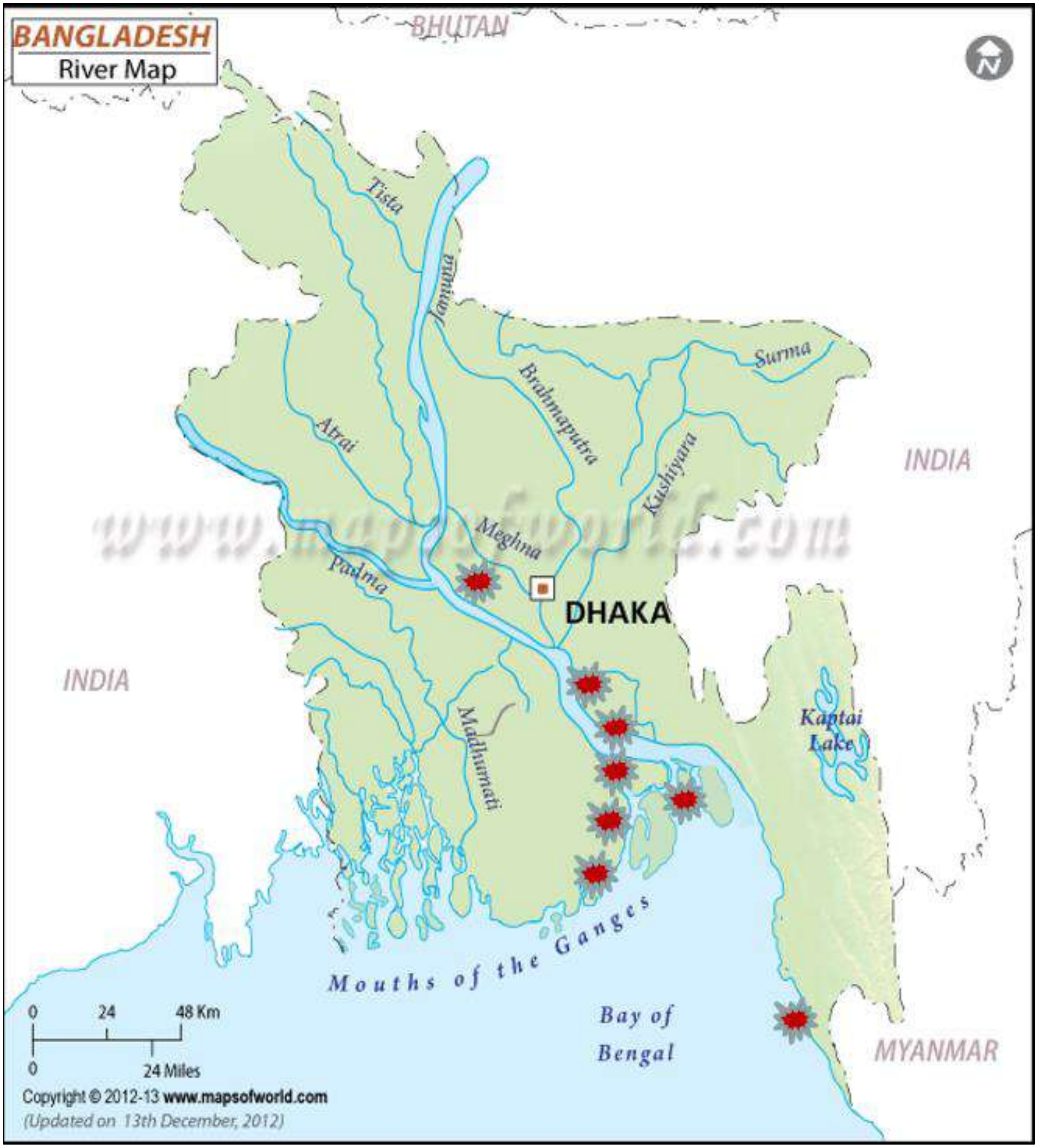

Figure 1. Sampling locations with asterisk mark on the map of Bangladesh.

The results of the analysis are furnished below:

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Salinity of Meghna River and Hilsa Production

Salinity intrusion, ranging from 1 to 12 parts per thousand (ppt) on average, was observed gradually advancing from the lower to upper reaches of the Meghna River, persisting for approximately half of the year. Throughout the study period, salinity levels were documented at Ramgoti (1-12 ppt avg.), Char Alexander (1-8 ppt avg.), and Char Ludhua (0.5-6.5 ppt avg.) sequentially during the dry season (December to May) of each sampling year in the Meghna River. This intrusion, occurring gradually from downstream to upstream, particularly during the dry season spanning December to May, could potentially create an inhospitable environment for freshwater fish over time.

The peak season for the increase in river water salinity typically occurs in February and March. Conversely, the ban on catching Jatka, a crucial stage in the hilsa fish's life cycle, is enforced during March and April. Consequently, the rising salinity levels within the sanctuary may impede the growth of Jatka, posing a threat to the overall hilsa production in the future.

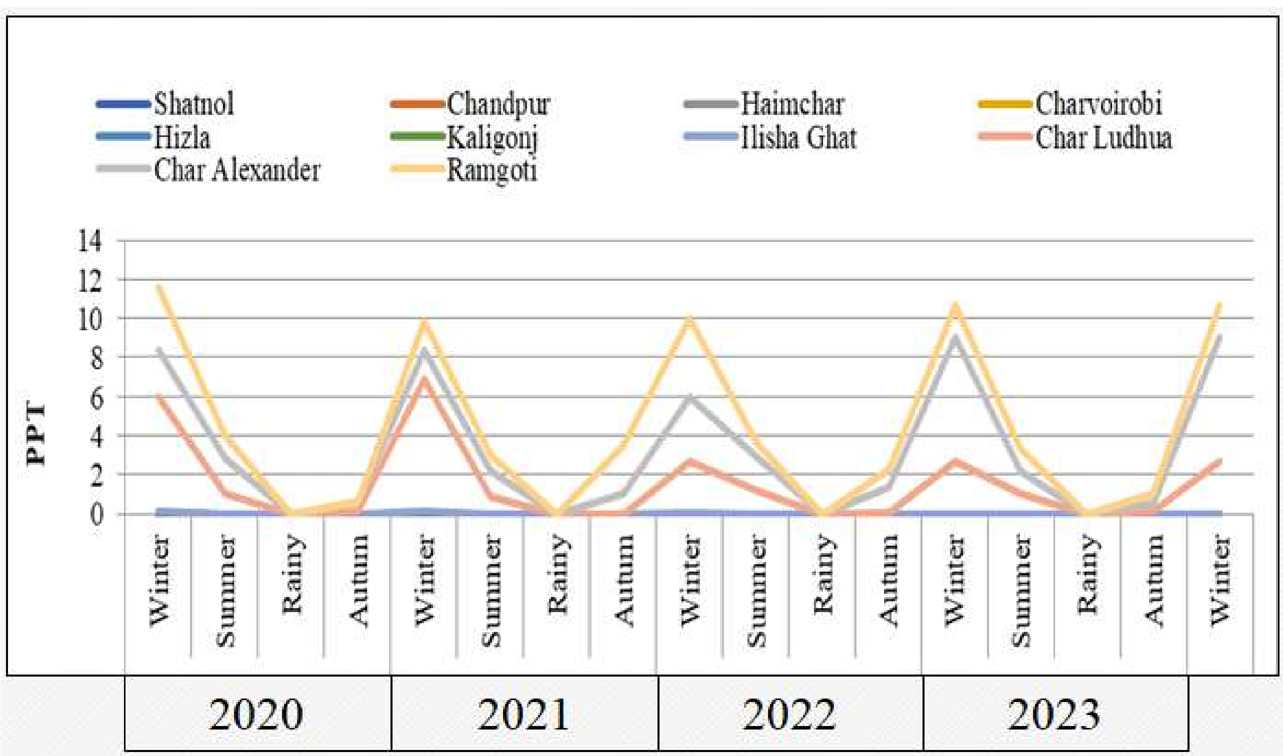


Figure.2. Average Salinity Intrusion of Meghna River (2020-23) Source: BFRI

Total nos. of hilsa in different lunar phases

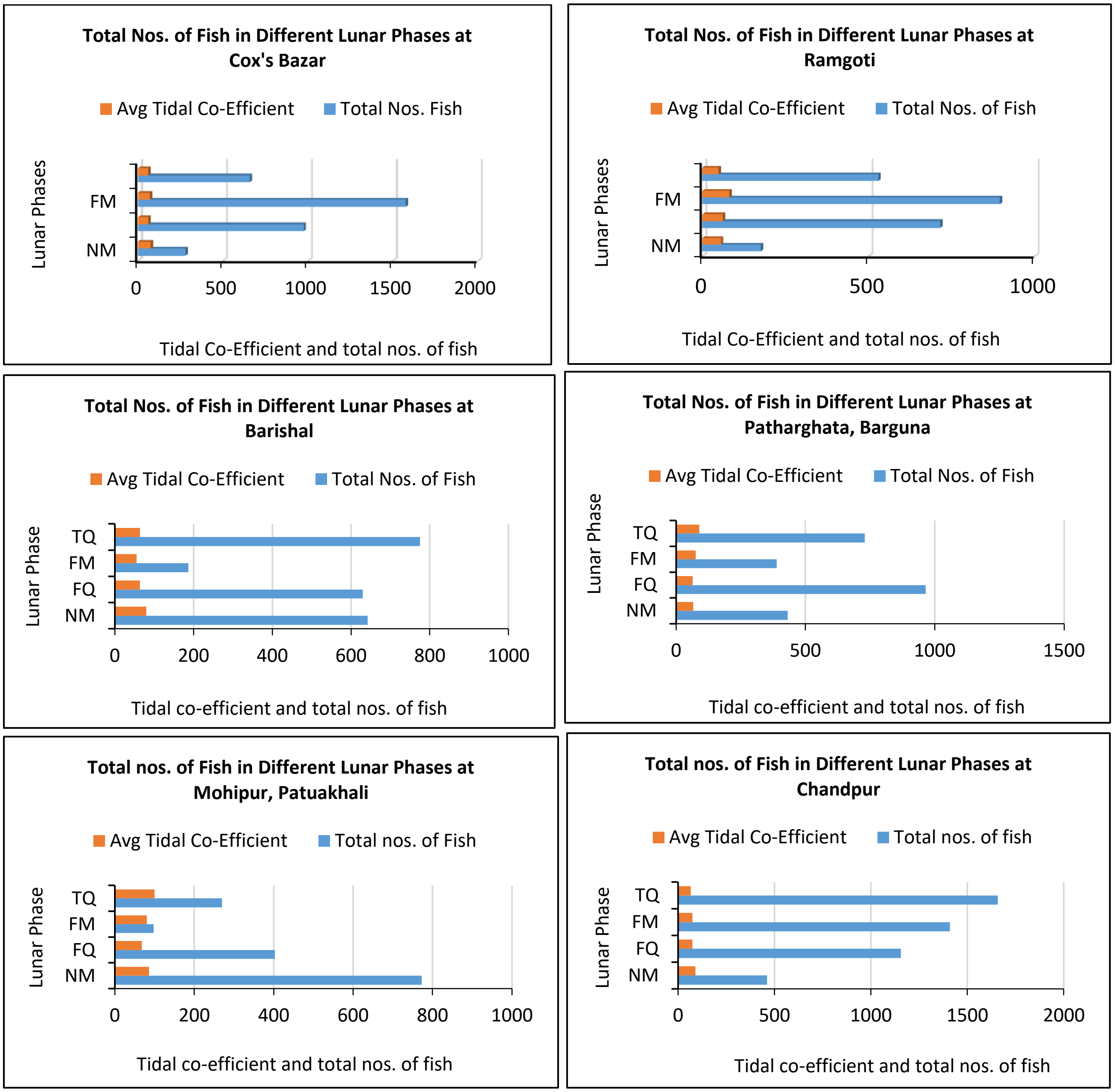


Figure 3. Total nos. of hilsa in different lunar quarters in relation to tidal co-efficient

Total nos. of hilsa captured in different lunar phases also varied considerably. At Cox's Bazar and Ramgoti, the highest total nos. of hilsa were captured during the FM and tidal co-efficient were also higher than other phases of moon. At Barishal and Chandpur, the highest nos. of hilsa were captured during the TQ of the moon whereas at Patharghata, Barguna and Mohipur, Patuakhali, the highest nos. of hilsa were captured during the FQ and NM, respectively. The results of the present study conducted till to date indicate that marine hilsa capture (Cox's Bazar, Ramgoti and Patuakhali) is influenced by lunar distance viz. full and new moon (Figure 3). Albeit, tidal co-efficient is directly influenced by the lunar distance, still is difficult opine that it has specific and direct influence on the total nos. of hilsa captured, more data collection is required to reach in that conclusion.

Hilsa breeding performance in different lunar phases

The percentages of male, female, spent and oozing hilsa were calculated in the present study. The result demonstrated that highest percentages of male, female, spent and oozing hilsa were found during the NM followed by the other quarters.

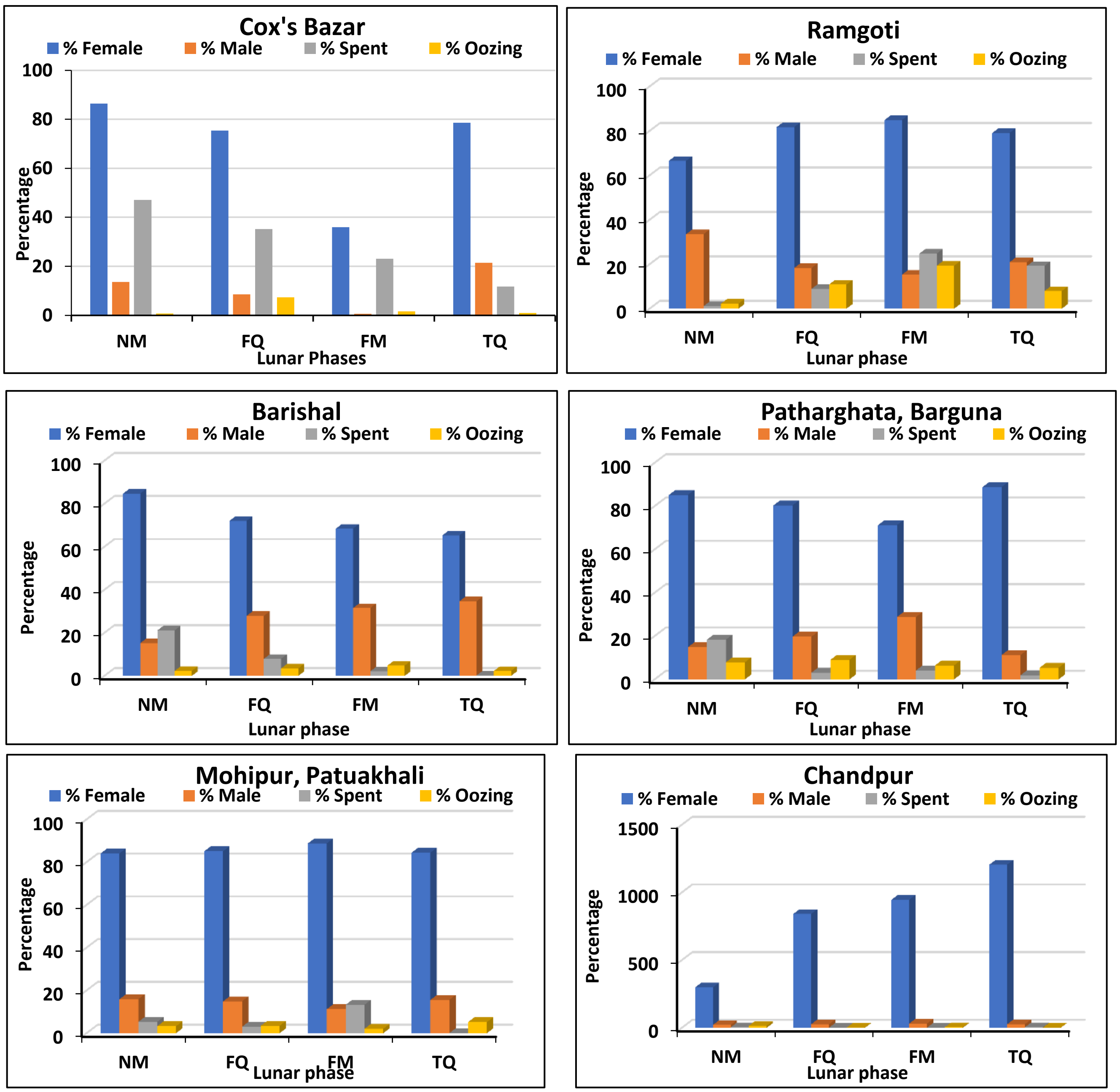


Figure 4. The percentages of male, female, spent and oozing hilsa during different lunar phases.

The gonadosomatic index (GSI) of hilsa collected from all sampling locations in different lunar phases were also calculated and the mean value of GSI exhibited considerable fluctuations. The highest GSI value was found during the NM as well, indicating that hilsa prefers to breed during the NM rather than the other quarters (Figure 4 and 5).

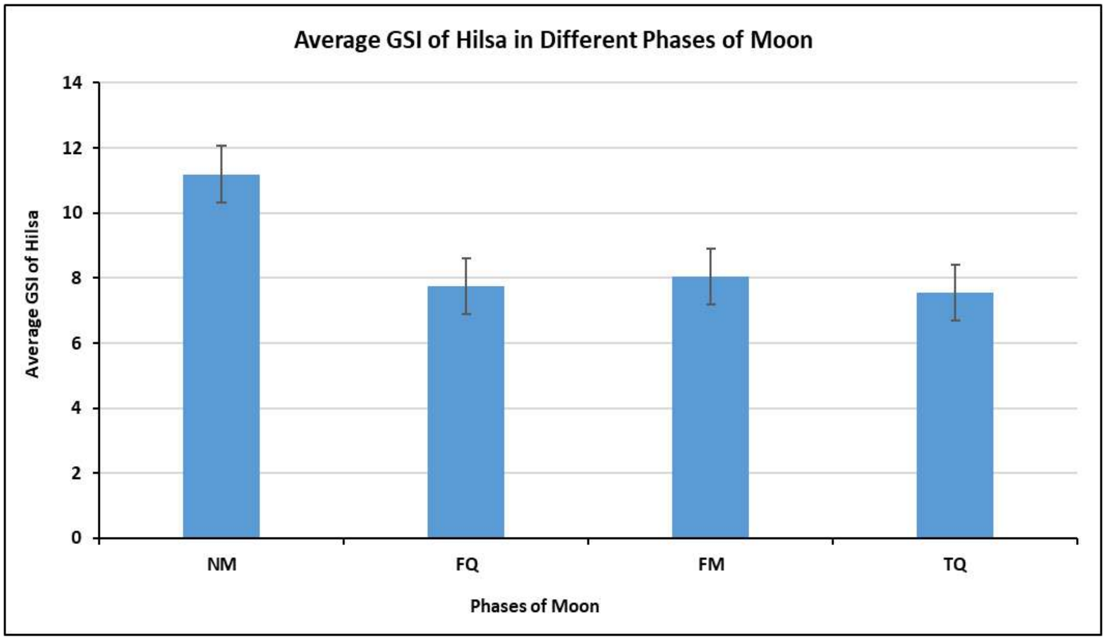


Figure 5. Gonadosomatic index of hilsa in different lunar phases

Oocyte Diameter

Annual increase in the oocyte diameter of *T.ilisha* was observed and compared with the GSI in Chandpur. Data of oocyte diameter showed significant correlation with GSI change, which suggest a synchronize development of gonads with the increasing size of eggs. Maximum oocyte diameter was in October (0.591±0.03mm) whereas minimum oocyte diameter was found in April (0.359±0.029mm) (Figure 6).

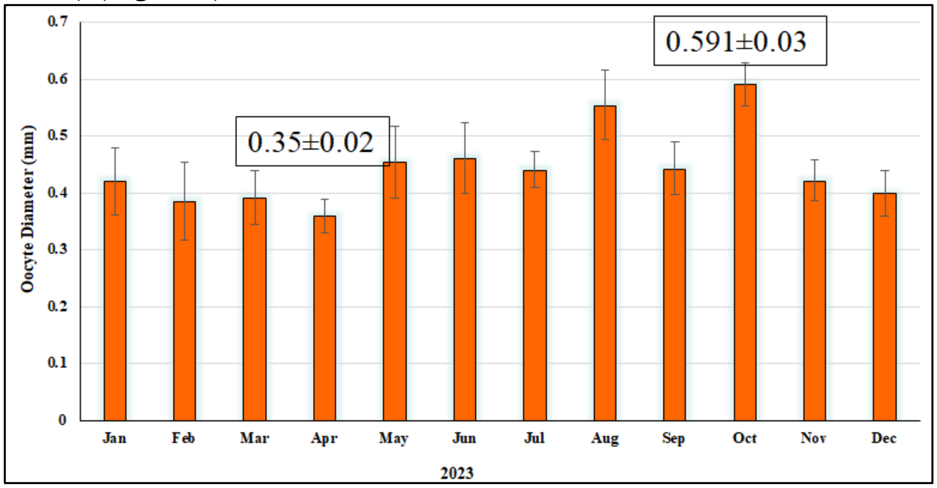


Figure 6. Oocyte diameter of *T.ilisha* collected from Chnadpur in 2023

Annual Rainfall and Hilsa Production

Production and catchability of fish in many aquatic ecosystems varies considerably as a result of seasonal, annual, inter-annual and decadal variability in rainfall. The historical rainfall data (1991-2022) was collected from the meteorological department and mean annual rainfall was calculated and correlated with year wish total hilsa production in Bangladesh (Figure 7). A regression model was constructed using mean annual rainfall and total hilsa production of the country (2010-2022) and the result showed weak linear association between these two variables (Figure 8). More data is required to reach in a conclusion in this regard.

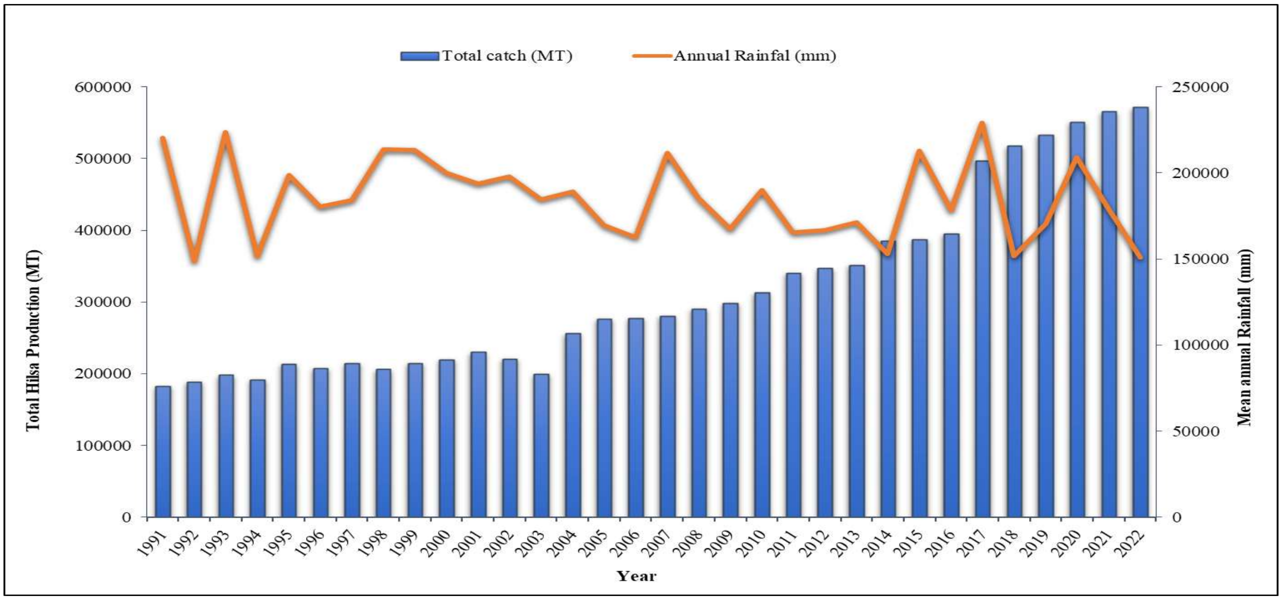


Figure 7. Mean annual rainfall and total hilsa production in Bangladesh (1991-2022)

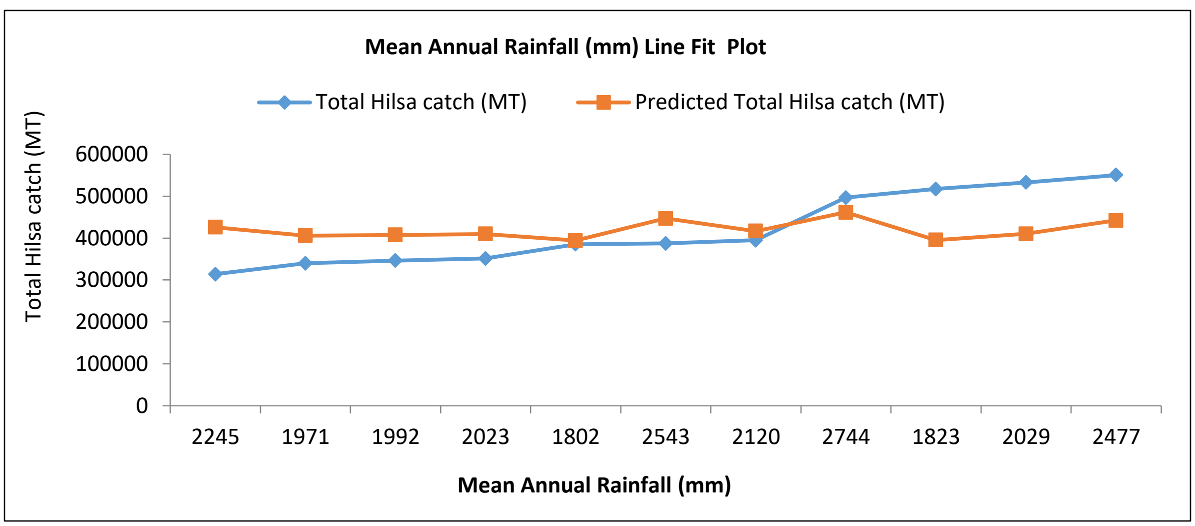


Figure 8. Linear regression model between mean annual rainfall and total hilsa production in Bangladesh (2010-2020).

Average Discharge and Hilsa Production

Discharge measurement has been conducted at Bhairab Bazar site of the Meghna by BWDB Surface Water Hydrological Circle (Figure: 9). Monthly maximum discharge of the Meghna at the Bhairab Bazar observatory came up to 14198.83 m^3/s in July 2021 and 9560.00 m^3/s in July 2022 respectively. While, there are no discharge during November to May in most of the years. This is due to combined results of scarce runoff and tidal effect. Annual Hilsa production of 2021 and 2022 in our sampling site were calculated and correlated with Average Discharge of the Meghna at Bhairab Bazar point of 2021 and 2022 (Figure: 10). The result showed considerable variations between these two variables. More data is required to reach in a conclusion.

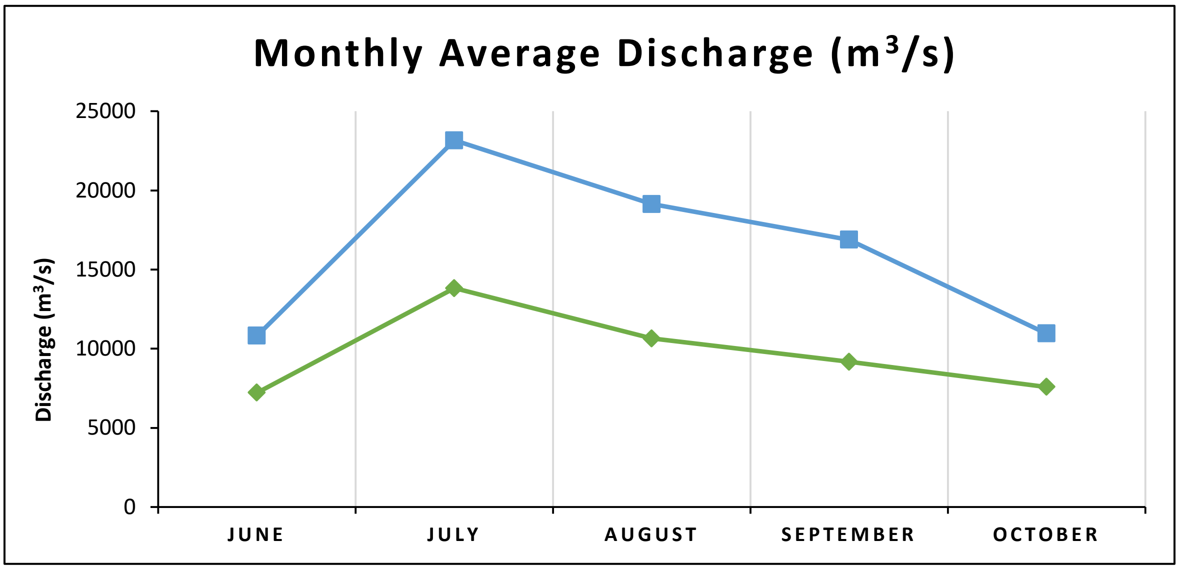
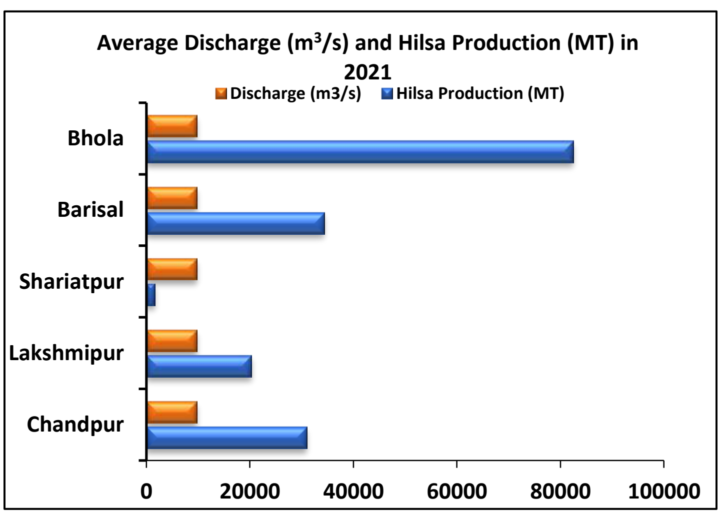


Figure 9. Monthly Average Discharge (m^3/s) of the upper Meghna River.



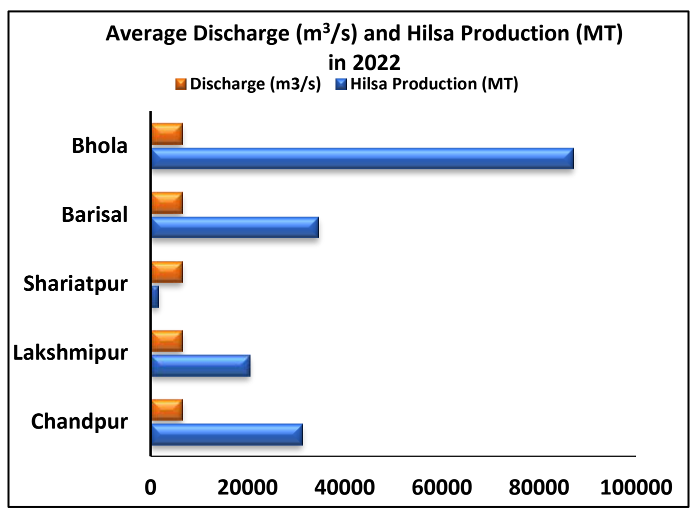


Figure 10. Hilsa production (MT) of different sampling site in relation to Monthly Average Discharge (m^3/s) of the upper Meghna River in 2021 and 2022

Physico-chemical parameters of water in the sampling locations

Analyses of various physico-chemical factors of the water quality from different sampling points are presented in Figure 11. The ranges of all studied water quality parameters were found within the acceptable limits for the growth of fishes.

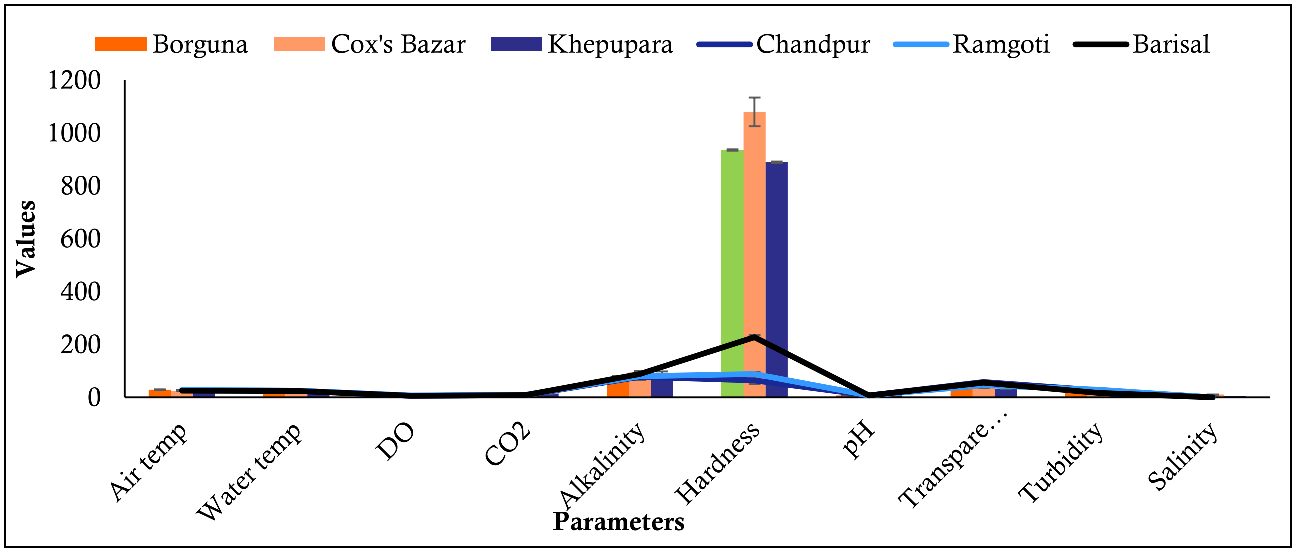


Figure 11. Physico-chemical parameters of the water quality from different sampling locations.

Parameters	Air temperature	Water temperature	DO	CO_2	Alkalinity	Hardness	pH	Transparency
Standard Values	20-30 (EQS,1997)	20-30 (EQS,1997)	4-6 (EQS,1997)	6 ppm (EQS, 1997)	>100 (Rahman, 1992)	200-500 (DOE, 2003)	6.5-8.5(EQS, 1997)	40 or less (Rahman, 1992)

Table 1: Plankton density in per liter water in Upper and lower Meghna River.

Sampling sites	Plankton (No./L)	Phytoplankton (No./L)	Zooplankton (No./L)
Upper	24×10^2	21×10^2	3×10^2
Lower	39×10^2	33×10^2	6×10^2

The data collection on more variables are going on and it will be reflected in the next report.

Estimation of nutrient flux and primary productivity in the major nursery grounds of hilsa

Researcher (s)

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: Dr. Md. Robiul Awal Hossain, PSO

: Md. Mozzammel Hoque, SSO

: Md. Abu Kawser Didar, SO

Objectives

- ➢ To assess the primary productivity of nursery grounds of hilsa
- ➢ To study the factors affecting primary productivity of nursery grounds of hilsa
- ➢ To assess the carrying capacity of nursery grounds of hilsa
- ➢ To assess the impact of hilsa management intervention on native pangus production.

Achievements

In order to address the aforesaid objectives of the project, monthly data from six sampling locations (Shatnol, Chandpur-Alexander, Laxmipur 100 km considered as station 1, Lower Meghna and Tarabunia, Shariotpur 20 km, Lower Padma considered as station 2, Hizla, Mehindigonj, Barishal (82 km) considered as station 3, Bheduria, Bhola, Char Rustom, Potuakhali (100 km, Tetulia River) considered as station 4, Char Ilisha-Char Pial, Bhola (90 km, Shahbazpur Channel considered as station 5, Kalapara Upazilla, Patuakhali (40km) considered as station 6 were collected and analyzed.

The annual production of *Pangasius pangasius*

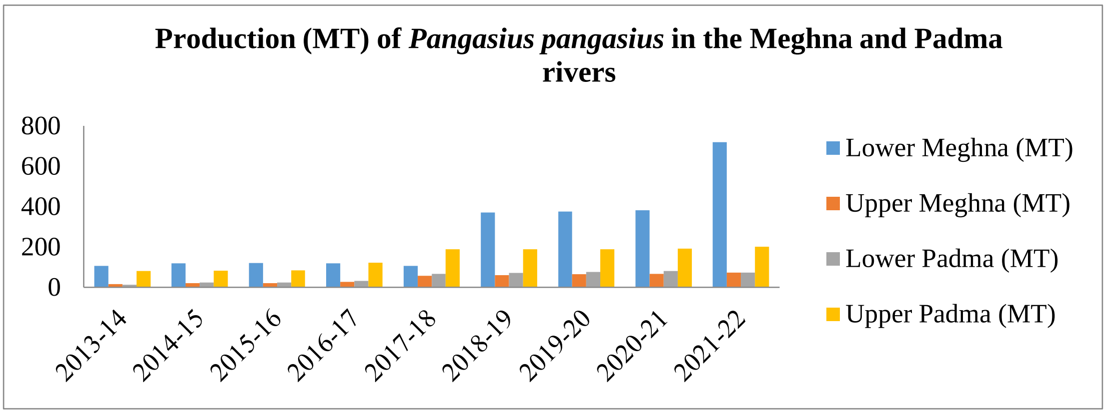


Figure 1: The annual production of *Pangasius pangasius* in the Meghna and Padma rivers

1. **Lower Meghna**: Production started at 107 metric tons in 2013-14 and gradually increased to 720 metric tons by 2021-22. There's a noticeable upward trend in production over the years, with some fluctuations, particularly from 2016-17 to 2019-20, where production remained relatively stable before a significant increase in 2020-21 and 2021-22 (Figure 1).

2. **Upper Meghna:** Production began at 16 metric tons in 2013-14 and experienced moderate growth to reach 74 metric tons by 2021-22. Similar to the Lower Meghna, there's a general upward trend in production, albeit at a slower pace. There are some fluctuations, but overall, production has increased over the years (Figure 1).
3. **Lower Padma:** Production started at 13 metric tons in 2013-14 and increased steadily to 82 metric tons by 2020-21, before decreasing slightly to 74 metric tons in 2021-22. The production trend shows consistent growth until 2020-21, followed by a slight decline in 2021-22 (Figure 1).
4. **Upper Padma:** Production began at 81 metric tons in 2013-14 and reached 202 metric tons by 2021-22. The production trend exhibits steady growth over the years, with some fluctuations, particularly in the earlier years, but overall showing an upward trajectory (Figure 1).

Overall, the production of native *pangasius* in both the Meghna and Padma rivers has generally increased over the years, albeit with some fluctuations and varying growth rates among different segments of the rivers. Data collection is currently underway and will be incorporated into the forthcoming report.

Length frequency distribution of juvenile and (b) adult *P. Pangasius* in Chandpur

The total length of juvenile and adult *P. pangasius* ranged from 8.80 - 20.20 cm and 68.50 - 105.90 cm, respectively, and weight ranged from 7.13 - 62.84 g and 3500 - 12078 g, respectively. The length-frequency distribution (LFD) of *P. pangasius* varied between juvenile and adult stages, where juvenile stage showed unimodal distribution; whereas, adults showed bimodal distribution. The highest frequency was obtained in 13.80 cm size group of juveniles accounted for 50 individuals (Figure 2) and 77.50 cm size group of adults accounted for 8 individuals (Figure 3). The data collections on more variables are going on and it will be reflected in the next report

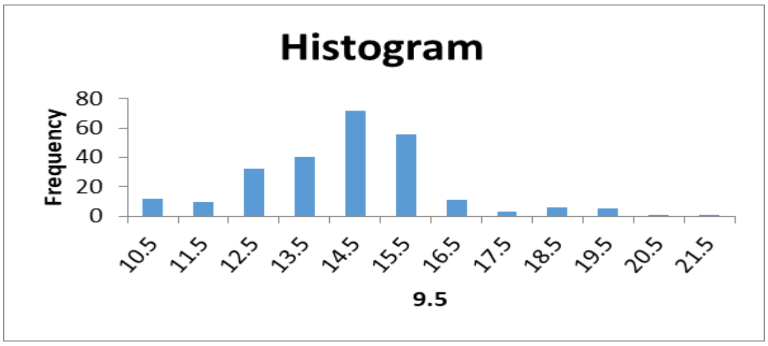


Figure 2: Histograms showing length frequency distribution of juvenile *P.* pangasius in Chandpur

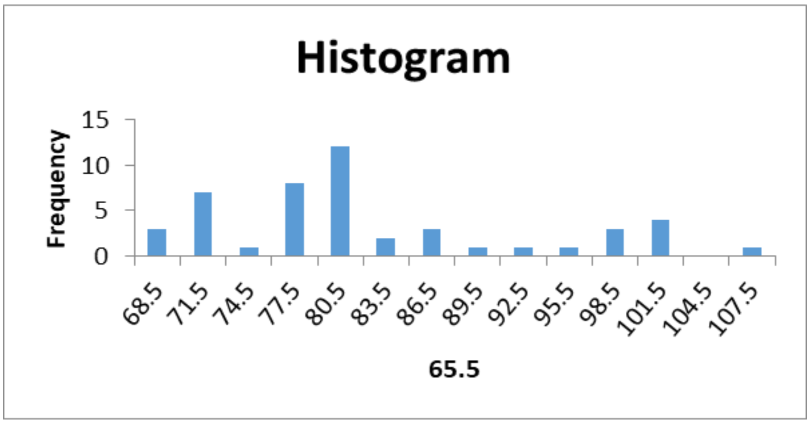


Figure 3: Histograms showing length frequency distribution of adult *P. pangasius* in Chandpur

Factors affecting the primary productivity

Physico-chemical parameters of water in the study areas

Water quality parameters of six stations exhibited considerable fluctuations. The maximum and minimum air temperature were found with mean value 29.4±1.3ºC and 26± 0.7ºCat (St-3) and (St-4) respectively (Table 2) while the maximum and minimum water temperature were found mean value 26.8±0.5ºC and 24.9± 0.8º Cat (St-3) and (St-4), respectively. The maximum and minimum values of water transparency were found 58.38±8.2 cm and 32±8.3 cm at St-1 and St-6, respectively. Average highest dissolved oxygen (mg/L) was found 7.2±0.08, 7.55±0.47, and 6.57±0.42 respectively at (S2, S5 and S6). Average CO_2 (mg/L) were in acceptable limits respectively. The maximum alkalinity was found 91±1.14 mg/l at S6 and hardness 220± 7.63 mg/l at S5 (Figure 4). The average pH was found just slightly above the neutral value in the studied sampling sites. In stations 1, 2 and 3 the ranges of all studied water quality parameters were found within the acceptable limits for the growth of fishes (Figure 4). In stations 4, 5 and 6 studied water quality parameters were slightly different but in acceptable limit (Figure 4).

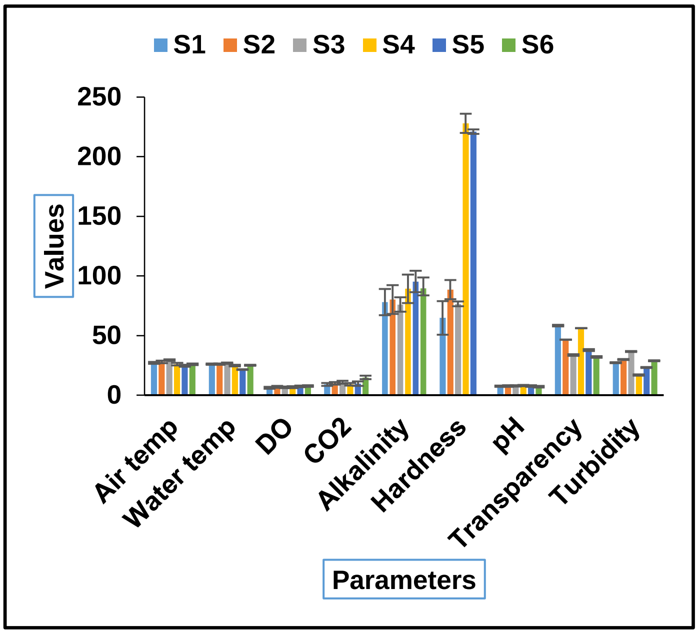
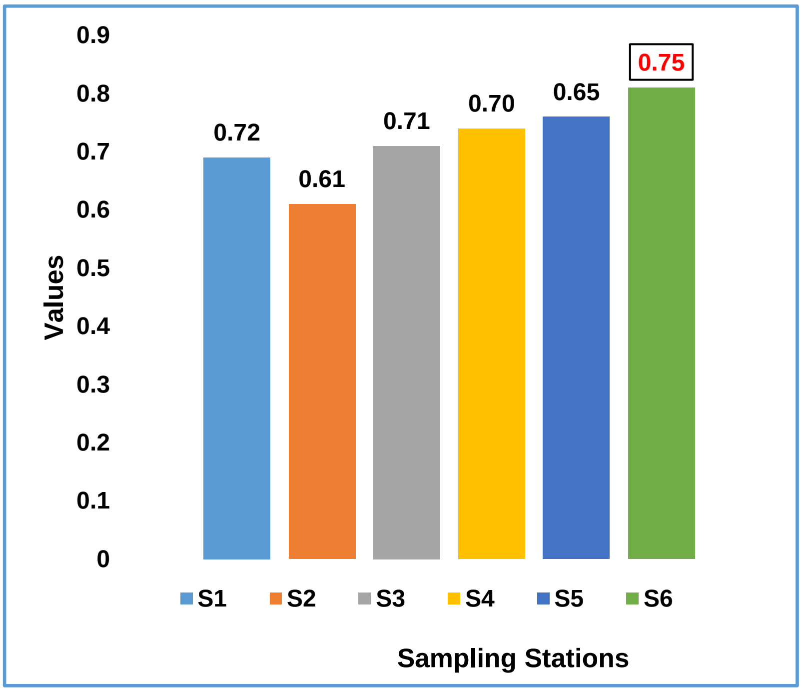


Figure 4: Water quality parameter of sampling stations

Parameters	Standard
Air temp	20-30 (EQS,1997)
Water temp	20-30 (EQS,1997)
DO	4-6 (EQS,1997)
CO_2	6 ppm (EQS,1997)
Alkalinity	>100 (Rahman, 1992)
Hardness	200-500 (DOE, 2003)
pH	6.5-8.5(EQS,1997)
Transparency	40 or less (Rahman, 1992)

Water quality index (WQI) is a dimensionless number that combines multiple water quality parameters into a single number by normalizing values to subjective score (Miller et al., 1986). Conventionally it has been used for evaluating the quality of water for water resources such as rivers, streams and lakes.



WQI Value	Category
<0.5	Excellent water
0.5-0.75	Good water
0.75-1	Moderately polluted water
>1	Highly polluted water

Figure 5. WQI at different stations

Parameter incorporated in WQI varies depending upon the designated water uses of the water body and local preferences. In the present study highest WQI values were found in S6 indicate moderately polluted water.

CA was carried out, using Bray Curtis Similarity, to show the similarity among the parameters. From the output of the cluster analysis, four clusters were found during different stations (Figure 6).

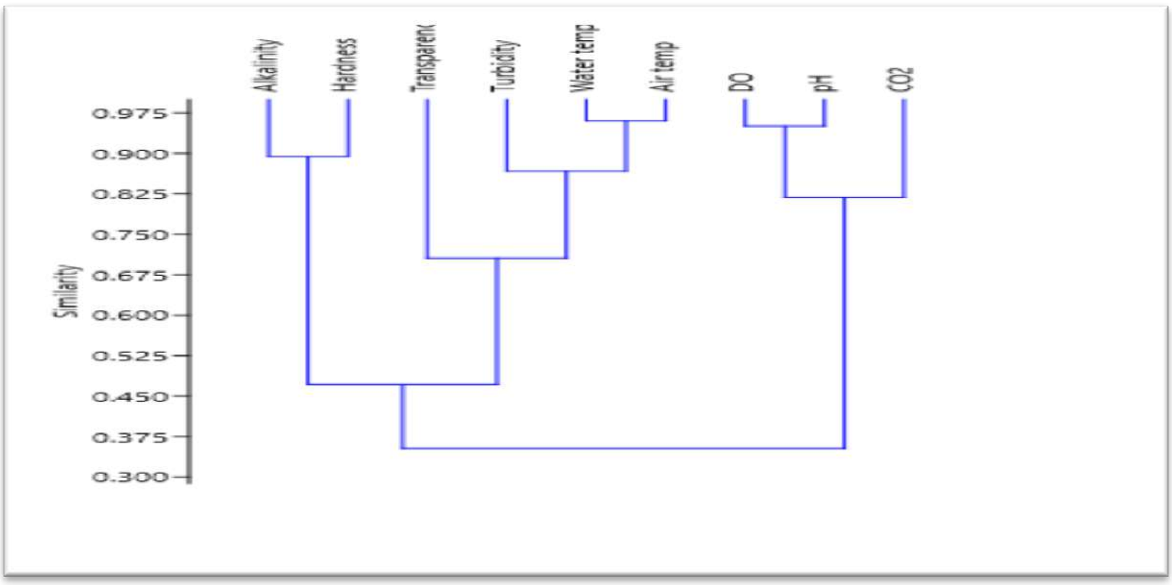


Figure 6. Dendrogram showing the percentage of similarity among water quality parameters.

4

Nitrogen (N) and phosphorus (P) are primary nutrients and vital for life processes such as protein synthesis, cellular growth and reproduction. However, in inordinate quantities, the two elements are also a major source of stream and river impairment. Large inputs of these limiting nutrients can cause deleterious algal growth with a myriad of negative ecosystem responses including eutrophication.

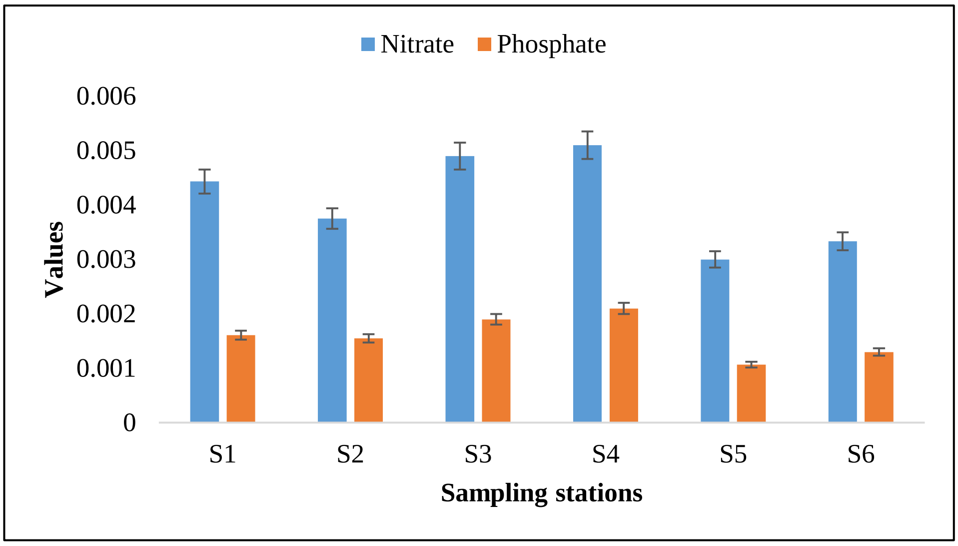


Figure 7: Concentrations of Nitrate (mg/l) and Phosphate (mg/l) in the selected sampling spots

Nitrate is important parameters of the water quality which trigger biological production in water bodies. Nitrate concentrations were found within the range 0.002 to 0.016 µg/L. The highest concentration (0.0051± 0.0037µg/L) was found at St-4 and the lowest (0.0033± 0.001µg/L) was found at St-6 (Figure 7).

Phosphate is a liming factor in almost all water bodies because in water, it remains in a very small amount, in most cases less than 0.1 ppm. Almost all of the phosphorus present in water is in the form of phosphate (PO_4) and in surface water mainly present as bound to living or dead particulate matter and in the soil is found as insoluble $Ca3(P0_4)_2$. Phosphate concentration were found 0.001 to 0.008 µg/L where the highest concentration (0.0021± 0.0025/L) was found in St-4 and the lowest (0.0015± 0.0006µg/L) in St-6 (Figure 7).

Concentration of nitrate 0.02-1.0 ppm is lethal to many fish species, > 1.0 ppm is lethal for many warm water fishes and < 0.02 ppm is acceptable (OATA, 2008). According to Stone and Thomforde (2004), the phosphate level of 0.06 mg/L -1 is desirable for fish culture. Bhatnagar *et al.* (2004), suggested 0.05-0.07 ppm is optimum and productive; 1.0 ppm is good for plankton and shrimp production. Thus, the nitrate and phosphate concentration in the present study was within the acceptable limit. The higher amount of contamination from fertilizers, municipal wastewaters, feedlots, septic systems in water increase the concentration of Nitrate, it refers that the higher (NO_2 and NO_3) the deviation the lower the quality of water for fish and other aquatic life and for common uses. The amount of nitrate could also be influenced by the growth of plankton.

The concentration of Chlorophyll a can act as an indicator of phytoplankton abundance in an aquatic ecosystem. One of the major objectives in analyzing photosynthetic pigments (Chlorophyll-a) in limnology is the estimation of phytoplankton biomass and its photosynthetic capacity. It is natural for levels of chlorophyll a to fluctuate over different seasons.

The concentration of Chlorophyll a can act as an indicator of phytoplankton abundance in an aquatic ecosystem. One of the major objectives in analyzing photosynthetic pigments (Chlorophyll a) in limnology is the estimation of phytoplankton biomass and its photosynthetic capacity. It is also reported in other research that chlorophyll a concentration remains high during low-water discharges (Devercelli and Peruchet, 2008). Chlorophyll-a concentrations ranged from 6.2 to 18 µg/L where the highest concentration (12.6±1.2 µg/L) was found in St-6 and the lowest (7.1±3.1 µg/L) in St-1 (Figure 8). Chlorophyll-a value is an indicator of productivity in the water body, which shows an inverse relationship with water transparency.

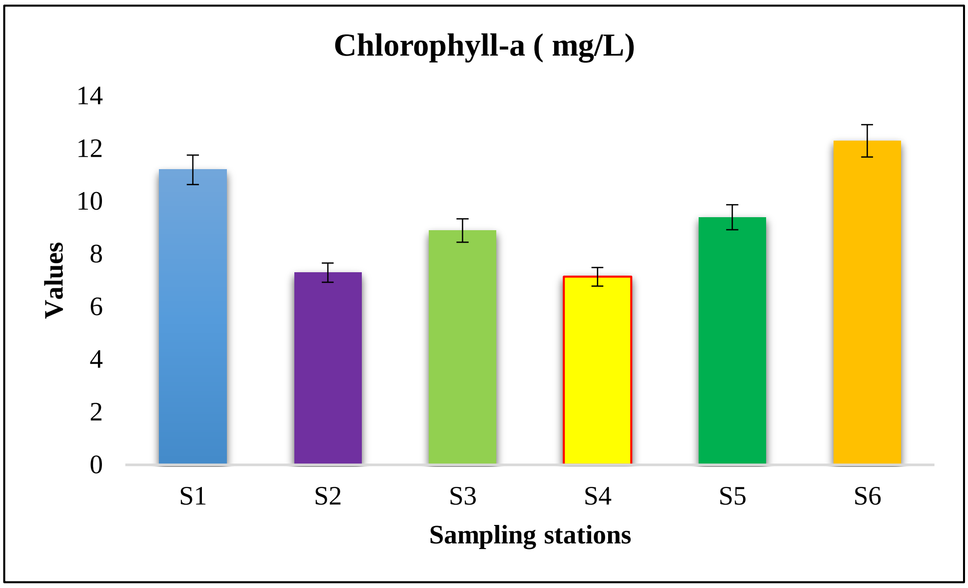


Figure 8: Concentrations of Chlorophyll-a (mg/L) in the selected sampling spots

Plankton abundance in the study areas

In station 1,20 taxa were identified in which 17 were phytoplankton and 3 were zooplankton. Phytoplankton belonged to the dominant groups *Zygnematophyceae*in all the sites in station 1 But in case of zooplankton the dominant groups was Nymphalidae (Figure 9)

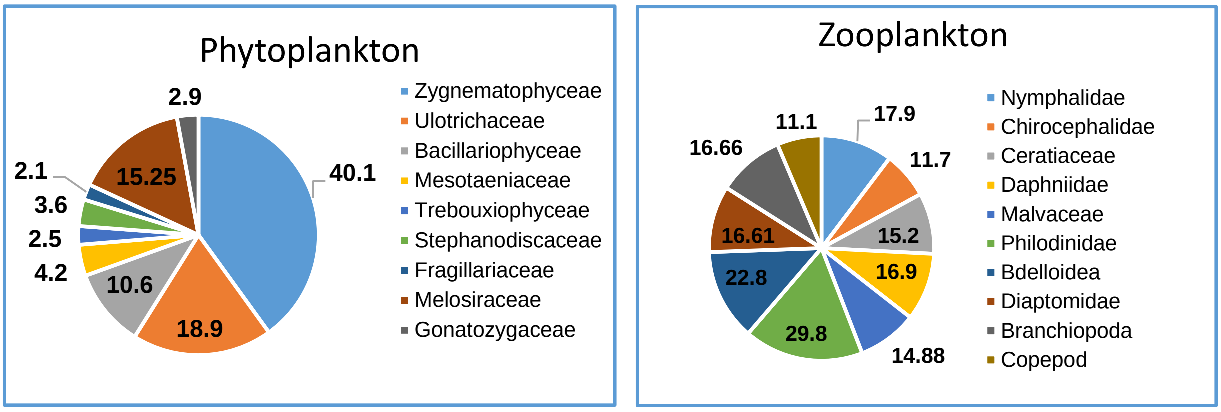


Figure 9: Phytoplankton and Zooplankton (%) of sampling station 1

In station 2, 14 taxa were identified among which 9 were phytoplankton and 5 were zooplankton. Phytoplankton belonged to the dominant groups *Zygnematophyceae* but in case of zooplankton the dominant groups was *Hexanauplia* (Figure 10)

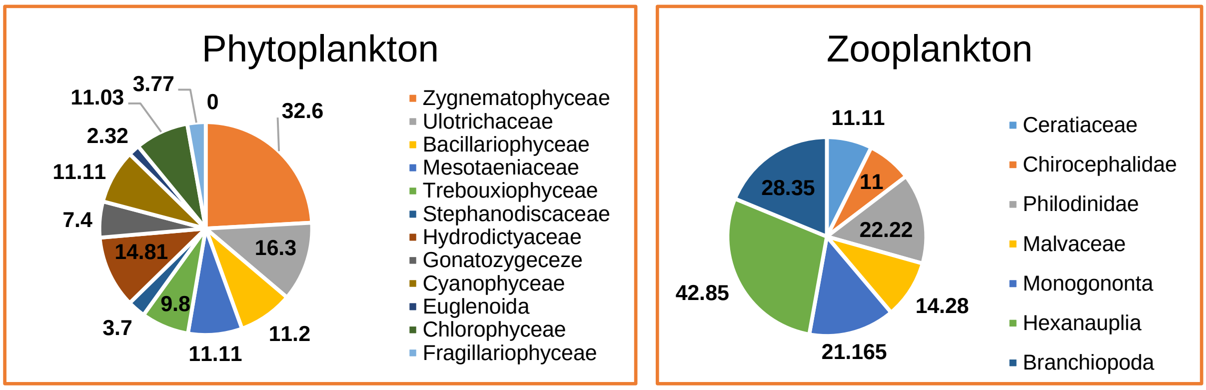


Figure 10: Phytoplankton and Zooplankton (%) of sampling station 2.

In station 3, 12 taxa were identified among which 7 were phytoplankton and 5 were zooplankton. Phytoplankton belonged to the dominant groups *Cholorophyceae* but in case of zooplankton the dominant groups was *Branchiopoda* (Figure 11)

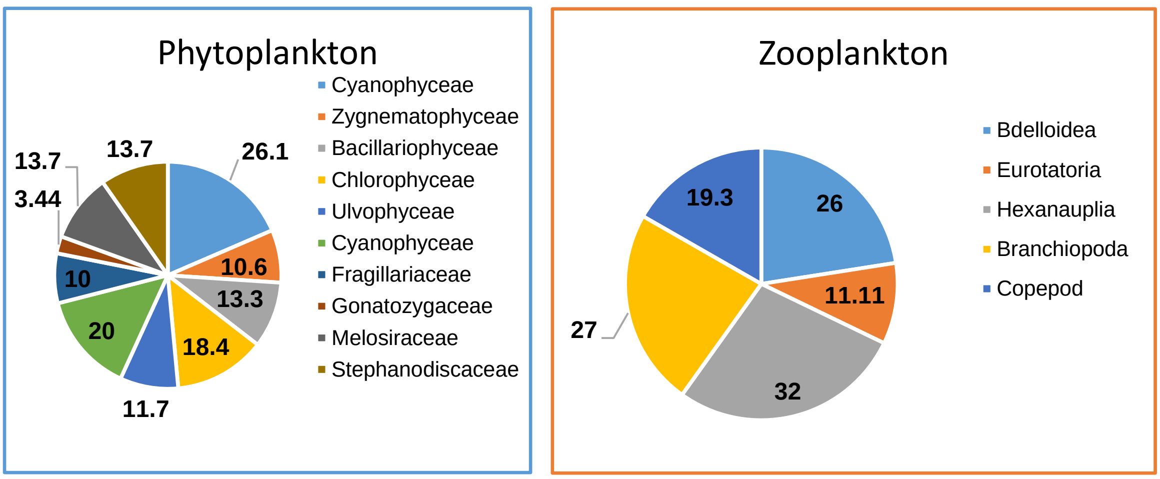


Figure 11: Phytoplankton and Zooplankton (%) of sampling station 3.

In station 4, 9 taxa were identified among which 6 were phytoplankton and 3 were zooplankton. Phytoplankton belonged to the dominant groups *Chlorophyceae* but in case of zooplankton the dominant groups was *Branchiopoda* (Figure 12)

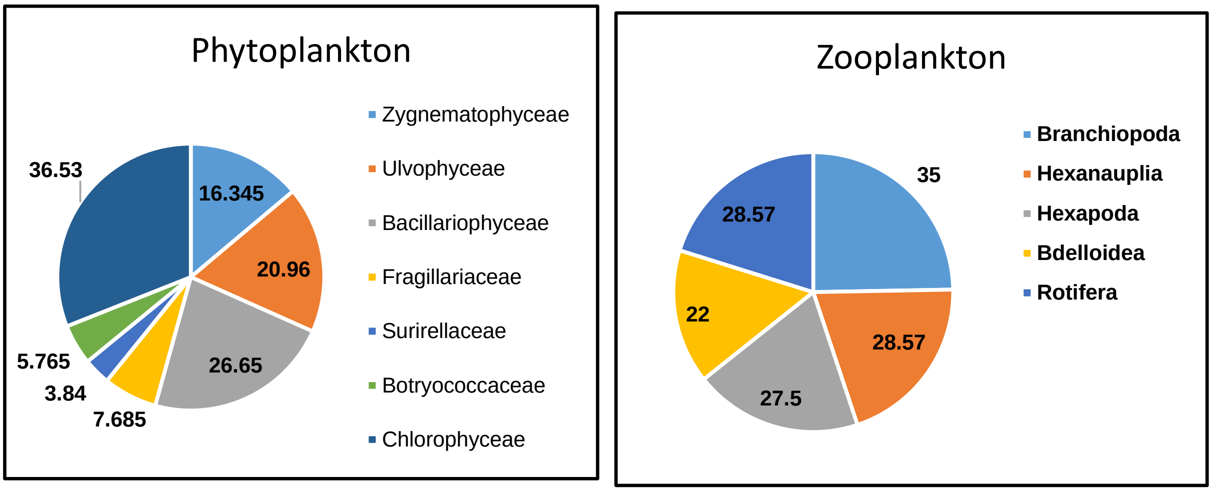


Figure 12: Phytoplankton and Zooplankton (%) of sampling station 4

In station5, 15 taxa were identified among which 9 were phytoplankton and 6 were zooplankton. Phytoplankton belonged to the dominant groups *Zygnematophyceae*, *Bacillariophyceae* and *Chlorophyceae*but in case of zooplankton the dominant groups were *Branchiopoda* and *Monogota* (Figure 13)

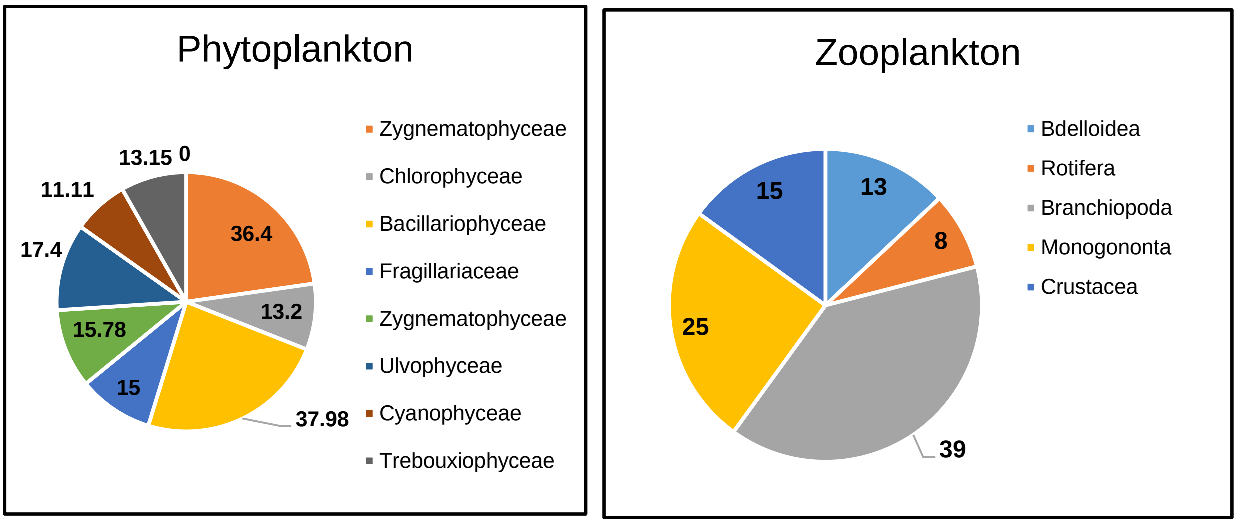
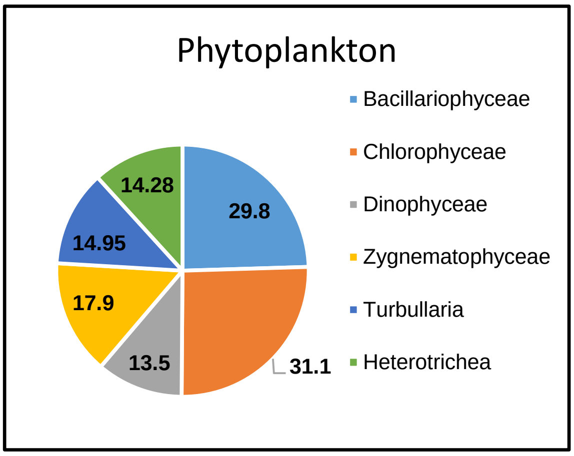


Figure 13: Phytoplankton andZooplankton(%) of sampling Station 5

In station 6, 17 taxa were identified among which 10 were phytoplankton and 7 were zooplankton. Phytoplankton belonged to the dominant groups *Zygnematophyceae*, *Bacillariophyceae* and *Chlorophyceae*but in case of zooplankton the dominant groups were *Monogononta* and *Branchiopoda* (Figure 14).



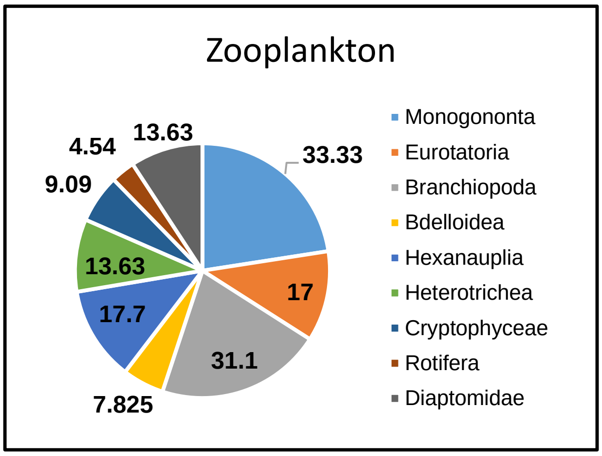


Figure 14: Phytoplankton and Zooplankton (%) of sampling Station 6

Table 1: Abundance of plankton at sampling stations

Sanctuary	**Total Plankton**	**Phytoplankton**	**Zooplankton**
Sanctuary 1	37×10^2	34×10^2	3×10^2
Sanctuary 2	35×10^2	33×10^2	2×10^2
Sanctuary 3	38×10^2	32×10^2	6×10^2
Sanctuary 4	37×10^2	33×10^2	4×10^2
Sanctuary 5	40×10^2	37×10^2	3×10^2
Sanctuary 6	32×10^2	23×10^2	9×10^2

Table 2: Class and Genus of Phytoplankton at sampling stations

Class	Genus
Chlorophyceae	*Pediastrum, Volvox, Scenedesmus, Acanthocystis*
Ulvophyceae	*Ulothrix*
Zygnematophyceae	*Spirogyra, Nitzschia, Netrium, Staurastrum, Gonatozygon*
Bacillariophyceae	*Navicula, Gomphonema, Asterionella, Diatoma, Frustulia, Stephanodiscus, Cyclotella*
Fragillariophyceae	*Tabellaria, Synedra*
Cyanophyceae	*Spirulina, Rivularia,Oscillatoria*
Trebouxiophyceae	*Protococcus, Botryococcus*
Dinophyceae	*Ceratium*
Euglenoida	*Euglena*

Table 3: Class and Genus of Zooplankton at sampling stations

Class	Genus
Branchiopoda	*Daphnia, Ceriodaphnia, Sida, Bosmina, Diaphanosoma, Leptodora, Eubranchipus*
Hexanauplia	*Cyclops*
Heterotrichea	*Spirostomum*
Diaptomidae	*Diaptomus*
Monogononta	*Filinia, Brachionus*
Bdelloida	*Nauplius, Rotaria*

Shannon-Wiener diversity index can be used as the pollution index in diatom communities. It is a commonly used diversity index that takes into account both abundance and evenness of species present in the community. Hendley (1977) put forward the following scale: of 0–1 for high pollution, of 1–3 for moderate pollution, and 3–4 for incipient pollution. In the present study, the highest Shannon-Wiener diversity index was found to be 3.143 at station 5 and a relatively low value (2.125) was observed at station 3 (Table 4 and fig.14). This means that station 5 has more abundance of plankton than the other stations. Balloch et al. found the Shannon Diversity Index to be a suitable indicator of water quality. Dash (1996) reported that the higher the Shannon-Wiener index (H′) in Odisha lake, the greater the planktonic diversity. Simpson diversity index varied from 0.872 (station 2) to 1.012 (station 5) during the present study (Table 4 and fig. 15). This indicates that the values are approaching 1, signifying that sites have high relative diversity due to their supporting surrounding components.

Table 4. Plankton diversity index of six sampling stations

Station	S1	S2	S3	S4	S5	S6
Shannon (H)	2.94	2.81	2.84	2.92	3.14	2.12
Simpson (1/D)	0.89	0.87	0.92	0.95	1.01	0.93
Margalef	2.42	2.39	2.31	2.27	2.51	1.78
Evenness	0.44	0.42	0.45	0.46	0.76	0.40

According to Ali et al. (2003), the values of Margalef's index ranging between 1 and 3 indicate moderately polluted water with values less than 1 indicating the heavily polluted environment, while values greater than 3 windows clean water. The Margalef diversity index values varied from 1.786 to 2.512, during the present study (Table 5 and fig. 14) which indicates that the system is threatened by pollution, which may be as a result of anthropogenic activities going on within the area. Pielou's evenness index refers to how close in number each species in an environment is. In the present study, the Pielou's evenness index was found to range from 0.401 to 0.765 (Table 4 and fig. 14); if the evenness index is high (approaching 1), there is no species dominance and vice versa. Pirzan et al. (2008) opined that if the evenness index approaches zero, the species evenness in the community was low, and inversely if the evenness index approaches 1 the species in the community is the same.

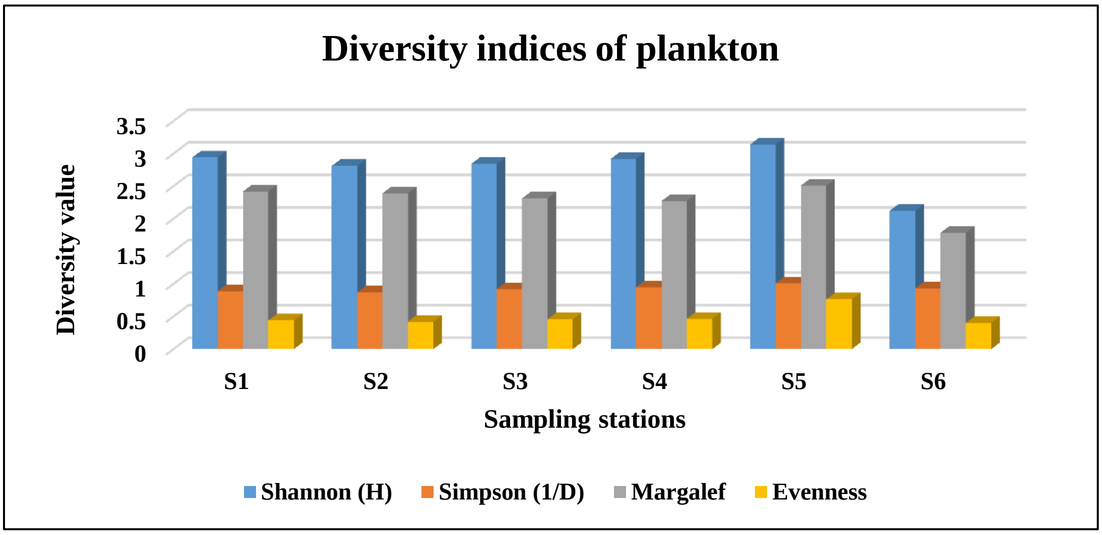


Fig 15. Diversity indices of plankton in the selected sampling stations

In the study period, this result also establishes coherence between the higher abundance of jatka and plankton density at S1 and S5 (Figure 16) compared to other sampling stations.

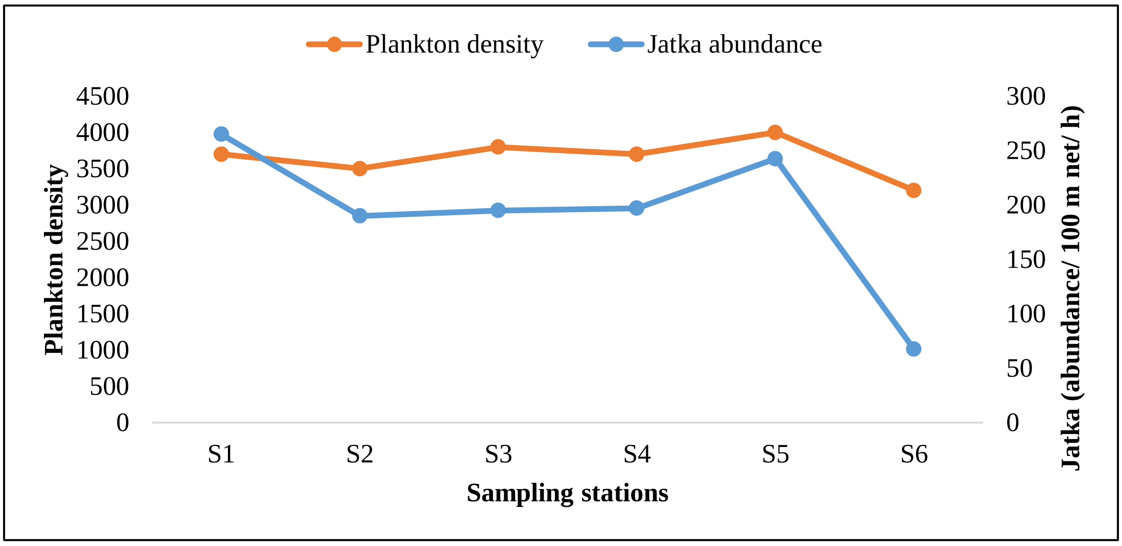


Figure 15: Relation between jatka abundance and plankton density at sampling stations

Table 5. Association between Primary productivity and Nutrient flux

	GPP vs. CPUE	GPP vs. Chlorophyll-a	GPP vs. Nitrate	GPP vs. Phosphate
95% confidence interval	0.059 to 0.981	0.451 to 0.991	0.178 to 0.985	0.101 to 0.982
R squared	0.690	0.854	0.747	0.7118
P (two-tailed)	0.040	0.008	0.026	0.034
P value summary	*	*	*	*
Significant? (alpha = 0.05)	Yes	Yes	Yes	Yes

In all four cases, the p-values are below the significance level (alpha = 0.05), indicating that there is a statistically significant relationship between GPP and the respective nutrient flux parameters (CPUE, Chlorophyll-a, Nitrate, and Phosphate). The R-squared values suggest that a substantial proportion of the variability in GPP can be explained by each of these nutrient flux parameters.

Niche Characterization of Meghna River Basin: Eco-morphological and Hydrodynamic Modelling

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Objectives

The main purpose of the study was to illustrate the present status of niche alteration using GIS for the better and sustainable management planning, strategy and decision-making.

- To formulate GIS-based models to picturize causal influence of stochastic and deterministic factors;
- To develop models of geo-morphological and hydrodynamic aspects using GIS;
- To generate spatio-temporal models of niche viability in context of meteorological alterations, interaction between different attributes and niche degradation;
- To produce GIS-based models for characterization of entire niche to assess suitability.

Methodology

The study was designed for characterizing and spatio-temporal modelling of causal influences on niche resources using deterministic stressor modelling. In order to obtain a raw description for characterization of the niche of the Meghna River basin, a grab sampling of water and soil quality parameter was performed at 40 sites (Figure 1). As many as 18 water and soil quality parameters were measured. All sampling were performed from approx. 1km away of the river bank with 3 replications. Approx. 1 km distance was maintained between each replicate. All Samples were collected from 45 cm below the surface of water. GPS (Geographic Positioning System) was recorded for all of 3 replicates. For the correction and validation and to support of existing historical map and determine the geographic position of sampling locations, GPS (eTrex Legend H, GARMIN) based field survey was carried out through the study area. Different field surveys were performed using standard methods. Secondary Data was collected from Satellite image, historical and reference map, Previous and present studies of Riverine Station, Chandpur, Different governmental organization and NGOs, different thematic information. Satellite image and Historical map was georeferenced to scaling, rotating, translating and de-skewing images to match a particular size and position and using map coordinates to assign a spatial location to map features. Sufficient number of effortlessly recognizable ground control points in the images were used to adjust the image to world's geographic co-ordinate system. Ground control points for geo-referencing the satellite images was taken from pre-added place marks. Every image and satellite data were resampled and transformed into a file referenced to the world datum, World Geodetic System (WGS84) of 1984. Procurement of a vector database encompassing all the data pertinent to the study was entailed the principal portion of the introductory work. Vector databases were established from historical maps and satellite images. By on-screen digitizing of the exhibited raster maps, the vector information was generated. The mean values of niche characters and stressors of individual sampling stations and individual season were used for GIS analysis. To generate feature wise distribution of all individual characteristics, each average value of those individual characteristics for a particular sampling station and individual season were monitored.

Achievements (2023-2024)

Study. 1. Characterization and Spatio-temporal Modelling of Causal Influences on Niche Resources using GIS Approach

The study was designed for characterizing and spatio-temporal modelling of causal influences on niche resources using deterministic stressor modelling. For this study different primary and secondary data (hydro-physico-chemical attributes, river bed attributes, data about bio-resources etc.) were collected and analyzed.

a) Virtual sampling site selection

The virtual sampling site was selected through analyzing Google Earth, 2021 observatory. Forty sampling stations with three sub-stations were selected along the Meghna River basin starting from near Brahmanbaria to Shahbazpur channel following the main river course downwards to the Meghna estuary including Hatiya and Monpura Island. The distance between sampling sites was approx. 10 km to cover the whole Meghna River basin from upper to lower Meghna. Satellite image of sampling sites is presented in Figure 1.

b) **Spatial distribution fluctuation and present senario of hydro-physico-chemical attributes with bio resources**

Spatial fluctuation of hydro-physico-chemical attributes *viz*. water temperature, Dissolved Oxygen, carbon-di-oxide, alkalinity, hardness, transparency, salinity, TDS, Conductivity, turbidity of old sampling sites were monitored. To till date all the hydro-physico-chemical parameters were found congenial in range (**Table 1**).

Table 1. Summary of observed hydro-physico-chemical attributes of old sampling sites

Parameters	**AT (°c)**	**WT (°c)**	**pH**	**DO (mg/l)**	**CO_2 (mg/l)**	**Alk (mg/l)**	**Har (mg/l)**	**Tra (cm)**	**Tur (NTU)**	**Con (μs/cm)**	**TDS (ppm)**
AVG	30.02	29.1	7.77	6.5	10.20	80.20	350.25	74.00	120.90	4.01	550.65
SD	3.30	3.35	0.19	1.37	4.12	45.00	-	20.68	-	2.17	-
CV	0.10	0.11	0.02	0.23	2.29	0.38	1.37	-	1.78	1.02	0.71
PE	0.44	0.47	0.02	0.18	0.76	4.42	69.88	96.48	38.06	0.57	62.83
MaxE	8.80	9.27	0.48	3.57	15.18	88.00	1389.94	0.11	756.97	11.31	1249.72
Precision	0.01	0.02	0.00	0.04	3.40	0.06	0.21	0.71	0.27	0.15	0.11

Spatial fluctuation of hydro-physico-chemical attributes *viz*. water temperature, Dissolved Oxygen, carbon-di-oxide, alkalinity, hardness, transparency, salinity, TDS, Conductivity, turbidity of new sampling sites was monitored and to till date all the hydro-physico-chemical parameters were found congenial in range (**Table 2**).

C) **Spatial distribution, changeability and present scenario of geo-physico-chemical attributes**

Spatial distribution, changeability and present scenario of geo-physico-chemical attributes *viz*. sediment type, pH, permeability, sloping, texture, acidity/alkalinity of all sampling sites were monitored. To till date all the geo-physico-chemical parameters were found congenial in range (**Table 3.1**).

Table 2. Summary of observed hydro-physico-chemical attributes of new sampling sites

Parameters	**AT °c**	**WT °c**	**pH**	**DO mg/l**	**CO_2 mg/l**	**Alk mg/l**	**Har mg/l**	**Tra cm**	**Tur NTU**	**Con μs/cm**	**TDS ppm**
AVG	32.63	30.37	7.62	6.43	16.00	142.75	139.75	34.95	131.02	5.30	600.43
SD	1.40	1.04	0.03	0.22	0.61	20.23	1.85	1.02	-	1.5	-
CV	0.11	0.13	0.04	0.13	2.41	0.31	1.25	-	1.87	1.00	0.68
PE	0.48	0.42	0.01	0.15	0.68	3.98	70.82	89.85	32.16	0.49	56.09
MaxE	6.20	8.09	0.37	2.99	13.10	79.10	990.89	0.13	567.06	9.22	1009.34
Precision	0.02	0.02	0.01	0.03	2.00	0.04	0.25	0.59	0.19	0.20	0.14

Table 3.1. Summary of observed River Bed Attributes

Parameters	type	pH	permeability	sloping	texture	acidity/alkalinity
Value/Attribute	NC to CA	6.9	Moderate to slow	<3%	Silt to clay	MA to N

Study 2: GIS-based Modelling of Effect of Stochastic Factors on Geo-Morphological and Hydrodynamic features in context of Meteorological Alteration

a) Spatio-temporal distribution and Changeability of Hydro-dynamic aspects of Meghna River basin

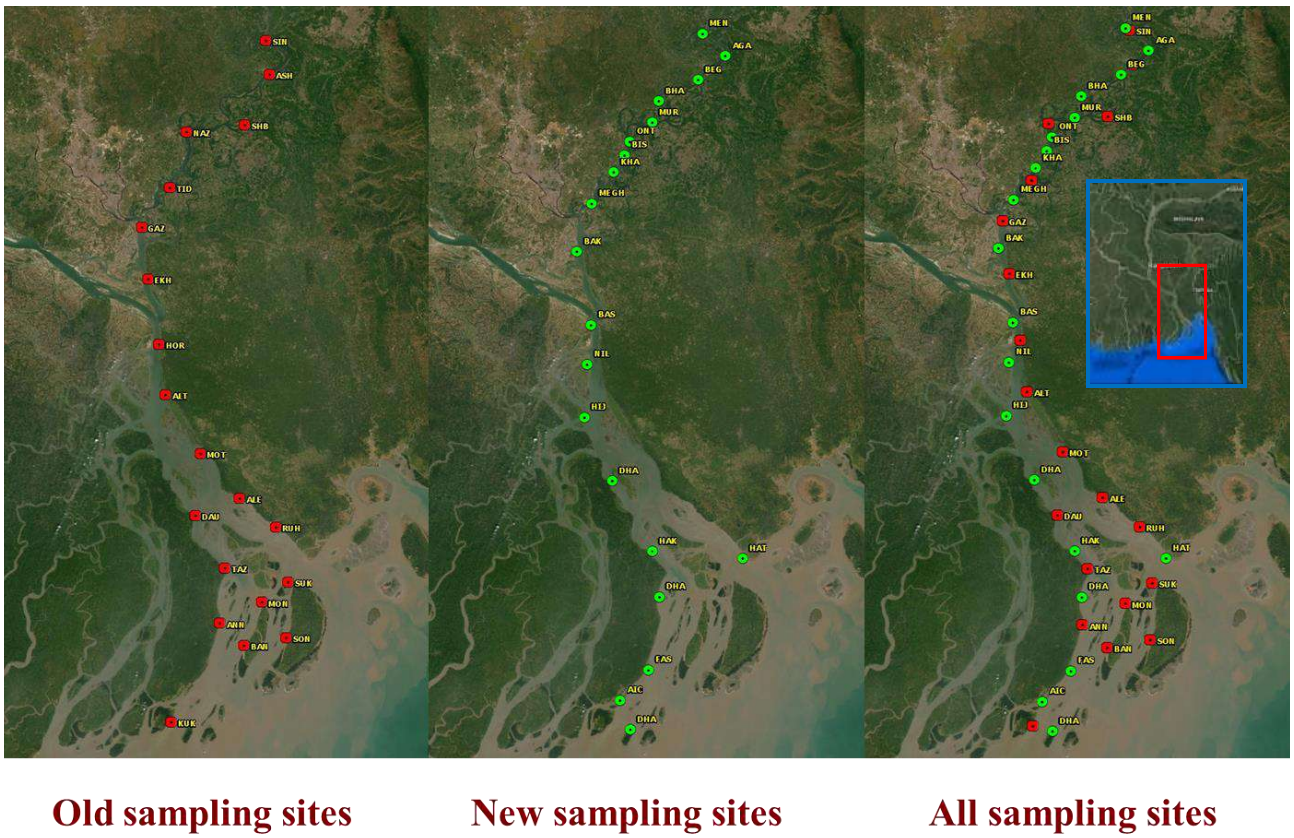

Figure: 1. Location of Sampling Stations

Spatio-temporal distribution and Changeability of Hydro-dynamic attributes of Meghna River basin viz. water Discharge, water level, depth, width, cross section, sediment load and maximum soil concentration of sampling sites were monitored and analyzed (**Table 3.2**).

Table 3.2. Summary of observed Hydro-dynamic aspect and Geo-Morphological aspect

Attribute	WL	WD	MSC	SL	RD	RW	CSA
Value	3.0	13,000.0	200.0	2000.0	20	30	0.8

b) Spatio-temporal distribution and Changeability of Geo-Morphological and Hydro-physical attributes of Meghna River basin

Spatio-temporal distribution and changeability of geo-morphological and hydro-physical attributes of Meghna River basin viz. depth, width and CSA were monitored and analyzed (**Table 3.2**).

c) **Spatio-temporal study of Meteorological Alteration along Meghna River basin**

In the years of 2017-2023, spatio-temporal fluctuation of meteorological attributes *viz*. max, min and average temperature, wind direction, wind speed, rainfall, water level of sampling sites were monitored.

Study. 3. GIS Based Modelling of Response and Adaptive Ability of Niche Bio-resources Against Stochastic and Deterministic Factors

a) **Spatial distribution and variation of bio resources (Planktonic community)**

A total of 70 genera of phytoplankton under 50 families within 14 groups and 32 genera of zooplankton under 24 families within 7 groups were abundant in the Meghna River basin. Among 14 groups of phytoplankton, Chlorophyceae was the most diverse group having 20 different genera of phytoplankton. For zooplankton, among 7 groups, Rotifera was the most diverse group having 16 different genera. During the study period it was recorded that 10 genera of Rotifera was the highest group available in all of the sampling stations. Relative abundance of zooplankton was about 4 times lower than phytoplankton. Among all phytoplankton groups, *chlorophycea* was the most abundant and *dinophycae* was the lowest abundant group. Among all seven zooplankton groups (35 species), *cladocera* was the most abundant and *oligohymenophycae* was the lowest abundant group (Table 4 &5).

Table 4. List of available phytoplankton

Group	Genus	No.
Cyanophyceae	*Spirulina, Microcystis, Polycystis, Anabaena, Nostoc, Oscillatoria, Phormidium, Rivularia*	8
Chlorophyceae	*Pediastrum, Closterium, Ankistrodesmus, Eudorina, Crucigena, Chlamydomonas, Coelosphaerium, Acanthocystis, Gonatozygon, Microspora, Genecularia, Pleodarina, Spirogyra, Volvox, Mougeotia, Oedogonium, Tetraspora, Penium, Coelastrum, Docidium, Tetrapedia, Scenedesmus*	22
Bacillariophyceae	*Naviculla, Melosira, Amphora, Tabellaria, Frustulia, Cyclotella, Ditoma, Nitzchia, Polycistis, Stphanodesmus, Gomphonema, Anomoeoneis, Asterionella, Campylodiscus, Gyrosigma, Stephanodiscus*	16

Group	Genus	No.
Zygnematophyceae	*Coscinodesmus Euastrum, Staurastrum, Netrium, Spirotenia, Cosmarium, Zygnema,*	7
Ulvophycea	*Ulothix*	1
Fragilariophyceae	*Fragilaria, Synedra*	2
Trebouxiophycae	*Botryococcus, Protococcus*	2
Dinophyceae	*Ceratium*	1

Table 5. List of available zooplankton

Group	Genus	No.
Protozoans	*Phacus, Difflugia, Euglepha, Spirostomum*	4
Rotifers	*Brachionus Sp., Polyarthra, Asplancha, Keratella, Filinia, Trichocera, Rotaria, Lindia, Mytilina, Eubranchiopus, Trichocera, Trichotria*	13
Copepods	*Nauplius, Diaptomus, Cyclops, Mesocyclops, Limnocalanus, Colpoda*	6
Cladocerans	*Daphnia, Diaphnosoma, Sida, Leptodora, Eubranchipus*	5
Diaphanosoma	*Chydorus, Bosmina, Moina, Sida, Ceriodaphnia*	5
Ostracods	*Cypridopsis*	1
Oligohymenophorea	*Paramecium*	1

b) Spatial distribution and variation of bio resources (Macrobenthic community)

A total of 123 genera of benthic macro-invertebrates belonging 59 families within 13 groups were recorded. Among 13 groups, Polychaeta was the highest abundant group following Gastropoda, Bivalvia, Malacostraca, Clitellata, Insecta, Chironomidae, Sphaeridae, Annelida, Lepidopteridae, Margaritiferidae, Pleuroceridae, and Branchiopoda. Among the species only *Perinereis sp.* and *Chaetogaster sp.* were observed in every stations.

c) Spatial distribution, variation and abundance of bio resources (Ichthyofauna)

A total of 85 fish spices under 35 families belonging to 13 groups were observed. The study revealed that among all groups, only Beloniformes and Mugiliformes were present in every stations. Among 85 species 33 were observed in every stations.

Study 4: GIS-based Modelling of the Impact of Structural and Functional Traits Interaction on Niche suitability and Viability

a) Spatio-temporal distribution and variation of abundance and CPUE

Spatio-temporal variation of CPUE in Winter was lowest and highest 3.0 and 4.0 kg/100 m net/hr respectively, CPUE in Fall was 7 and 15 kg/100 m net/hr respectively, CPUE In Summer was 2.0 and 3.5 kg/100 m net/hr respectively. From the distribution plot, CPUE was symmetrically distributed in the winter and asymmetrically distributed in the fall.

b) Spatio-temporal divergence of gear selectivity

A total of 10 types of fishing nets belonging five major groups were found to be used by the fishers´ for harvesting of fish. The main compositions of the nets were polyamide monofilament, polyamide multifilament, and polypropylene or nylon rope and polyamide tier cord.

c) Impact of Structural and Functional Traits Interaction on Niche suitability and Viability

For GIS-based Modelling of Effect of Stochastic Factors on Geo-Morphological, Hydrodynamic and Meteorological Alteration, effect of precipitation, change in flooding pattern, drought, erosion and accretion data was collected and its effect on niche was analyzed through in situ spatial clustering and in situ PCA. Among the three clusters, S2-S4, S8-S16 and S20 formed the biggest cluster with maximum similarity. Among 16 diver factors Plankton and CPUE were found as a major driver factor; TDS and conductivity were found as a second driver factor. CPUE was positively associated with CO_2, alkalinity and hardness and negatively associated with transparency and available plankton composition.

d) Interaction between Functional Traits and fish fauna of Meghna River basin

There was no association between air temperature and fish production of the Meghna River basin. There was also no association between rainfall and fish production. Strong negative association (-0.8) was found between water level and fish production of Meghna River.

Adoption of Culture Techniques and Bioactive Compound Analysis of Commercially Important Seaweeds in the Mid-Southern Coast of Bangladesh

Researchers

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Objectives

- To demonstrate the culture techniques of commercially important seaweeds on the Kuakata coastline.
- To analyze the proximate composition and mineral content of commercially important seaweeds in Bangladesh.
- To determine and isolate bioactive compounds of commercially important seaweeds of Bangladesh.

Achievements (2023-24)

Experiment-1. Potentiality of seaweed, *Gracilaria tenuistipitata* culture in the Kuakata coastline

Methodology

Site selection

The site was selected at the Gangamati and Nizampur estuary on the Kuakata coastline of the Bay of Bengal, Bangladesh.

Seaweed culture method

Young, growing fragments of *Gracilaria tenuistipitata* were collected from Cox's Bazar, and culture was started at the Gangamati and Nizampur estuary in December 2023. At each site three horizontal nets of 22 cm of mesh size were used for floating raft culture and the area of each net was 16 m^2. The initial seaweed weight was 1±0.05 Kg fresh weight/m^2.

Water quality parameters monitoring

Water temperature, salinity, transparency, pH, and dissolved oxygen (DO) were recorded every 15-day interval at the cultivation sites using a multiparameter (HANNA, HI98194).

Daily growth rate

Every 15 days of culture, the daily growth rate (DGR) %/day was computed using the

Hung *et al*., (2009) formula.

$$DGR = [(W_t / W_0)^{1/t} - 1] \times 100 \text{ \%/day}$$

where W_0 is the initial fresh weight, W_t is the final fresh weight, and t is the days of culture.

Biomass yield

Seaweed biomass is expressed as the fresh weight of seaweed per unit culture area (Kg/m^2) and computed with the following modified formula of Doty (1986):

$$Y = (W_t - W_0)/A$$

where: Y = biomass production; W_t = fresh weight at day t; W_0 =initial fresh weight; A = area of net.

Statistical analysis

Statistical Package for Social Sciences (SPSS) software version v25.0 and Microsoft Office (Excel 365) were used to analyze the data. For comparisons, t-test was used for analysis. In this investigation, the level of significance was set at $P < 0.05$.

Results

Water quality parameters

The results of water quality parameters in the culture sites of the Kuakata coast, Bangladesh are shown in Table 1. Water salinity, transparency, and total dissolved solids (TDS) were significantly higher ($P < 0.05$) in the Gangamati estuary than Hazipur estuary.

Table 1. Water quality parameters (mean±SD) of seaweed culture sites at Kuakata, Bangladesh.

Parameters	Gangamati estuary	Hazipur estuary
Water temperature (^oC)	23.30±1.36	22.50±1.31
Salinity (ppt)	14.75±4.89[a]	6.10±2.17[b]
Water transparency (cm)	56.50±8.57[a]	24.60±5.83[b]
pH	7.85±0.32	7.58±0.29
DO (mg/L)	8.10±0.51	7.72±0.48
TDS (ppm)	6154.90±692.50[a]	4326.54±317.74[b]

Daily growth rate (DGR)

The maximum daily growth rate was found on the 45th day in the Gangamati estuary (Figure 1). In contrast, the minimum daily growth rate was found on the 15th day in the Hazipur estuary (Figure 1) which could be due to the changes in water quality parameters of the culture sites.

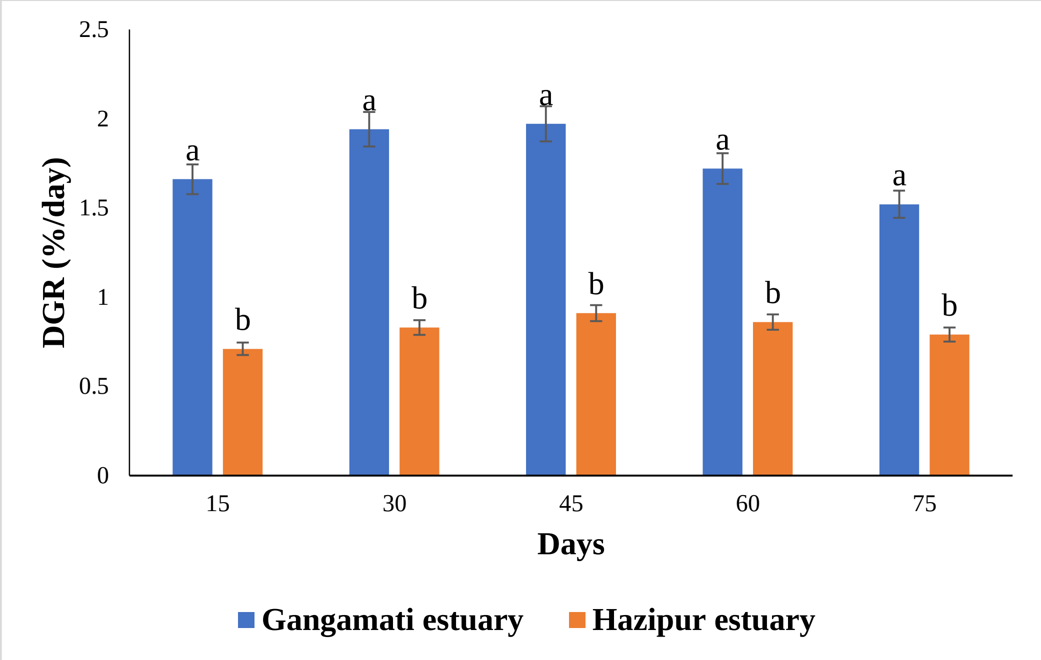


Figure 1. Daily growth rate (%/day) of *Gracilaria tenuistipitata* in different locations.

Production

The production (Kg/m^2) of *Gracilaria tenuistipitata* was significantly higher in the Gangamati estuary. Production of *Gracilaria tenuistipitata* in different locations is given in Table 2.

Table 2. Production of *Gracilaria tenuistipitata* in different locations.

Production	Gangamati estuary	Hazipur estuary
Kg/m^2	3.09±0.18^a	1.81±0.14^b

Experiment-2. Proximate composition and mineral content analysis of commercially important seaweeds in Bangladesh

Methodology

Seaweed species

Gracilaria tenuistipitata, *Ulva lactuca*, and *Sargassum oligocystum* species were used for the study.

Proximate analysis

For proximate composition, the protein content was determined by converting the nitrogen content obtained by Kjeldahl's method (AOAC, 2000). The total lipid was determined by Bligh and Dyer's method using chloroform/methanol (1/1, v/v) (Bligh and Dyer, 1959). The ash content was measured after 20-hour burn at 550°C (AOAC, 2000). The moisture content was determined using the hot air oven by drying the sample at 105°C ± 2°C until a constant weight was obtained (AOAC, 2000). Total carbohydrates were determined by subtracting the sum of fat content, protein content, ash content, and moisture from 100 (Onyeike et al., 2000).

Mineral analysis

The spectrophotometer was used to determine sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), and sulfur (S) using appropriate filters. The respective metal standard, blank, triplicate, and continuing calibration verification were conducted in each batch throughout the mineral analysis (AOAC, 2000).

Statistical analysis

All data were analyzed using Microsoft Excel and SPSS version 25 software. The samples were analyzed in triplicate.

Results

Proximate composition

All the proximate composition i.e., moisture, protein, lipid, ash, fiber, and carbohydrate contents showed significant variations (P < 0.05) among the three different seaweed species. The proximate composition of three seaweed species collected from the Bay of Bengal, Bangladesh coast is given in Table 3.

Table 3. Proximate composition of three commercially important seaweeds in Bangladesh.

Parameters	**Seaweed species**		
	G. tenuistipitata	***U. lactuca***	***S. oligocystum***
Moisture	90.59 ± 0.52^{b}	89.05 ± 0.48^{c}	91.96 ± 0.64^{a}
Protein (% DW)	25.06 ± 0.90^{a}	17.34 ± 0.37^{b}	8.72 ± 0.54^{c}
Lipid (% DW)	1.22 ± 0.05^{a}	0.71 ± 0.08^{b}	0.46 ± 0.06^{c}
Ash (% DW)	28.28 ± 1.10^{c}	37.58 ± 1.18^{a}	31.28 ± 1.27^{b}
Fiber (% DW)	7.03 ± 1.26^{b}	12.14 ± 1.65^{a}	3.45 ± 0.93^{c}
Carbohydrate (% DW)	38.41 ± 0.80^{b}	32.23 ± 1.62^{c}	56.09 ± 1.36^{a}

Mineral content

The mineral contents of the three studied seaweeds showed significant variations (P < 0.05). All the studied mineral contents were significantly higher in *G. tenuistipitata* than *U. lactuca* and *S. oligocystum*. Table 4 lists the mineral content (%) of three studied seaweed species gathered from the Bay of Bengal, Bangladesh coast.

Table 4. The mineral content of three commercially important seaweeds in Bangladesh.

Parameters	**Seaweed species**		
	G. tenuistipitata	***U. lactuca***	***S. oligocystum***
Sodium (%)	0.78 ± 0.02^{a}	0.34 ± 0.02^{c}	0.58 ± 0.02^{b}
Potassium (%)	2.69 ± 0.05^{a}	0.71 ± 0.03^{c}	1.62 ± 0.04^{b}
Calcium (%)	2.48 ± 0.04^{a}	1.42 ± 0.02^{b}	1.37 ± 0.04^{b}

Magnesium (%)	1.95±0.02[a]	1.70±0.01[b]	1.09±0.01[c]
Phosphorus (%)	0.26±0.03[a]	0.05±0.00[b]	0.06±0.01[b]
Sulfur (%)	0.72±0.05[a]	0.09±0.03[c]	0.28±0.06[b]
Na/K	0.29±0.01[c]	0.48±0.01[a]	0.36±0.01[b]

Experiment-3. Determination of phenolic and flavonoid content of commercially important seaweeds of Bangladesh

Methodology

Seaweed species

Gracilaria tenuistipitata, Ulva lactuca, and *Sargassum oligocystum* species were used for the study.

Preparation of seaweed extract

The collected seaweed samples were washed thoroughly with clean seawater to remove dirt, sand, and other impurities. Fresh samples were washed thoroughly with distilled water for further removal of any other remaining impurities. Then the cleaned seaweed was kept in a freeze dryer (LFD-BT-101, Labocon, UK) for 48 h at -80 ℃ to remove the moisture. Dried samples were sealed in plastic bags and stored in a refrigerator at 4 ℃ for further analysis in the laboratory.

The dried seaweed sample was grounded to make fine powder; the finer the powder, the more efficient the extraction. Four (4) grams of seaweed fine powder was soaked in 100 mL of solvent (water, methanol, ethanol, and acetone) by maceration for the preparation of an extract by solvent extraction. Then the samples were kept in the dark for 24 h with intermittent shaking for better extraction. After incubation, the solution was filtered with Whatman filter paper No 1 filter paper for retaining hygienic conditions. After filtration, the remaining wet powder was extracted again in their respective solvents for 12 h through sporadic shaking and filtered to get the maximum out of the sample powder. The methanol, ethanol, and acetone extracts were then concentrated using a Rotary Vacuum Evaporator (SCI100-Pro, SCILOGEX, USA) at 36 °C and the water solvent was dried by the Freeze Dryer (LFD-BT-101, Labocon, UK) at -80 ℃. Finally, working solutions were prepared for each extract.

Total phenolic content (TPC) determination

The concentration of total phenols in the crude extracts was determined using Folin-Ciocalteu Phenol reagents and external calibration with Gallic acid followed by Karydas et al. (2020) with slight modification. Briefly, 0.5 ml extract solution was added with 0.1 ml of FC reagent solution. After 15 min, 2.5 ml of saturated Na_2CO_3 (75 g/L) was added in the solution and allowed to stand for 30 min at RT and absorbance was measured at 760 nm using the spectrophotometer (UV-Visible Spectrophotometer double beam, model C7200, USA). The concentration of total phenolic was calculated as mg of Gallic acid equivalent per gram.

Total flavonoid content (TFC) determination

The total flavonoid contents of crude extracts were determined using the aluminum chloride colorimetric method, as described by Padhan et al. (2020) with slight modifications. Briefly, 1 ml extract solution was mixed with 3 ml methanol, 0.2 ml 10% aluminum chloride, and 0.2 ml 1 M potassium acetate. The solution was then incubated at RT for 30 min and absorbance was measured at 420 nm. The concentration of total flavonoids was calculated as mg of quercetin equivalent per gram.

ABTS (2, 2'-azino-bis3-ethylbenzthiazoline-6-sulfonic) radical cation decolorization assay

The ABTS radical anion scavenging assay will be carried out by the method of Reet al. (1999) with some modifications. The ABTS reagent will be prepared by mixing 5 mL of 14 mM ABTS with 5 mL of 4.9 mM $K_2S_2O_8$. The mixture will be kept in the dark at room temperature for 16 h. The absorbance will be adjusted with distilled water to 0.700 ± 0.02 at 734 nm. To determine the scavenging activity, 1 mL ABTS reagent will be added to 10 µL of different concentrations of seaweed extract and absorbance will be measured at 734 nm at 3 min intervals of Trolox will be used as standard. Percentage inhibition of the sample will be calculated by the following equation:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

A_0 expresses the absorbance of control; A_1 expresses the absorbance of the tested seaweed extract. The ABTS radical anion scavenging assay will be expressed as Trolox equivalent antioxidant capacity (TEAC) and defined as mg of Trolox equivalents per 1 g of sample.

DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) radical-scavenging assay

The DPPH radical-scavenging activity assay will be performed after Hou et al. (2001) with some modifications. 1.2 mL extract of different concentrations will be added to 0.1 mL of 1 M Tris-HCl buffer (pH 7.9) and mixed with 1.2 mL of 5 mM DPPH in MeOH and kept in the dark at room temperature for 30 min. The absorbance of the resulting solution will be measured at 517 nm. Gallic acid, a phenolic organic acid, was used as a standard. The decrease of absorbance at 517 nm will be calculated as the percentage of inhibition by the same equation of the ABTS assay.

Statistical analysis

All data were analyzed using Microsoft Office, and SPSS version 25 software. The samples were analyzed in triplicate.

Results

Total phenolic and flavonoid content

The total phenolic and total flavonoid content of the three studied seaweeds of Bangladesh differ significantly ($P < 0.05$) and significantly higher amounts of total phenolic and total flavonoid content were found in *Gracilaria tenuistipitata*. The total phenolic content and total flavonoid content of the three commercially important seaweeds of Bangladesh are shown in Table 5.

Table 5. Total phenolic content of the three commercially important seaweeds of Bangladesh.

Seaweed species	Total phenolic content (mg GAE/g dry weight)	Total flavonoid content (mg QE/g dry weight)
Gracilaria tenuistipitata	75.38±1.75[a]	49.57±0.92[a]
Ulva lactuca	24.51±0.83[c]	14.29±0.56[b]
Sargassum oligocystum	62.97±1.19[b]	46.10±0.84[c]

ABTS and DPPH content of the three studied seaweeds of Bangladesh differ significantly ($P < 0.05$) and significantly higher amounts of ABTS and DPPH content were found in *Sargassum oligocystum*. The ABTS and DPPH content of the three commercially important seaweeds of Bangladesh are shown in Table 6.

Table 6. ABTS and DPPH content of the three commercially important seaweeds of Bangladesh.

Solvent	ABTS scavenged (%)	DPPH scavenged (%)
Gracilaria tenuistipitata	63.91±0.85[b]	61.40±0.78[b]
Ulva lactuca	52.30±1.76[c]	57.14±1.76[c]
Sargassum oligocystum	71.08±2.19[a]	74.35±2.19[a]

Domestication and Captive Breeding of Brackishwater Finfish Species of Bangladesh

Researchers

: Md. Monjurul Hasan, SSO
: Dr. Mohammed Ashraful Haque, SSO
Aovijite Bosu, SSO
Md. Rahamat Ullah, SO
Farhana Yasmin, SO

Objectives

- To study the food and feeding habit and reproductive biology of *Otolithoides pama, Gagata gagata, Sillaginopsis panijus* and *Coilia dussumieri.*
- To domesticate and brood development of *Otolithoides pama, Gagata gagata, Sillaginopsis panijus* and *Coilia dussumieri*.
- To develop the breeding and larval rearing techniques of *Otolithoides pama, Gagata gagata, Sillaginopsis panijus* and *Coilia dussumieri.*

Achievement (2023-2024)

Name of the experiment/study:

To study the food and feeding habits and reproductive biology of *Otolithoides pama, Gagata gagata, Sillaginopsis panijus* and *Coilia dussumieri.*

Methodology:

The study was conducted at Bangladesh Fisheries Research Institute, Riverine Sub-station, Khepupara, Patuakhali from July 2023 to June 2024.

Food and feeding habit:

The study was conducted for the period of 10 months to estimate the monthly food items in the stomach contents of *Otolithoides pama, Gagata gagata, Sillaginopsis panijus* and *Coilia dussumieri* was collected from major rivers like, Andhermanik, Payra, Khaprabhanga, Sonatola and surrounding areas. The total length, body weight, stomach length and weight of specimens were measured. The collected specimens were dissected for food analysis in the RSS laboratory. Data were recorded monthly basis.

Index of preponderance:

The index of preponderance was calculated by using the following formula (Natarajan and Jhingran, 1961)

$$Ii = (Vi * Oi / \sum Vi * Oi) \times 100$$

where ''Vi'' and ''Oi'' are the volume and occurrence index of food items in percentage respectively.

Relative length of gut (RLG):

RLG was calculated as the ratio of full unstretched gut length to the total length of the fish. RLG value will be measured by using the formula (Al-Hussaini, 1949).

$$RLG = \text{Total Length of gut/Total body length}$$

Gastrosomatic index (GaSI):

Gastrosomatic index (GaSI) was calculated by using the formula (Bhatnagar and Karamchandani, 1970).

$$GaSI = \frac{\text{Weight of the Gut}}{\text{Weight of the Fish}} \times 100$$

Gonadosomatic index:

Gonadosomatic index (GSI) of the male and female were calculated separately by using the formula (Lagler, 1962).

$$GSI = \frac{\text{Weight of the Gonad}}{\text{Weight of the Fish}} \times 100$$

Fecundity estimation:

Fecundity of the collected fish species was estimated by the gravimetric method as described by Blay (1981). The fecundity was calculated using the following formula.

$$F = N \times \text{gonad weight/sample weight}$$

Where, F is the fecundity and N is the number of eggs in the sample

Histology:

The microscopic slides were prepared by the following procedure followed by Agarwal (1996).

Fixation (Bouins solution: 48 hrs.)
⇩
Dehydration (Ethanol)
⇩
Clearing (Xylene)
⇩
Embedding (Paraffin)
⇩
Trimming (5 µm)
⇩
Staining (Haematoxylene & Eosin)
⇩
Mounting (Dibutylphthalate Polystyrene Xylene)
⇩
Microscopic observations

Results:

➢ Relative length of gut (RLG):

Table 1: RLG value of collected fish species

Species	Relative length of gut (RLG))
Otolithoides pama	0.92±0.06
Gagata gagata	0.87±0.04
Sillaginopsis panijus	0.80±0.09
Coilia dussumieri	0.43±0.11

The RLG value of four fish species implies that all the species are carnivorous in nature (<1).

➢ Gastrosomatic index (GaSI):

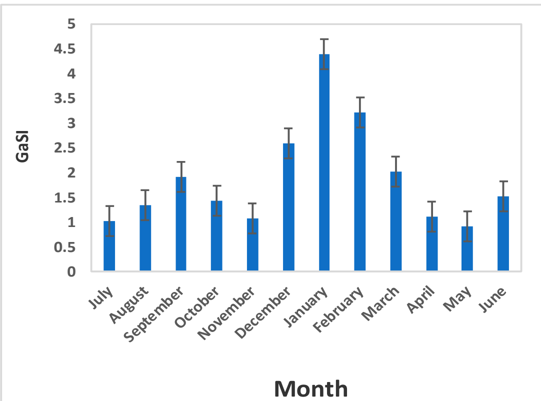


Fig. 1. Month wise GaSI of *Gagata gagata*

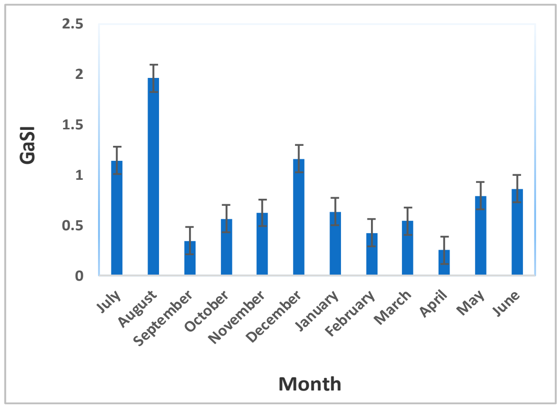


Fig. 2. Month wise GaSI of *Otolithoides pama*

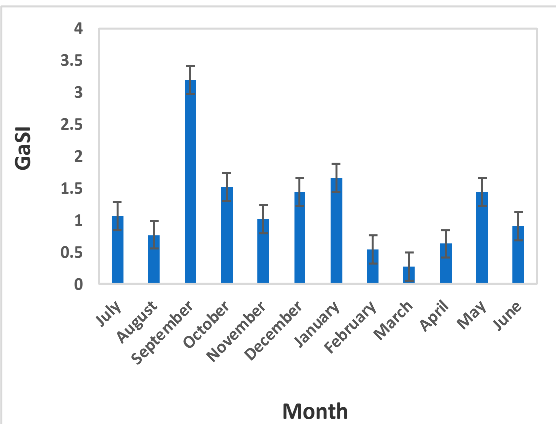


Fig. 3. Month wise GaSI of *Sillaginopsis panijus*

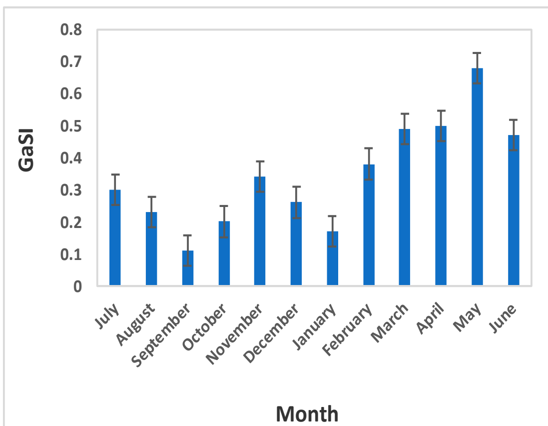


Fig. 4. Month wise GaSI of *Coilia dussumieri*

Table 2: Prey consumed by *O. pama, G. gagata, S. panijus, C. dussumieri*

Groups	*O. pama*	*G. gagata*	*S. panijnus*	*C. dussumieri*
Fish	*Trypauchen vagina* *Glossogobius giuris* *Coilia Dussumieri* *Otolithoides Pama* *Setipinna sp.* *Polynemus paradiseus*	Fish larvae	*Trypauchen vagina* *Glossogobius giuris* *Coilia dussumieri* *Setipinna sp.* *Polynemus paradiseus*	Fish larvae
Crustacean	*Squilla* sp. *Actes* sp. *Scylla* sp.		*Actes* sp. *Scylla* sp.	*Actes* sp.
Others	Fish scale/Fish bone Shrimp shell	Sand Debris	Fish scale Crab shell	

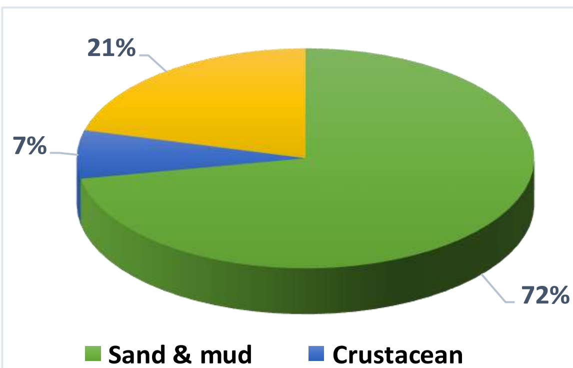


Fig.5 Index of preponderance of different prey groups of *Gagata gagata*

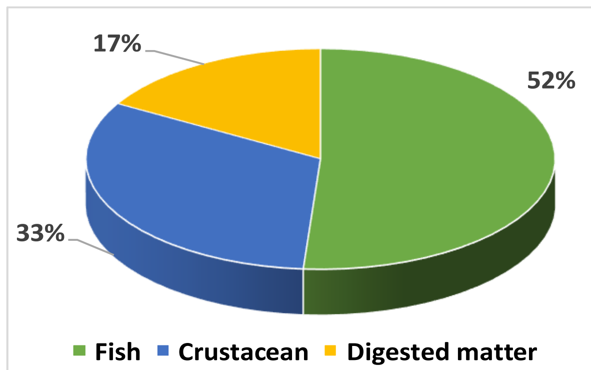


Fig.6 Index of preponderance of different prey groups of *Otolithoides pama*

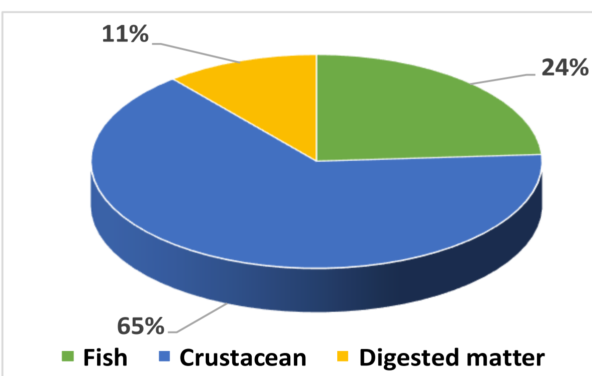


Fig.7 Index of preponderance of different prey groups of *Sillaginopsis panijus*

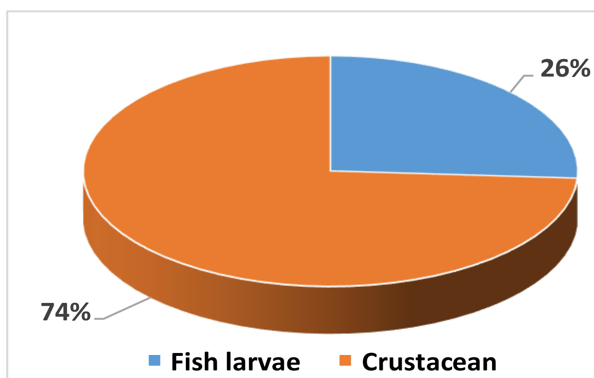


Fig.8 Index of preponderance of different prey groups of *Coilia dussumieri*

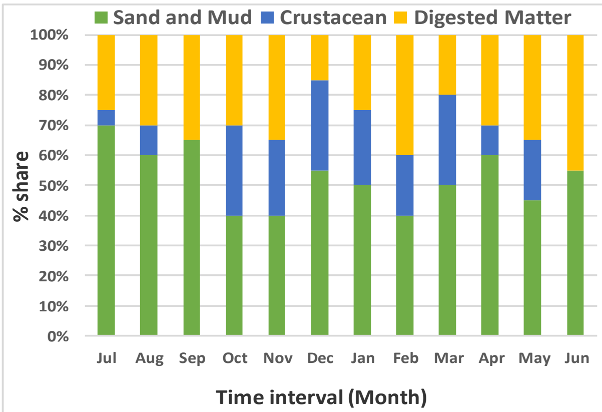


Fig.9 Month-wise percentage composition of different food items of *Gagata gagata*

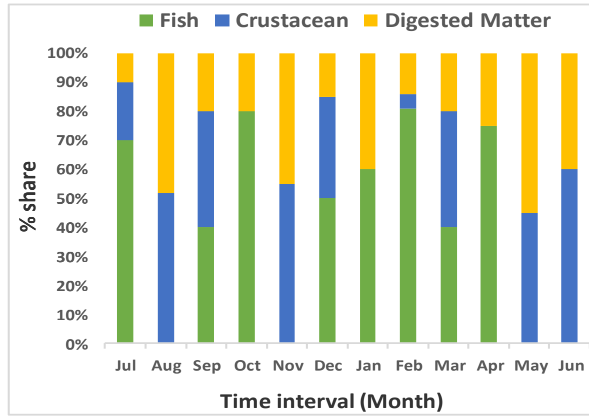


Fig.10 Month-wise percentage composition of different food items of *Otolithoides pama*

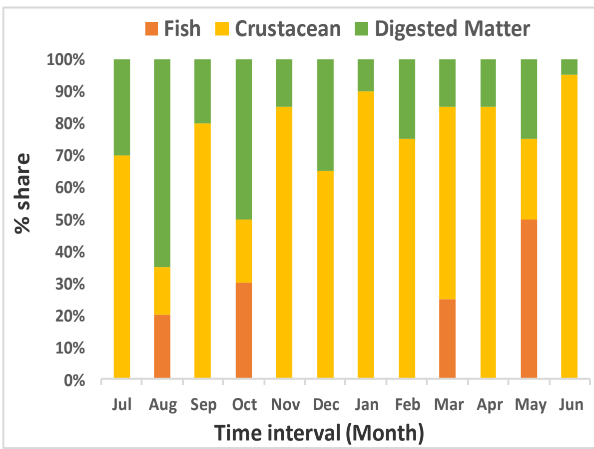


Fig.11 Month-wise percentage composition of of different food items of *Sillaginopsis panijus*

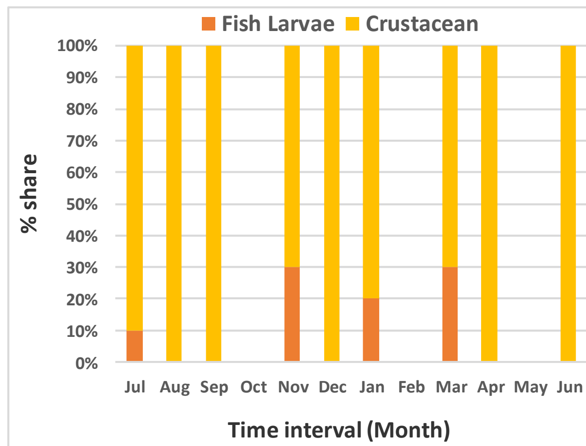
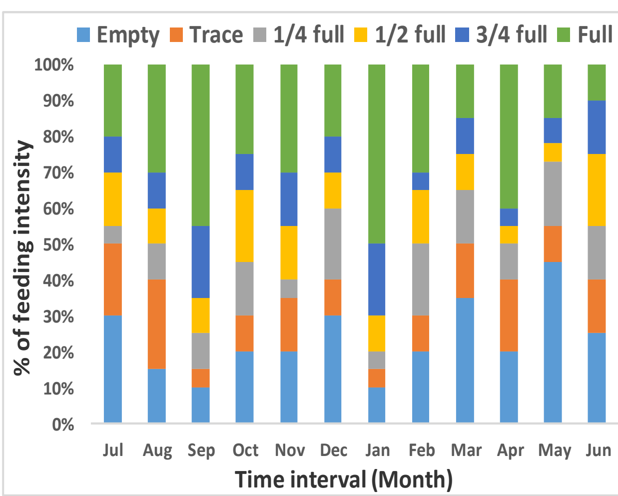


Fig.12 Month-wise percentage composition different food items of *Coilia dussumieri*



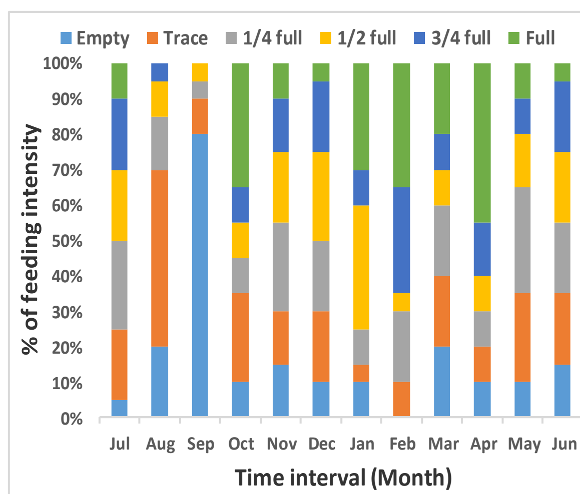
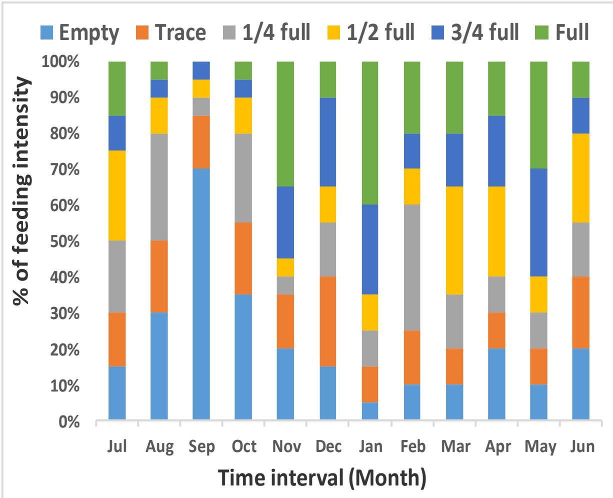


Fig.13 Month-wise percentage of feeding intensity of intensity of *Gagata gagata*

Fig.14 Month-wise percentage of feeding intensity of *Otolithoides pama*



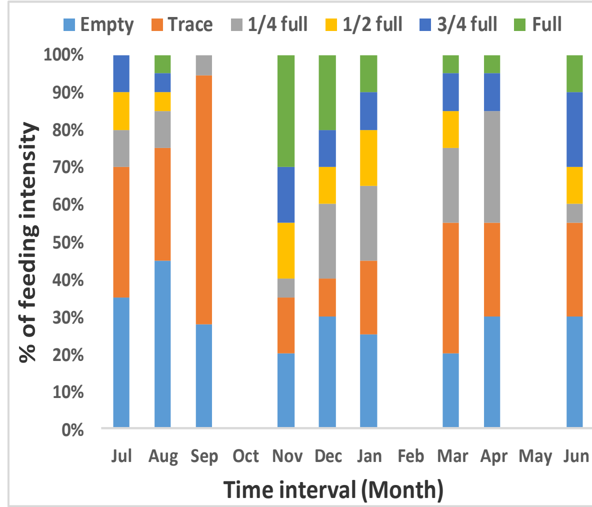


Fig.15 Month-wise percentage of feeding intensity of *Sillaginopsis panijus*

Fig.16 Month-wise percentage of feeding intensity of *Coilia dussumieri*

- **Gonadosomatic Index (GSI):**

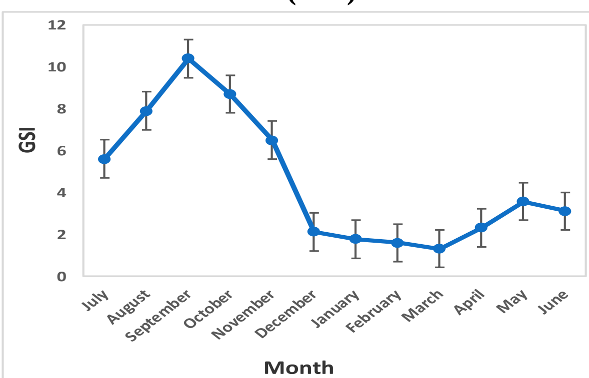


Fig.17 Month wise GSI of *Gagata gagata*

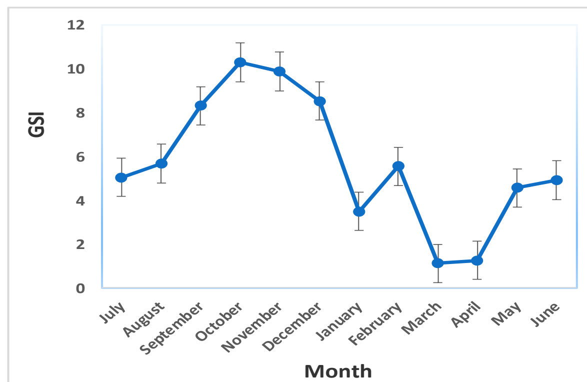


Fig.18 Month wise GSI of *Otolithoides pama*

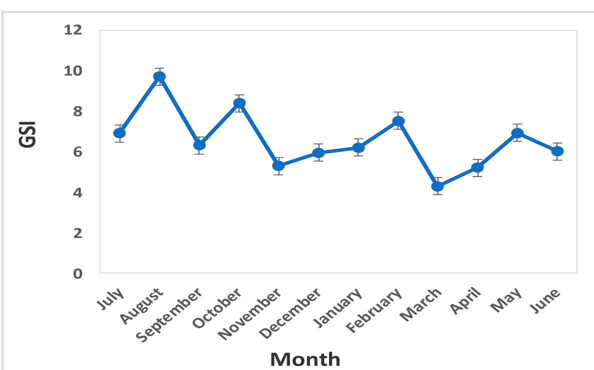


Fig.19 Month wise GSI of *Sillaginopsis panijus*

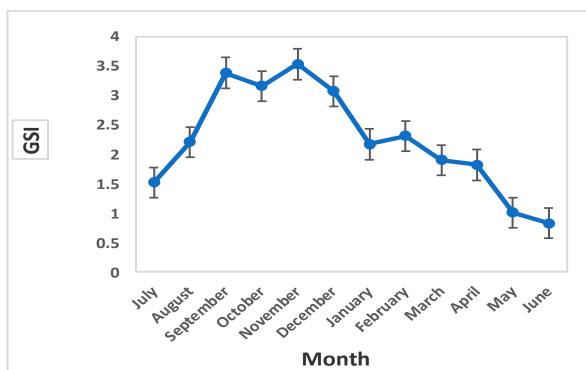


Fig.20 Month wise GSI of *Coilia dussumieri*

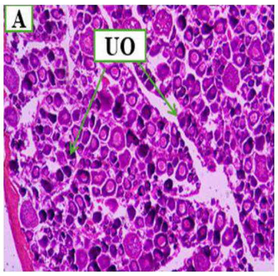
6

- **Fecundity:**

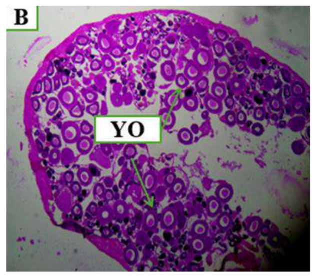
Table 3: Fecundity of collected fish species

Species	Fecundity (range)
Gagata gagata	2672-38234
Otolithoides pama	27676-346482
Sillaginopsis panijus	65072-597780
Coilia dussumieri	6100-12500

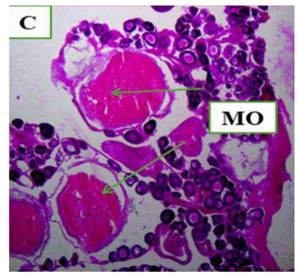
- **Histology:**



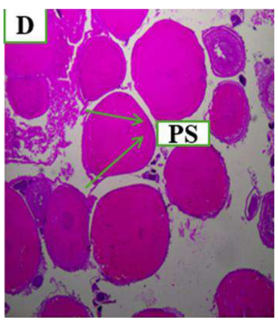
A. Immature Stage (UO= Unyolked oocyte)



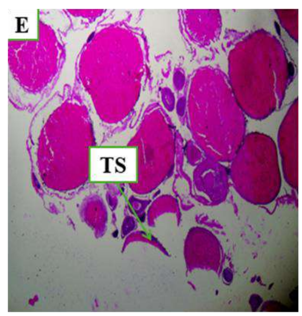
B. Maturing stage (YO= Yolked oocyte)



C. Mature stage (MO=Mature oocyte)



D. Ripe stage (PS=Partially spent)



E. Spent stage (TS=Totally spent)

Fig.21 Photomicrographs of different ovarian stages of the female *Gagata gagata* at various stages in gamete development.

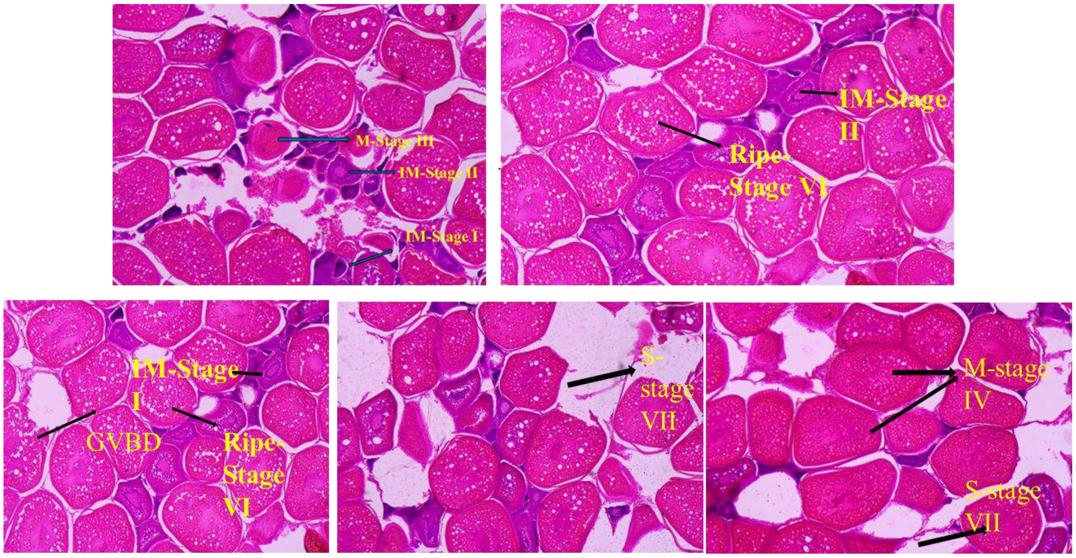


Fig.22 Photomicrographs of different ovarian stages of the female *Otolithoides pama* at various stages in gamete development

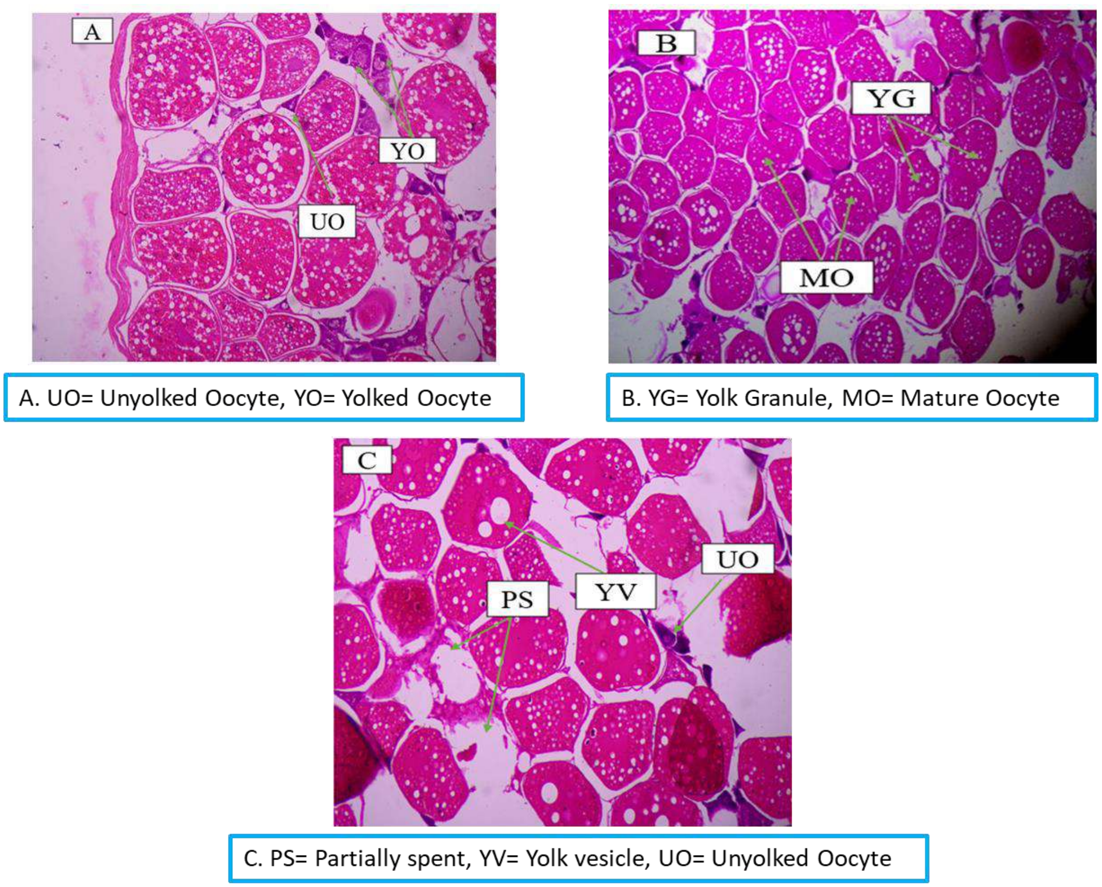
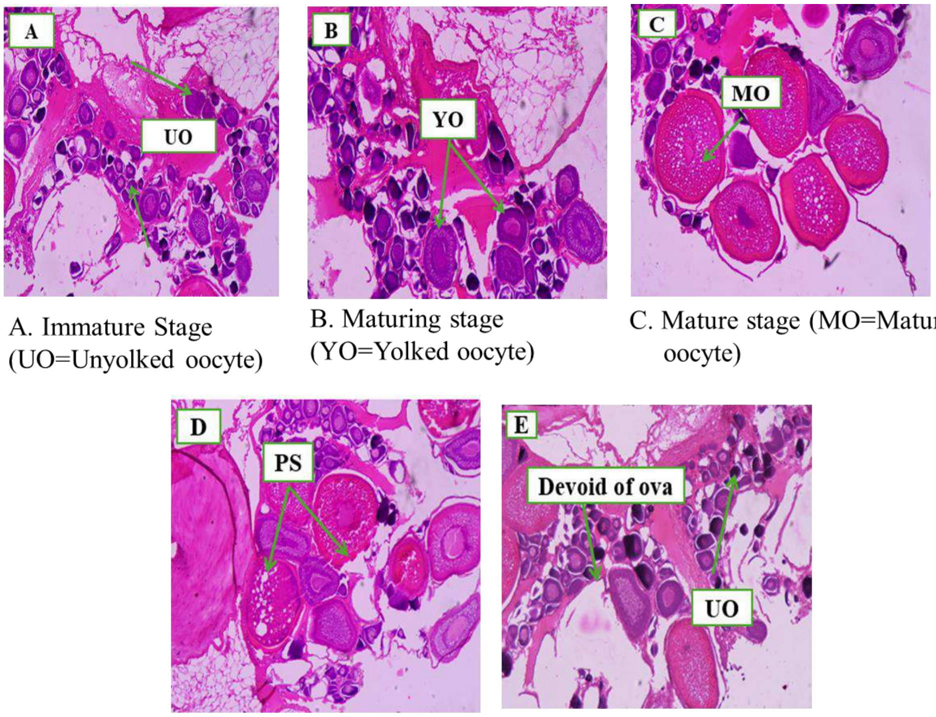


Fig.23 Photomicrographs of different ovarian stages of the female *Sillaginopsis panijus* at various stages in gamete development



A. Immature Stage (UO=Unyolked oocyte)

B. Maturing stage (YO=Yolked oocyte)

C. Mature stage (MO=Mature oocyte)

D. Ripe stage (PS=Partially spent)

E. Spent stage

Fig.24 Photomicrographs of different ovarian stages of the female *Coilia dussumeri* at various stages in gamete development.

Name of the experiment/study:

Domestication and brood development of *Otolithoides pama, Gagata gagata*, *Sillaginopsis panijus* and *Coilia dussumieri*

Methodology:

The study was conducted at Bangladesh Fisheries Research Institute, Riverine Sub-station, Khepupara, Patuakhali for a period of 07 months during July 2022 to June 2023.

Pond preparation:

Ponds were prepared following standard method before stocking of selected finfish fry. Ponds bottoms were dried out to get rid of unwanted fish species. Selected ponds were 2 to 3 feet higher than the ponds highest water level. While excavating or de-mudding, this was achieved quickly and effortlessly. Ponds were facilitated with efficient inlet and outlet system through which pond water can be exchanged with river water regularly. Lime was applied @500g/decimal/pond and Urea and TSP was applied@150g/decimal/pond and @75g/decimal/pond respectively during pond preparation.

Collection and stocking of brackish water finfish fry:

Fingerlings and sub-adults of *Gagata gagata*, *Otolithoides pama* and *Sillaginopsis panijus* were collected from the adjacent river Andharmanik and stocked in the RSS, Khepupara ponds.

Feed and feeding:

The fish were fed with live feed (fish larvae, shrimp larvae) @ 3-5% of their body weight, twice daily.

Growth performance monitoring:

Growth performances were calculated as following Bagenal (1978).

Length-weight relationship (LWR) was estimated by using the equation: $W = aL^b$

where, W is the weight of fish (g), a is the regression constant or intercept, b is the regression coefficient or slope. The equation was linearized by a logarithmic transformation into:

$\text{Log } W = \text{Log } a+b$

Log L was computed to estimate the "a" and "b" values. The condition factor was calculated using equation: $K = 100\ W/L^3$

where, K is the condition factor, W is the wet body weight (g) and L is the total length (cm). The linear relationship between the length and weight was estimated by calculating the correlation coefficient (R^2).

Water quality parameters monitoring:

Monthly monitoring of water quality parameters (Water Temperature, pH, Dissolve Oxygen, Salinity, TDS, Conductivity, ORP) was conducted.

Results:

- **Pond preparation:**

Pond was prepared following standard method before stocking of selected finfish fry.

- **Collection and stocking of brackish water finfish fry:**

Fingerlings and sub-adults of *Otolithoides pama*, and *Gagata gagata* were collected from the adjacent river Andharmanik and stocked in the RSS, Khepupara pond.

Fig.25 Collection and stocking of Brackishwater finfish species

➢ Feed and feeding:

The fish were fed with live feed (fish larvae, shrimp larvae) @ 3-5% of their body weight, twice dail

Fig.26 Live & formulated feed application

Fig.27 Monitoring of the domesticated fish

➢ Length-weight relationship:

L-W relationship shows negative allometric growth for domesticated species.

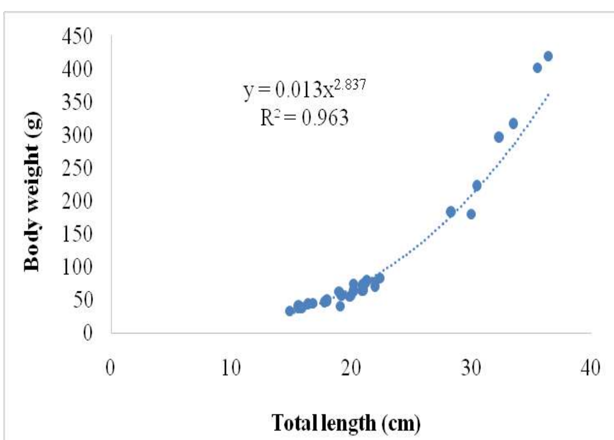


Fig.28 Length-weight relationship of cultured *O. pama*

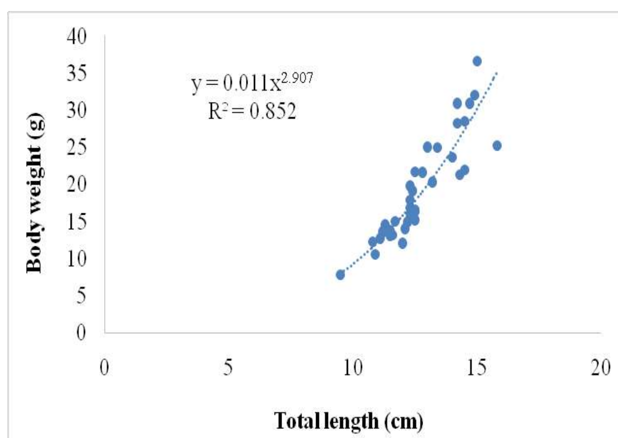


Fig.29 Length-weight relationship of cultured *G. gagata*

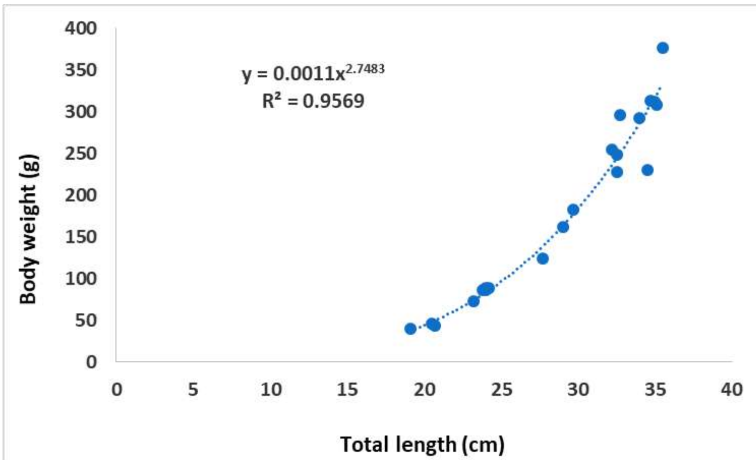


Fig.30 Length-weight relationship of cultured *S. panijus*

➢ **Water quality parameters monitoring:**

Monthly monitoring of water quality parameters (Water Temperature, pH, Dissolve Oxygen, Salinity, TDS, Conductivity, ORP) was conducted. All the parameters were found congenial in range.

Table 4: Water quality parameters in earthen pond

Month	Water Temperature (°C)	pH	DO (ppm)	Salinity (ppt)	TDS (ppm)	Conductivity (µs/cm)	ORP (mv)
July	31.2	7.4	5.2	1.48	1442	2883	89.9
August	28.3	7.3	5.4	0.62	627	1354	111
September	32.2	7.4	5.7	0.38	394	798	123
October	31.6	7.6	5.6	0.32	331	662	143.4
November	25.2	7.1	5.8	0.52	543	1083	124
December	22.3	6.9	5.7	0.8	798	1592	77.3
January	19.4	6.8	5.5	1.83	1735	3468	76.2
February	23.7	7.7	5.4	1.19	1163	2329	108.4
March	30.9	7.5	5.5	3.01	2810	5618	113.6
April	33.3	7.2	5.4	4.2	3872	7846	107
May	35.4	6.9	5.5	7.8	6880	13620	95
Jun	33.9	7.5	5.7	5	4438	8852	132.8
Mean±SD	28.5±5.12	7.3±0.3	5.53±0.2	2.26±2.2	2086±2039	4175±4046	106.8±23.5

Development of Mariculture Practice of Seabass (*Lates calcarifer*) in the South-West Coast of Bangladesh (Component-C)

Researchers : Aovijite Bosu, SSO
: Dr. Mohammed Ashraful Haque, SSO
: Md. Monjurul Hasan, SSO
: Md. Rahamat Ullah, SO
: Farhana Yasmin, SO

Objectives

- To develop cage culture technique of Seabass in coastal water of Bangladesh
- To study the growth and survival rate of Seabass in net cages and pond

Achievements (2023-2024)

Experiment I: Study on Growth and Survival of Seabass in Cage Based on Weight of Fish

Selection of study area

Experiment was carried out in protected area of the Andharmanik River, near to the Riverine Sub-Station, Khepupara, Patuakhali. The site located in an area where influence of tidal fluctuation is not pronounced with salinity ranging from 05-15 ppt and is suitable for Seabass culture. The site is far from the area where biofoulers abound and far from the sources of domestic, industrial and agricultural pollution and other environmental hazards.

Cage culture management and techniques

Prior to stocking Seabass juvenile in cages, fish was acclimatized to the ambient temperature and salinity prevailing in the cages. Weight ranges from 50-150g of wild Seabass fry was stock in the early mornings (0600–0800 hours) or late in the evening (2000-2200 hours), when the temperature is cooler. Stocking was done through following design.

Table 1: Experimental design with stocking rate of sea bass

SL No.	Treatment Name	Weight(g)	Stocking density (Nos/m^3)
1	T_1	50	1.5fish/m^3
2	T_2	100	
3	T_3	150	

*All treatments were 3 replications

Feeds and feeding

Feed is the major constraint confronting the Seabass culture industry. Different types of feed were applied. Feed was provided twice daily in the morning at 08:00 hours and afternoon at 17:00 hours as overall rate of 3-5% of total biomass.

Fig. Pictorial view of feeding of seabass and feeding biology observation

Fish cage management

Regular observation of cages was done. Since fish cages were immersed under water all the time, they are vulnerable to destruction by aquatic animals such as crabs, otter, etc. If damaged, they 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 is 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.

Water Quality and growth parameters monitoring:

Water quality parameters viz., pH, Dissolve Oxygen, Salinity, Ammonia, Alkalinity etc of different cage were monitored weekly.

Fish Growth parameters

Growth of Seabass was monitored monthly. Length of fish were measured by measuring scale and weighed by digital weighing machine. The following parameters were used to calculate the growth of fish such as length gain (cm), weight gain (g), percent weight gain, food conversion ratio (FCR), survival rate (%) and production of fish (Kg/m^3).

Length gain

Length gain was calculated using the following formula:

Length gain (cm) =Mean final length (cm)-Mean initial length (cm)

Weight gain

Weight gain was calculated using the following formula:

Weight gain (g) = mean final Wight (g) – mean initial weight (g)

Feed conversion ratio

FCR was expressed by the rate of food consumed to weight gain was determined by the following formula:
FCR= Feed fed / live weight gain

Survival rate

Survival rate of fish was calculated from the number of fish of particular species harvested at the end of the experiment. The survival rate was estimated by the following formulae:
Survival rate= (no. of fish harvested / no. of fish stocked) x100

Specific growth rate (% per day)

Specific growth rate was calculated by the following formulae:
Specific growth rate (SGR %)=(ln W_2-ln W_1 / culture period)x100
Whereas, W_2= mean final weight (g), W_1= mean initial weight (g)

Fish Production

The production of fish was determined by multiplying the mean increased weight (g) of fish by the total number of caught fish. Production was calculated by the following formula:
Fish production= No. of fish caught × Mean increased weight (g)

Data analysis:

Statistical Package for Social Sciences (SPSS) software version v25.0 and Microsoft Office 365 were used to analyze the data.

Results

Study on Growth and Survival of Seabass in Cage Based on Weight of Fish

Rearing of Seabass fry in net cages

Selection of study area

Experiment was carried out in protected area of the Andharmanik River, near to the Riverine Sub-Station, Khepupara, Patuakhali. The site should preferably locate in an area where influence of tidal fluctuation is not pronounced with salinity ranging from 02-15 ppt and is suitable for seabass culture.

Collection and stocking of Seabass fry

Prior to stocking seabass in cages, fish was acclimatized to the ambient temperature and salinity prevailing in the cages. Different size (50-150gm) wild seabass fry stocked in the early mornings (0600–0800 hours) when the temperature is cooler. Stocking was done through following design.

Table 2: Experimental design with stocking rate of Seabass

SL No.	Treatment Name	Weight(g)	Stocking density(Nos/m^3)
1	T_1	50	1.5 fish/m^3
2	T_2	100	
3	T_3	150	

*All treatments have 3 replications

Water quality parameters of the cage site of the Andhermanik River

Water quality parameters of the cage site of the Andhermanik River are shown in Table 3. Water quality parameters viz., pH, Dissolve Oxygen, Salinity, Ammonia, Alkalinity etc of different cage was monitored bi-weekly.

Table 3. Water quality parameters of Andharmanik River

Month	Water Temperature (°C)	P^H	Ammonia (ppm)	Salinity (ppt)	DO (ppm)
July 2023	31.2	7.5	0.02	1.83	5.83
Aug 2023	28.3	7.43	0.02	1.71	5.62
Sep 2023	32.4	7.14	0.03	2.23	6.21
Oct 2023	30.7	7.26	0.01	4.74	5.36
Nov 2023	26.7	7.75	0.02	6.65	5.32
Dec 2023	23.8	7.43	0.01	8.82	6.21
Jan 2024	21.5	7.61	0.02	11.54	5.47
Feb 2024	22.84	7.8	0.02	12.65	5.53
Mar 2024	29.43	7.41	0.03	11.78	6.32
Apr 2024	33.6	7.62	0.02	9.62	6.27
May 2024	35.8	7.50	0.01	6.9	6.58
June 2024	31.1	7.50	0.01	5.12	5.76
Mean±SD	28.94±3.93	7.49 ± 0.23	0.01±0.005	6.965±4.64	5.873±0.47

Fig. Pictorial view of Water quality parameter monitoring

Growth Parameters Monitoring

Growth of seabass was monitored monthly. Length of fish was measured by measuring scale and weighed by digital weighing machine.

Fig. Pictorial view of length-weight measurement of seabass

Growth performances of Seabass in the net cages

From the results, it was observed that different size fish had an effect on the growth of Seabass (Table 4). The highest Length gain, Weight gain, SGR, Survival rate and Production was obtained in T_3 than in other treatments.

Table 4. Growth performances of Seabass in the net cages in the Andharmanik river

Parameters	T_1	T_2	T_3
Culture period (days)	240	240	240
Total stocked fingerling	96	96	96
Initial avg. length (cm)	10.2±0.82	13.7±2.7	17.6±2.6
Final avg. length (cm)	31.8±4.56	37±5.12	41.2±4.56
Initial avg. weight (g)	50.6±0.56	101.7±1.84	150.6±7.5
Final avg. weight (g)	1397.09±105.38	1553±115.78	1806.69±321.84
Length gain (cm)	21.6±3.25^{b}	23.3±2.96^{a}	24.6±3.25^{a}
Weight gain (g)	1346.49±65.38^{c}	1452.40±97.72^{b}	1656.09±105.38^{a}
SGR (%)	1.63±0.07^{c}	1.72±0.09^{b}	1.81±0.11^{a}
Survival rate (%)	81.37±1.25^{c}	88.13±2.5^{b}	90.65±1.4^{a}
Production (Kg/m^3)	1.64±0.08^{c}	1.92±0.14^{b}	2.25±0.18^{a}

Assessment of Natural Breeding Grounds of Commercially Important Carps, Chitol, Boal and Baim in Kaptai Lake

Researcher(s) : Md. Lipon Mia, SO
: Md. Istiaque Haidar, SSO
: B. M. Shahinur Rahman, SSO
: Md. Khaled Rahman, SO

Objectives

- To know the current state of the previously identified natural breeding ground of commercially important carps in Kaptai Lake
- To identify the specific breeding locations

Achievement (2023-24)

The below four are the main sampling site in kaptai Lake. Beside these local market and fish landing centers in Rangamati were also included as sampling site for collecting carp juveniles. Monthly sampling is going on.

- Kasalong channel: Mynimukh and upwards
- Barkal channel: Jagannathchari and upwards
- Chengi channel: Naniarchar and upwards
- Riankhang channel: Bilaichari to Chakrachari and upward

Physicochemical Analysis

Physicochemical parameters such as air temperature, water temperature, hardness, transparency, salinity, dissolved oxygen, ammonia, free carbon dioxide, alkalinity and primary productivity were analyzed in different sampling stations and recorded on monthly basis. Data of water quality parameters were furnished in table-1.

Fig 1: Pictorial view of water quality analysis (A: Chengi river and B: Karnaphuli river).

Table 1: Physico-chemical factors with range, mean values (*x*) and standard deviation (±SD) of the existing carp natural breeding grounds of Kaptai Lake

Parameter	Kasalong channel	Barkal Channel	Chengi Channel	Riankhang Channel
Air Temp (℃)	27.33±4.497	21.67±2.624	26±3.559	22.67±0.942
Water Temp (℃)	26.01±2.463	24.55±1.305	27.03±2.597	26.47±0.942
Dissolved oxygen (mg/l)	4.13±1.309	5.43±0.758	3.87±1.897	4.17±1.376
Free CO_2 (mg/l)	15.67±6.548	7±2.449	11±2.449	24.67±10.370
pH	7.55±0.062	7.83±0.181	7.47±0.121	7.93±0.094
mVpH	-33.23±3.437	-54.03±6.399	-32.37±7.994	-56.47±2.310
Conductivity (µS/cm)	156±14.966	172.67±11.145	147.33±27.884	354.33±43.369
Absolute Conductivity (µS/cma)	159.67±20.434	172.33±14.839	146±28.296	368±42.426
Oxidation Reduction Potential (mVORP)	109.3±45.774	137.03±210192	134.93±36.496	140.17±38.372
Resistivity (K/MΩ cm)	6.47±0.579	5.8±0.374	7.23±1.329	2.867±0.377
Salinity (ppt, psu)	0.03±0.004	0.08±0.008	0.07±0.012	0.17±0.0188
Secchi disc (cm)	24.67±8.178	79.57±22.994	65.67±30.404	27.33±4.478
Alkalinity (mg/l)	169.33±13.299	149.33±58.931	201.67±29.465	429.47±78.441
Total dissolved solids (mg/l)	78±7.483	86.33±5.734	72±12.192	177±21.213

All the water quality parameters were recorded in suitable range for different locations of Kaptai Lake.

A B

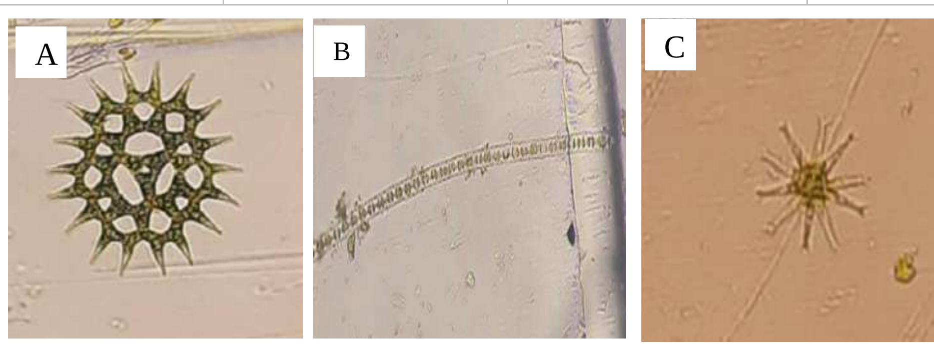
Fig 2: A: Pictorial view of the collection of plankton (Myni river) and B: determination of water transparency using a Secchi disk in Chengi Channel.

Identification and Quantification of Plankton

A plankton net of 20 µ mesh size was used for filtering water and collecting plankton samples for qualitative (preferably up to generic level) and quantitative analysis. We have recorded 21 genera of phytoplankton under 9 classes and 9 genera of zooplankton under 4 classes so far.

Table 02. List of identified phytoplankton from the existing breeding grounds of carp in Kaptai Lake.

SN	Class	Genus		
01	Cyanophyceae	*Aphanocapsa*	*Aphanozomenon*	*Aphanothece*
		Gloeocapsa	*Nodularia*	*Oscillatoria*
02	Bacillariophyceae	*Tabellaria*	*Nitzschia*	*Navicula*
		Pseeudo-nitzschia	*Synedra*	
03	Zygnematophyceae	*Staurastrum*	*Cosmarium*	
04	Dinophyceae	*Gymnodinium*	*Cyclotella*	*Conscinodiscus*
05	Chlorophyceae	*Chlorella*	*Kirchnerilla*	
06	Euglenophyceae	*Euglena*	*Phacus*	*Monas*



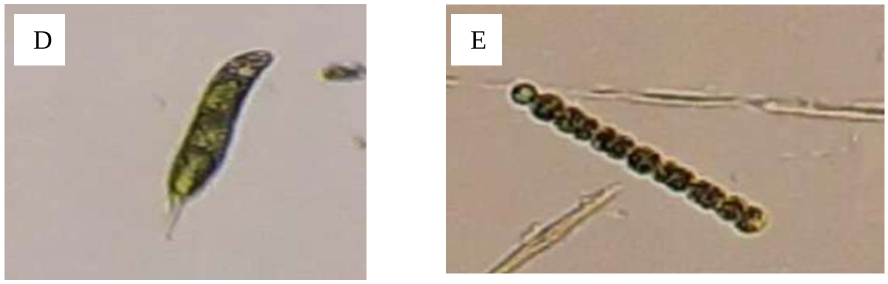


Fig 3: Phytoplankton observed in microscope (A. *Pediastrum.*, B. *Ulothrix*, C. *Straustrun*, D. *Euglena*, E. *Nostoc)* in different sampling stations of Kaptai Lake.

Table 03. List of identified zooplanktons from the existing carp breeding grounds of kaptai lake

SN	Class	Genus		
01	Rotifera	*Gastropus*	*Dicranophorus*	*Polyarthra*
02	Copepoda	*Nauplius*	*Canthocamptus*	*Hexarthra*
		Polyar		
03	Cladocera	*Bosmina*		
04	Amoebozoa	*Lecane*		

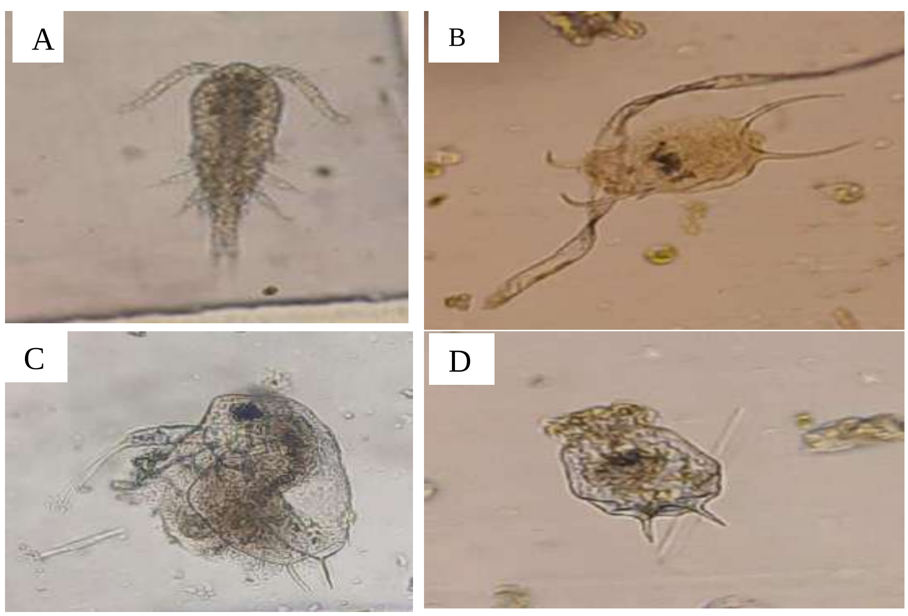


Fig 4: Zooplankton observed in a light microscope (A. *Cyclops*, B. *Bosmina*, C. *Ceriodaphnia sp.* D. *Lecane)* collected from different sampling stations of Kaptai Lake.

Collection of juvenile fishes

During the post-breeding season, juveniles of different carp species were collected from fishers catch and local markets to know the availability of carp juveniles in Kaptai Lake.

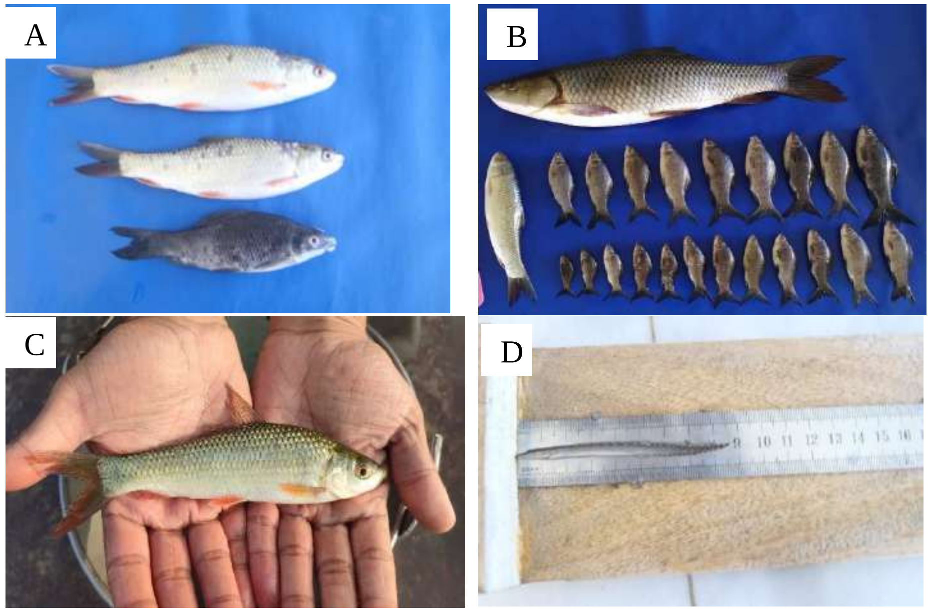


Fig 5: A: *L. calbasu* and *Cirrhinus mrigala* fry collected from the local market; *B:* Carp species collected from Chengi River; C: Live *L. rohita* fry collected from kattoli beel; D: Live *Mastacemblus armatus* fry collected from Kaptai Lake.

Present condition of breeding channel

Existing breeding grounds of Kaptai lake:

- Kasalong channel : Mynimukh and upwards; (N $22^{0}58^{/}42.73356^{//}$ and E $92^{0}11^{/}18.51288^{//}$)
- Barkal channel : Jagannathchari and upwards(N $22^{0}43^{/}38.89830^{//}$ and E $92^{0}20^{/}13.68535^{//}$)
- Chengi channel : Nanierchar and Upwards (N $22^{0}51^{/}27.11664^{//}$ and E $92^{0}4^{/}42.65112^{//}$)
- Riankhang channel : Bilaichari and upwards (N $22^{0}29^{/}10.49964^{//}$ and E $92^{0}20^{/}10.26384^{//}$)

Existing breeding grounds water depth (m)

Water depth was measured at different points of the breeding grounds and their migratory routes. The highest water depth is similar in January and March 2024 in Kasalong channel (Myni River) due to dredging being done at the Myni River.

Table 4: Monthly variation in water depth (m) in the existing breeding grounds of carp.

Location/Month	Kasalong channel	Barkal channel	Chengi channel	Riankhang channel
January/2024	4.80-6.56	3.4-22.5	5.33-7.24	4.14-5.87
February/2024	2.54-4.72	2.53-17.67	4.36-6.04	3.09-4.37
March/2024	1.727-6.42	1.17-12.64	1.37-2.64	1.16-2.489
April	1.08-5.54	0.22-8.64	0.46-1.95	0.38-1.78
May	0.76-4.85	0.00-7.42	0.25-1.63	0.00-1.18
June	0.86-5.0	0.6-7.92	0.85-2.03	0.54-1.9

Fig 6: A: Chengi channel filled due to siltation; B: Canal beside Chengi channel March 2024; C: Riankhang channel filled due to siltation (March 2024); D: Forest ghat (mostaner tila) Myni River March 2024.

Identifications of new breeding grounds

Natural breeding ground is the major source of natural recruitment in a water body. During the monsoon periods, the feeder rivers and the upper reaches of Kaptai Lake become inundated and prevail current. All these factors provide a favorable environment for the natural breeding of major carp. During the rainy season, these marginal areas get inundated with heavy flash floods, causing turbulence and upwelling currents as well as counter-flow currents along the bends, forming a favorable place for carp spawning. Under natural conditions, carp typically undergo hatching during the months of May and June. This coincides with heavy flood water runoff from the upper river, resulting in elevated tides and significant changes in water level, turbidity, and current velocity, which is a common phenomenon for carp spawning.

On 2nd July 2024, a researcher team have been identified new breeding grounds of carp at Maladip point, Longodhu upazila in kaptai lake. For confirmation a group of fishermen have been operated knotless filter net to collect egg. After egg collection, then transfer to the pit to obtain fingerling. Physico-chemical parameters of water have been recorded and found in a suitable range of all recorded parameters.

Fig 7: A: Pictorial view of egg collection; B and C: Collected eggs; D: Microscopic view of fertilized egg.

13. List of scientific Publications: Not applicable

14. Problems and Constraints encountered (if any): Not applicable

15. Signature of Principal Investigator(s):

16. Signature of the Chief Scientific Officer:

Refinement and validation of culture technology of cuchia in hill tract districts

Researcher(s) : Md. Khaled Rahman, SO
: Md. Istiaque Haidar, SSO
: B. M. Shahinur Rahman, SSO
: Md. Lipon Mia, SO

Objectives

The overall objective of this proposed project is to understand the Cuchia fry rearing, enhancement and ranching of this endangered species and to introduce this exportable fish into the aquaculture of Bangladesh for enhancement of income by the fish farmers. The specific objectives are -

a) To disseminate *M. cuchia* culture in Chittagong hill tract districts

b) To popularize Cuchia culture in Hill tract area

Achievement (2023-24)

Site selection

Site was selected from three different districts such as Rangamati, Bandarban and Khagrachhari. The experiment was conducted with two treatments in Rangamati and Bandarban; one treatment in Khagrachhari. To fulfill the objectives of the experiment, the following design was followed.

Table 1: Design of the experiment

Study area	Feed type	Stocking density
Rangamati	SIS (1.5% of BW) 3 days interval and Vermi Compost (1.5% of BW) Every day	$10/m^2$
Bandarban		
Khagrachori		


Figure 1. Pictorial view of site selection.

Pond Preparation

The experiment was conducted in 06 ponds with water area was $40m^2$ each of ponds. Ponds were dug with (30×15×3.5) ft^3. Pond bottom were covered by Polythene, knotless nylon net and triple then fill-up with 08-12 inch clay mud. The ponds were protected by fencing with nylon net. The ponds were prepared by treating soil with quick lime at rate of 2kg per decimal. Ponds was filled-up with 0.6-0.8m water and then use of dolomite at the rate of 15 ppm for strengthening buffer capacity of water. After three days, the pond water was fertilized with Urea, TSP and MoP at the rate of 2.5 ppm, 3.0 ppm 1.0 ppm respectively to accelerate primary productivity. Water hyacinth and PVC pipe was used as shelter.

Figure 2. Pictorial view of pond preparation

Stocking cuchia fingerlings

After sufficient plankton production, Cuchia fingerlings were stocked at a density of 10 individual/m^2. Feeding and sampling is in progress.

Figure 3. Stocking of Cuchia fingerling in five (5) sampling sites.

Feeding

Fingerlings and vermi compost were fed according to design. Feed was supplied at night up to satiation level. Feed was supplied by a feeding tray to check the waste of feed.

Figure 04. Vermi compost

Water quality parameters

Water quality parameters of all the sampling sites were monitored monthly by using a multiparameter. All the water quality parameters were in a suitable range for Cuchia culture (Table 2).

Table 2. Water quality parameters in culture ponds.

Water quality parameters	Rangamati		Bandarban		Khagrachhari
	P_1	P_2	P_1	P_2	P_1
Water temp. (ºC)	25.38±2.5	23.2±1.2	22.44±1.7	25.77±1.1	24.75±1.5
DO (mg/l)	5.6±0.7	5.8±0.4	5.8±0.4	4.4±0.6	6.8±0.8
pH	8.27±0.4	8.82±0.6	7.75±0.6	7.43±0.35	8.16±0.7
TDS (mg/l)	46.5±1.5	91.3±2.6	25.0±3.2	24.4±2.3	64±2.5
Alkalinity (mg/l)	65.3±1.2	71.3±2.67	88.2±2.6	76.0±2.4	60.2±2.5
Ammonia (mg/l)	0.1±0.01	0.1±0.01	0.2±0.01	0.3±0.02	0.1±0.01
Hardness (mg/l)	58±1.6	52±1.3	22.9±2.05	35.7±1.4	55.5±2.4

Growth performance of Cuchia

Cuchia were harvested after six months of culture period by dewatering the ponds. Highest final weight, survival rate and production were recorded in Khagrachhari district, 198.5±4.6 g, 79.5±3.4% and 47.6±3.4 kg/decimal respectively. The lowest final weight, survival rate and production were recorded in Rangamati district, 183.15±4.85 g, 73.8±4.5% and 43.4±3.2 kg/decimal respectively (Table 3).

Table 3. Production performance of cuchia (*Monopterus cuchia*).

Location	Pond	Initial		Final		Survival (%)	Production (Kg/decimal)
		Length (cm)	Weight (g)	Length (cm)	Weight (g)		
Rangamati	P_1	38.2±3.6	55.7±3.3	56.5±5.7	177.2±5.3	75	42.3
	P_2	37.7±2.5	53.6±4.7	58.1±4.2	189.1±4.4	72.6	44.5
	Mean±SD	**37.95±3.7**	**54.65±2.8**	**57.3±3.5**	**183.15±4.85**	**73.8±4.5**	**43.4±3.2**
Bandarban	P_1	35.2±2.67	50.3±3.5	57.7±5.3	187.1±5.3	74.3	44.1
	P_2	33.5±4.2	49.6±2.4	57.2±6.5	183.6±4.9	73.7	44.5
	Mean±SD	**34.35±3.44**	**49.95±2.9**	**55.45±6.6**	**185.35±5.5**	**74±2.9**	**44.3±2.9**
Khagrachhari	P_1	39.3±3.8	62.4±3.9	60.9±5.4	198.5±4.6	79.5±3.4	47.6±3.4
	Mean±SD	**39.3±3.8**	**62.4±3.9**	**60.9±5.4**	**198.5±4.6**	**79.5±3.4**	**47.6±3.4**

Figure 5. Growth monitoring of *Monopterus cuchia*

Population Dynamics and Stock Assessment of Two Long-whishkered Catfish Species (*Sperata seenghala* and *Sperata aor*) in the Kaptai Lake

Researcher(s) : Md. Khaled Rahman, SO
: Md. Istiaque Haidar, SSO
: B. M. Shahinur Rahman, SSO
: Md. Lipon Mia, SO

Objectives

a) To estimate the population parameters of two long-whishkered catfish (Sperata seenghala and Sperata aor) in Kaptai lake

b) To assess the stock of two long-whishkered catfish (Sperata seenghala and Sperata aor) in Kaptai lake

Achievement (2023-24)

Estimation of population parameters of *S. seenghala*

A total of 1695 specimens of *S. seenghala* were collected from BFDC fish landing centers, local fish markets and direct catch observation survey from fishermen in Kaptai lake during the study period. Descriptive statistics on the length and weight measurements are given in Table 1. During the study period, minimum total length (9.4 cm) and maximum total length (63.4 cm) were found in October 23 and January 24 respectively. Minimum body weight (5 g) and maximum body weight (1300 g) was also found in October 23 and January 24 respectively. The lowest average total length (23.34±10.56) and weight (125.86±20.38) was found in November 23, while highest average total length (33.06±9.62) and weight (270.59±85.03) was found in April 24 and January 24.

Table 1. Length and weight data of *S. seenghala* collected from Kaptai Lake.

Month	Sex	n	Total length (cm)			Body weight (g)		
			Min	Max	Mean±SD	Min	Max	Mean±SD
Oct, 23	C	260	**9.4**	57	26.01±12.41	**5**	1300	212.3±25.62
Nov, 23	C	260	11.8	54.8	**23.34±10.56**	9	1074	**125.86±20.38**
Dec, 23	C	230	14.1	52.1	30.88±9.64	14	888	226.55±96.05
Jan, 24	C	265	14.2	**64.3**	32.14±10.22	13	**1378**	**270.59±85.03**
Feb, 24	C	267	13.1	55.6	25.43±8.72	12	1156	137.07±78.32
Mar, 24	C	225	13.8	54.2	31.96±8.05	12	1127	221.12±188.52
Apr, 24	C	148	15.9	54.1	**33.06±9.62**	20	1003	268.78±236.62
May, 24	C	40	20.6	54.3	29.49	38.4	1035	159.13±105.2
June, 24	C	-	-	-	-	-	-	-

C: Combined; n: sample size; Min: minimum; Max: maximum; cm: centimeter; g: gram

The sample size (n), regression parameters *a* and *b* of the LWRs, coefficient of determination (r^2), and growth type of *S. seenghala* are given Table 2. All relationships were highly significant (P<0.01), with r^2 values ranged from 0.921 to 0.996. The calculated coefficient *b* ranged from 2.984 (for TL) to 3.295 (for TL). The *b* values of LWR indicate that, growth pattern of *S. seenghala* are positive allometric except in October (negative).

Table 2. Descriptive statistics and estimated parameters on the length-weight relationships (BW = a×TL^b) of *S. seenghala* from the Kaptai Lake

Month	Sex	n	Regression parameter		r^2	Growth type	P value
			a	*b*			
Oct, 23	C	260	0.006	2.984	0.921	-A	*
Nov, 23	C	260	0.0041	3.09	0.992	+A	*
Dec, 23	C	230	0.0033	3.16	0.988	+A	*
Jan, 24	**C**	**265**	**0.0032**	**3.17**	**0.97**	**+A**	*****
Feb, 24	C	267	0.0035	3.14	0.986	+A	*
Mar, 24	C	225	0.0025	3.23	0.97	+A	*
Apr, 24	C	148	0.0024	3.244	0.978	+A	*
May, 24	C	40	0.0019	3.295	0.996	+A	*
June, 24	C	-	-	-	-	-	-

The relationships between length-weight, TL vs SL and TL vs FL of *S. seenghala* with the estimated parameters of the length-weight relationship, length-length relationship and the coefficient of determination r^2 are presented in Fig 1 and 2. All LWRs and LLRs were highly significant (P<0.05), with r^2 values higher than 0.95. In addition, Fig 1 revealed positive allometric growth pattern of *S. seenghala*.

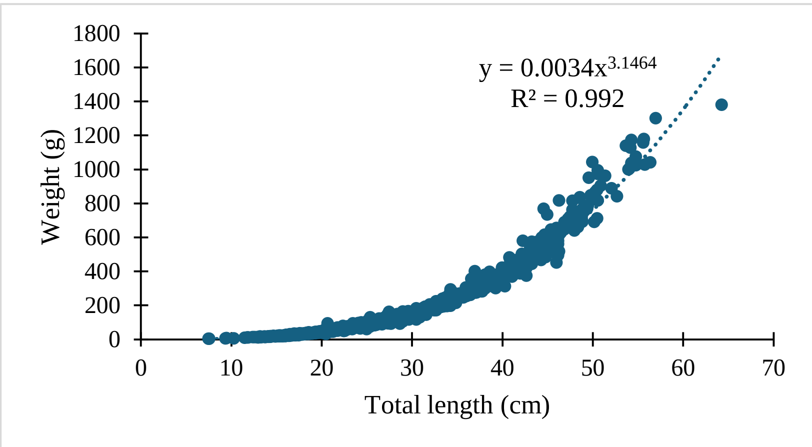


Fig 1. Length-weight relationship of *S. seenghala*

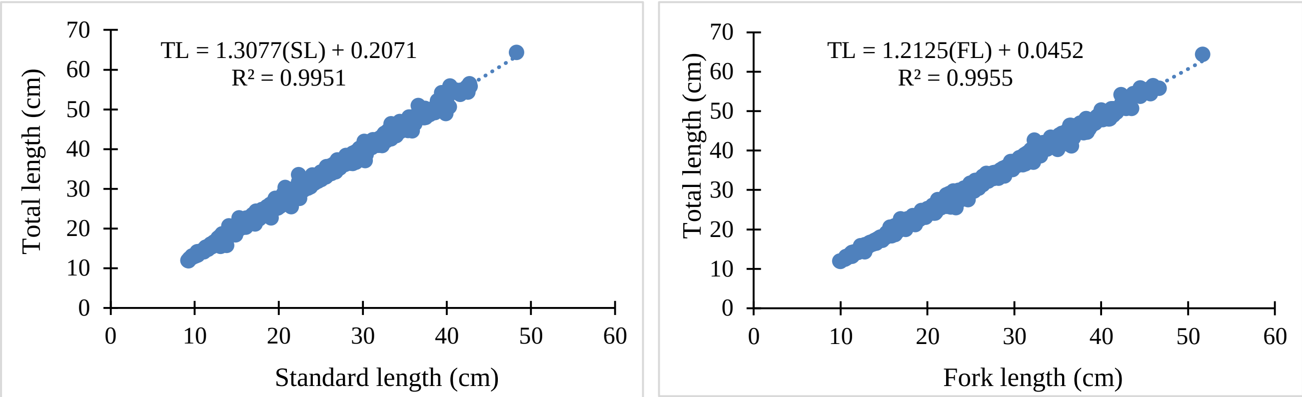


Fig 2. Length-length relationship of *S. seenghala*

Fulton's condition factor (K_F) and Relative condition factor (K_R) of *S. seenghala* during the study period are summarized in Table 3. The relative condition factor ranged from 0.48 to 1.96, lower in April 24 and higher in January 24. In addition, the estimated Fulton's condition factor ranged from 0.28 to2.68, lower in April 24 and higher in February 24.

Table 3. Fulton's (K_F) and Relative (K_R) condition factor of *S. seenghala* from the Kaptai Lake.

Month	Sex	n	Fulton's condition factor (K_F)			Relative condition factor (K_R)		
			Min	Max	Mean±SD	Min	Max	Mean±SD
Oct, 23	C	260	0.38	0.702	0.574±0.07	0.68	1.25	1.006±0.12
Nov, 23	C	260	0.46	0.77	0.547±0.064	0.83	1.39	1.01±0.11
Dec, 23	C	230	0.43	0.71	0.572±0.055	0.797	1.26	1.008±0.08
Jan, 24	C	265	0.37	1.049	**0.588±0.08**	0.67	**1.96**	**1.03±0.12**
Feb, 24	C	267	0.45	**2.68**	0.566±0.14	0.832	1.23	1.035±0.269
Mar, 24	C	225	0.35	0.708	0.544±0.06	0.614	1.18	0.987±0.09
Apr, 24	C	148	**0.28**	0.82	0.55±0.83	**0.48**	1.34	1.00±0.1
May, 24	C	40	0.43	0.65	0.51±0.05	0.87	1.2	0.99±0.07
June, 24	C	-	-	-	-	-	-	-

Estimation of population parameters of *S. aor*

A total of 1086 specimens of *S. aor* were collected from BFDC fish landing centers, local fish markets and direct catch observation survey from fishermen in Kaptai lake during the study period. Descriptive statistics on the length and weight measurements are given in Table 4. During the study period, minimum total length (7.6 cm), minimum body weight (5.1 g), lowest average total length (23.7±7.2) and weight (212.7±18.5) were found in October 23. Additionally, maximum total length (73.4 cm), and maximum body weight (1979 g) were found in January 24. The highest average total length (32.28±13.37) and weight (275±47.09) were found in April 24 and February 24, respectively.

Table 4. Length and weight data of *S. aor* collected from the Kaptai Lake

Month	Sex	n	Total length (cm)			Body weight (g)		
			Min	Max	Mean±SD	Min	Max	Mean±SD
Oct, 23	C	165	**7.6**	46.6	**23.7±7.2**	5.1	1072	**212.7±18.5**
Nov, 23	C	160	10.8	55.3	25.3±9.56	9	1023	216.6±20.7
Dec, 23	C	130	19.8	61	30.9±14.38	40	1088	255.5±26.3
Jan, 24	**C**	165	15.5	**73.4**	32.0±11.71	**19**	**1979**	268.9±32.03
Feb, 24	C	167	12.3	70.3	27.76±12.43	10	1743	**275±47.09**
Mar, 24	C	175	12.6	46.6	30.11±8.91	11.3	1162	262.7±16.2
Apr, 24	C	124	12.4	61	**32.28±13.37**	9	1094	238.98±44.45
May, 24	C	-	-	-	-	-	-	-
June, 24	C	-	-	-	-	-	-	-

C: Combined; n: sample size; Min: minimum; Max: maximum; cm: centimeter; g: gram

The sample size (n), regression parameters *a* and *b* of the LWRs, coefficient of determination (r^2), and growth type of *S. aor* are given Table 5. All relationships were highly significant (P<0.01), with r^2 values ranged from 0.974 to 0.997. The calculated coefficient *b* ranged from 2.75 (for TL) to 3.141 (for TL). The *b* values of LWR indicate that positive allometric growth was found in October 23, January and April 24, negative allometry in December 23, February and March 24 and isometric growth in November 23.

Table 5. Descriptive statistics and estimated parameters on the length-weight relationships (BW = a×TL^b) of *S. aor* from the Kaptai Lake

Month	Sex	n	Regression parameter		r^2	Growth type	P value
			a	*b*			
Oct, 23	C	165	0.004	3.141	0.996	+A	*
Nov, 23	C	160	0.0047	3.014	0.974	I	*
Dec, 23	C	130	0.0052	2.96	0.997	-A	*
Jan, 24	C	165	0.0038	3.17	0.9926	+A	*
Feb, 24	C	167	0.0063	2.9	0.9954	-A	*
Mar, 24	C	175	0.0116	2.75	0.9748	-A	*
Apr, 24	C	124	0.0034	3.0812	0.9819	+A	*
May, 24	C	-	-	-	-	-	-
June, 24	C	-	-	-	-	-	-

The relationships between length-weight, TL vs SL and TL vs FL of *S. aor* with the estimated parameters of the length-weight relationship, length-length relationship and the coefficient of determination r^2 are presented in Fig 3 and 4. All LWRs and LLRs were highly significant (P<0.05), with r^2 values higher than 0.95. In addition, Fig 3 revealed negative allometric growth pattern of *S. aor*.

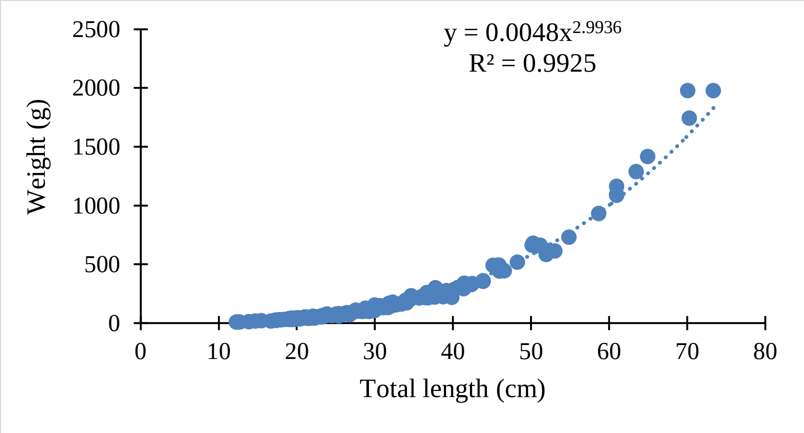


Fig. 3. Length-weight relationship of *S. aor*.

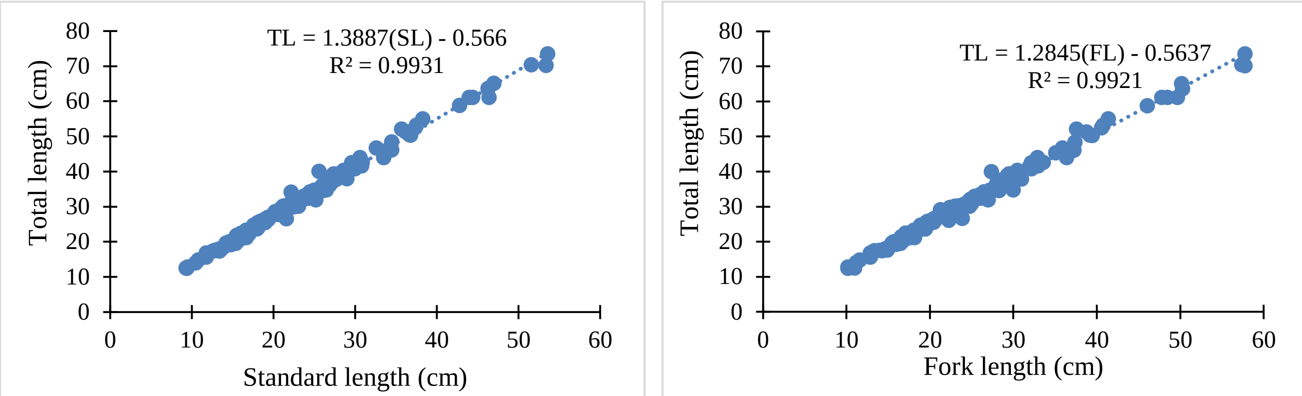


Fig. 4. Length-length relationship of *S. aor*.

Fulton's condition factor (K_F) and Relative condition factor (K_R) of *S. aor* during the study period are summarized in Table 6. The relative condition factor ranged from 0.81 to 2.01, lower in November 23 and February 24 and higher in March 24. Besides, the estimated Fulton's condition factor ranged from 0.34 to 0.71, lower in January 24 and higher in March 24.

Table 6. Fulton's (K_F) and Relative (K_R) condition factor of *S. aor* from the Kaptai Lake.

Month	Sex	n	Fulton's condition factor (K_F)			Relative condition factor (K_R)		
			Min	Max	Mean±SD	Min	Max	Mean±SD
Oct, 23	C	165	0.38	0.69	0.498±0.1	0.87	1.11	1.06±0.12
Nov, 23	C	160	0.47	0.67	0.547±0.041	**0.81**	1.39	1.02±0.14
Dec, 23	C	130	0.46	0.62	0.52±0.06	0.87	1.6	1.09±0.08
Jan, 24	C	165	**0.34**	0.57	0.47±0.04	0.89	0.87	0.71±0.07
Feb, 24	C	167	0.37	0.55	0.55±0.05	**0.81**	1.23	1.004±0.269
Mar, 24	C	175	0.39	**0.71**	0.554±0.08	0.83	**2.01**	1.007±0.186
Apr, 24	C	124	0.38	0.62	0.45±0.06	0.86	1.35	1.02±0.13
May, 24	C	-	-	-	-	-	-	-
June, 24	C	-	-	-	-	-	-	-

Population Dynamics of important fish and shell fishes in the Sundarbans Mangrove of Bangladesh

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: Md. Abu Naser, SO
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Objectives:

1. To assess the abundance and to estimate growth parameters of important fish and shell fish species;
2. To calculate the mortality rate and exploitation level of selected species;
3. To identify vulnerable size groups of a fish species in the Sundarbans;
4. To assess ichthyofaunal diversity in the major river of the Sundarbans; and
5. To recommend some fish stock management measures on the basis of stock assessment.

Achievement in the past (2023-24):

Experiment/study-1: Estimation of abundance and growth parameters of selected fish/crustacean species in the Sundarbans mangrove river

Site selection

The Sundarbans mangrove territory consist of three districts namely Bagerhat, Khulna and Shatkhira. The forest lies a little south to the Tropic of Cancer between the latitudes 21°30'N and 22°30'N, and longitudes 89°00'E and 89°55'E (Figure 1). A total of 13 major rivers flow through the Sundarbans and met to the Bay of Bengal. Among 13 rivers, the Shibsha, the Arpangasia (lower stream of the Kholpetua) and the Pashur river of Khulna, Satkhira and Bagerhat district, respectively were selected for sampling. Single sampling station/spot from each river was chosen and those are, Hodda in the Sibsha river; Nildumur and Gabura at the joint between the Arpangasia and the Kholpetua river, and Karamjol spot of the Pashur river (Figure 1).

Species selection

At this phase, three commercially important species were considered from two groups i.e., fish and mollusk of the mangrove aquatic species. From the groups, white grunter (*Pomadasys hasta*), ramcarat grenadier anchovy (*Coilia ramcarati*) and telescope snail (*Telescopium Telescopium*) were opted.

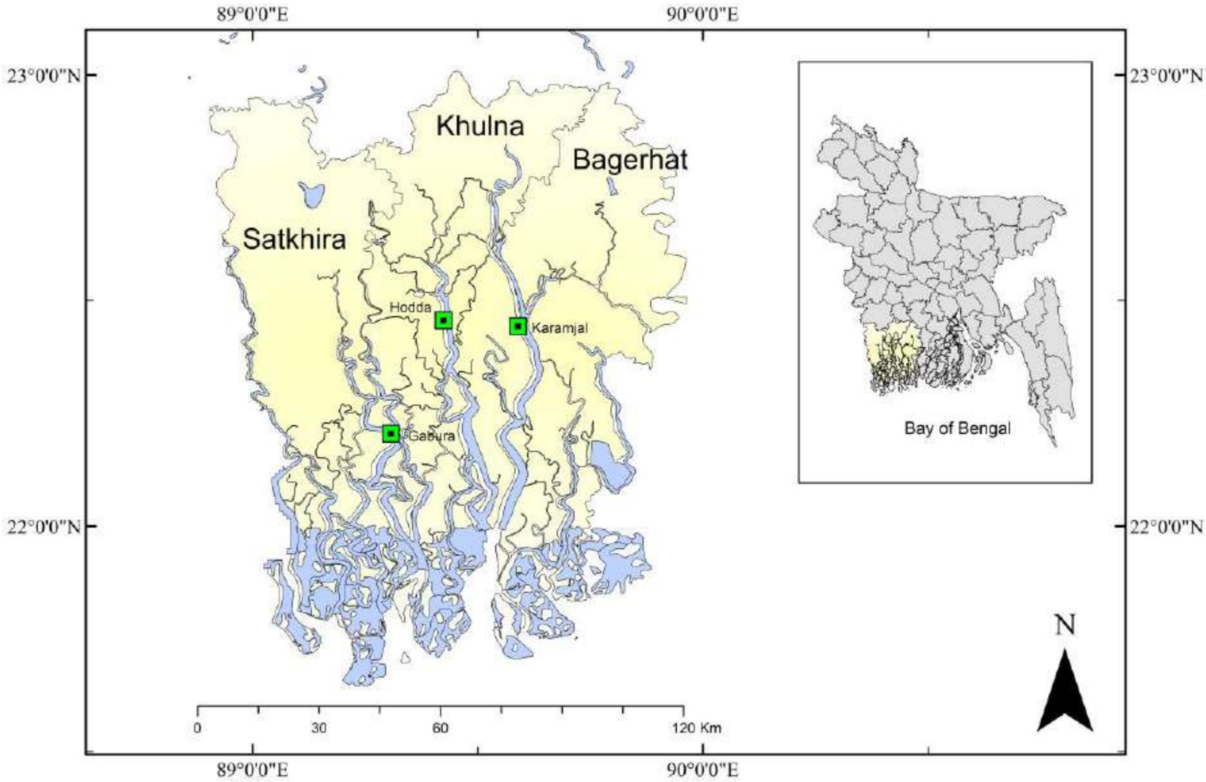

Figure 1: The sampling sites in the map

According to the responses of stakeholders some species have appeared for conducting study on population dynamics in the Sundarbans fisheries. In 2023-24, above mentioned species were considered such as white grunter (*P. hasta*), ramcarat grenadier anchovy (*C. ramcarati*) and telescope snail (*T. Telescopium*) for the assessment to get the important population parameters. In 2023-24, baseline data were collected regarding the three species from different literatures and respondents of FGDs (PAR).

Sampling procedure and frequency

Sampling has been done monthly basis either during full moon or during new moon period (considering lunar cycle) for a period of 12 months from July 2023 to June 2024 using three types of gear such as Hooks, nets and traps were mostly used in this area for harvesting of fish and shell-fish. A day long fishing operation was operated for understating the abundance at rivers in the Sundarbans.

Total length (TL) in cm and total body weight (BW) in g for each individual was measured using measuring scale and an electronic balance, respectively. For shellfishes, total length estimation were observed as shell length with a vernial scale. We calculated Length-Weight Relationship (LWR) using the equation:

$BW=a\ xTL^b$ …..…………………………..………………….…………………………….. (1)

Where, BW is the total body weight (g), and TL is the total length (cm). The estimation of parameters a and b was done by linear regression analyses, which follows equation such as ln(W) = ln(a) + bln(L). Additionally, 95% confidence interval was calculated for parameters a and b. We were also calculated the coefficient of determination (r^2). Regression analyses were performed to eliminate outliers (Froese, 2006). Statistical Product and Service Solution (SPSS) software was used to perform statistical analyses. The statistical difference from the isometric value (b = 3) for LWRs were determined by t-test. All statistical analyses were considered at 5% significance level ($P < 0.05$).

Generally, catch per unit effort (CPUE) is estimated by dividing annual fish landing amount by a total number of fishing trips in a year. Moreover, CPUE can also be calculated by considering fishing days and vessel numbers. CPUE is one of the important indices of species abundance (Chen and Chiu, 2009). However, it is not a firm indicator of stock abundance since it can be influenced by some factors (Harley et al., 2001). Usually, these factors affect fish harvest from the sea during fishing operation (Maunder et al., 2006). Like other factors, vessel's capacity in gross registered tonnage (GRT) was found as a significant contributor to CPUE (Parente, 2004). In this study, a standard formula was used to estimate the abundance of a species as follows-

$$C_t = P_t / T_t \quad \ldots\ldots\ldots\ldots (2)$$

Where, C is the catch per unit effort (CPUE) for fish species ($gm.day^{-1}.person^{-1}$). C_t is the CPUE for the year t. P_t represents fish catch for a particular season t. T_t indicates number of days of fishing with a particular fishing craft in the same season t.

Incorporated ELEFAN-I (Electronic Length Frequency Analysis) in FiSAT-II program was assigned to estimate the value of asymptotic length and growth co-efficient (K) from formula (3) of the von Bertalanffy;

$$L_t = L_\propto \left(1 - e^{-K(t-t_0)}\right) \quad \ldots\ldots\ldots\ldots (3)$$

Where, t indicates the age of a fish species (yr), L is the mean total length at age t (cm), t_0 is the hypothetical age when L is zero, K represents a growth coefficient (yr^{-1}).

From K and $L_\propto$ the Growth performance index (ϕ') of species were derived according to the formula of Pauly and Munro (1984);

$$\phi' = \text{Log } K + 2 \text{ Log } L_\propto \quad \ldots\ldots\ldots\ldots (4)$$

This section was concluded with some finding such as Abundance, length-weight relationship, asymptotic length, growth coefficient, growth performance index of a stock and status of stock.

Length-Weight Relationship

White grunter (*Pomadasys hasta*)

The relationship between total length (TL) and body weight (BW) of *P. hasta* for both sexes has been displayed in Table 1. Logarithmic form of the equation ($BW = a \times TL^b$) was considered to establish TL-BW relationship.

Table 1. TL-BW association of sampled *P. hasta* from the Sundarbans of Bangldesh

Species	Size (N)	*a*	*b*	*r*	R^2	Allometry	p-Value
P. hasta	1012	0.017	3.04	0.90	0.99	Isomerism	0.00

All values of total lengths were plotted against the values of respective body weights to complete the scatter diagram for getting a curvilinear relationship (Figure 2). Parabolic curves were made by plotting the calculated value of the body weight against the total length of *P. hasta*. In contrast, the values of log total TL against their log calculated BW were plotted to get a linear line.

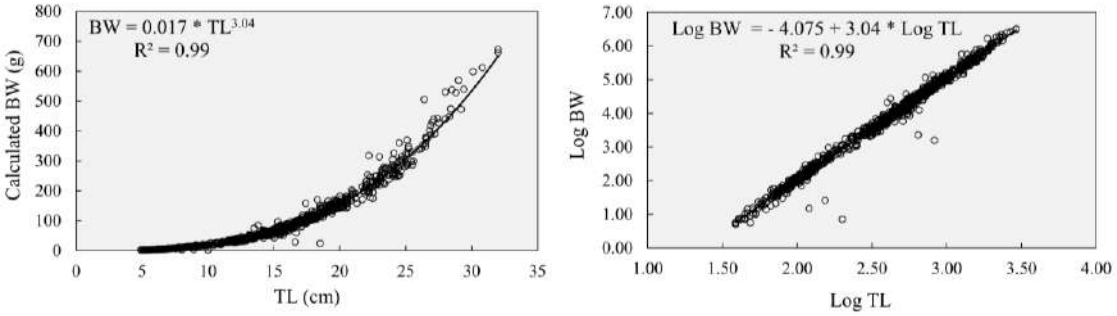

Figure 2. The relationship between Total lenth (TL) and body weight (BW) of *P. hasta* in the Sundarbans mangrove forest of Bangladesh

The estimated *b* value was calculated as 3.04. *P. hasta* showed isometric growth. The Pearson correlation co-efficient (*r*) values were estimated as 0.90 for *P. hasta*. It indicates highly significant relationships (p = 0.00) between TL and BW of this species.

Ramcarat grenadier anchovy (*Coilia ramcarati*)

In Table 2, the association between total length (TL) and body weight (BW) of *C. ramcarati* has been shown. A TW-BW relationship was established in a form of the equation, BW = $a \times TL^b$. All values of total lengths (TL) were plotted against the values of respective body weights (BW) to complete the scatter diagram for getting a curvilinear line (Figure 3). Parabolic curves were made by plotting the calculated value of the body weight against the total length of *C. ramcarati*. In contrast, the values of log total TL against their log calculated BW were plotted to get linear lines.

Table 2. TL-BW association of sampled *C. ramcarati* from the Sundarbans of Bangldesh

Species	Size (N)	*a*	*b*	*r*	R^2	Allometry	p-Value
C. ramcarati	2151	0.0023	3.11	0.93	0.95	Positive	0.00

The number of total sampled *C. ramcarati* was 2151. The estimated *b* value was calculated as 3.11. This species showed positive allometry growth but almost isometric. The Pearson correlation co-efficient (*r*) value was estimated as 0.93. It reveals highly positive and significant relationships (p = 0.00) between TL and BW of this species.

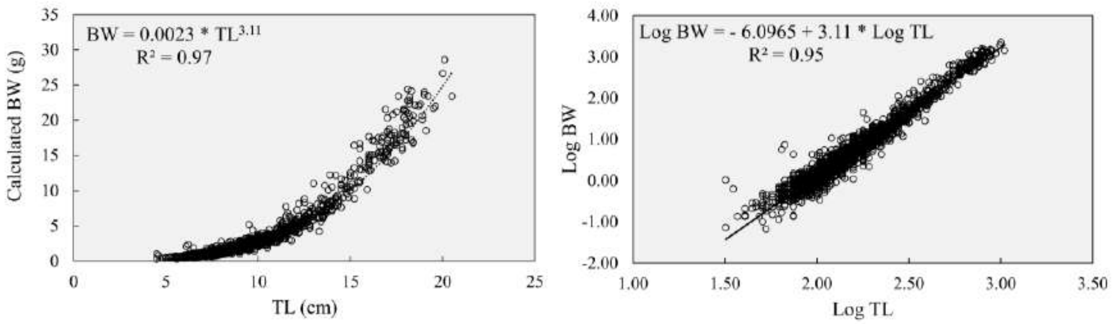

Figure 3. The relationship between Total lenth (TL) and body weight (BW) of *C. ramcarati* in the Sundarbans mangrove forest of Bangladesh

Telescope snail (*Telescopium telescopium*)

The relationship between shell length (SL) and body weight (BW) of *T. Telescopium* has been displayed in Table 3. Logarithmic form of the equation (BW = $a \times SL^b$) was considered to establish

SL-BW relationship. All values of shell length were plotted against the values of respective body weights to complete the scatter diagram for getting a curvilinear relationship (Figure 4). Parabolic curves were made by plotting the calculated value of the body weight against the shell length of the *T. Telescopium.* In contrast, the values of log total SL against their log calculated BW were plotted to get a linear line.

Table 3. SL-BW association of sampled *T. Telescopium* from the Sundarbans of Bangldesh

Species	Size (N)	*a*	*b*	*r*	R^2	Allometry	p-Value
T. Telescopium	3411	0.24	2.45	0.92	0.95	Negative	0.00

The number of sampled *T. Telescopium* was 4019. The estimated *b* value was 2.45 for the species. *T. Telescopium* showed negative growth allometry. The Pearson correlation co-efficient (*r*) values were estimated as 0.92 for *T. Telescopium*. It showed a positive and highly significant relationships (p = 0.00) between SL and BW of this species.

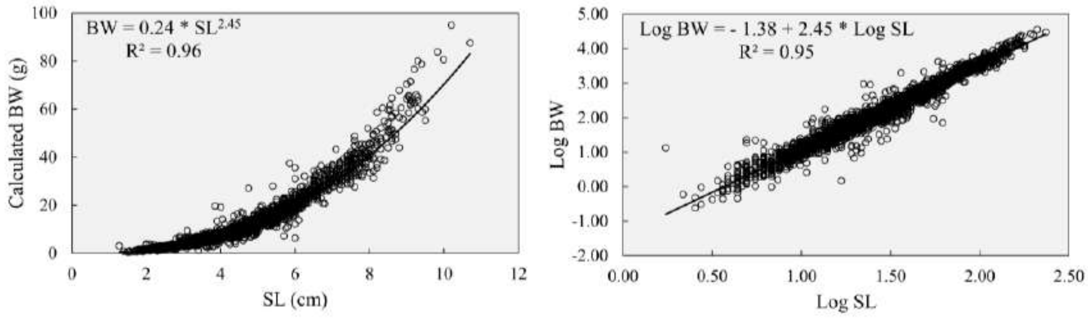

Figure 4. The relationship between shell length (SL) and body weight (BW) of *T. Telescopium* in the Sundarbans mangrove forest of Bangladesh

Species-wise abundance

Abundance of White grunter (*P. hasta*), Ramcarat grenadier anchovy (*C. ramcarati*) and Telescope snail (*T. telescopium*) were 1,506.66 gm, 1,330.00 gm and 22,210.00 gm per person per day (Table 4).

Table 4. Species-wise abundance in the Sundarbans

Species	CPUE (weight/person/day)
White grunter (*P. hasta*)	1,506.66 gm/person/day
Ramcarat grenadier anchovy (*C. ramcarati*)	1,330.00 gm/person/day
Telescope snail (*T. telescopium*)	22,210.00 gm/person/day

Growth parameters

The von Bertalenffy asymptotic lengths were 34.65 cm, 22.05 cm and 11.55 cm, however, the K were 0.78 yr^{-1}, 0.74 yr^{-1} 0.56 yr^{-1} for White grunter (*P. hasta*), Ramcarat grenadier anchovy (*C. ramcarati*) and Telescope snail (*T. telescopium*), respectively (Table 5). The estimated growth performance index (ϕ') of White grunter, Ramcarat grenadier anchovy and Telescope snail were observed to be 2.97, 2.55, and 1.87.

Table 5. Growth parameters of selected species from the Sundarbans

Species	L_∞	K	ϕ'
White grunter (*P. hasta*)	34.65	0.78	2.97
Ramcarat grenadier anchovy (*C. ramcarati*)	22.05	0.74	2.55
Telescope snail (*T. telescopium*)	11.55	0.56	1.87

Experiment/Study-2:

Estimation of mortality rates and exploitable level of the selected species

Mortality is a key component for understanding the population dynamics of fish species. Total mortality is often estimated from the sequential decline observed in cohorts of fish. Length converted catch curve method of Beverton and Holt (1956) was applied to determine total mortality (Z). The formula of the total mortality as follows;

Z = F/M………...……………………………………………………………………… (5)

Where, Z indicates total mortality of the stock, F is the fishing mortality and M is the natural mortality. Natural mortality is the removal of fish from the stock due to causes not associated with fishing. Such causes can include disease, competition, cannibalism, old age, predation, pollution or any other natural factor that causes the death of fish. In fisheries model's natural mortality is denoted by (M). Natural mortality (M) was estimated according to Pauly (1980) as follows in the formula 6;

$\log10M = -0.0066 - 0.279 \log10L\infty + 0.6543 \log10K + 0.4634 \log10T$ ……………..…..….. (6)

where, M indicates natural mortality of the stock, L∞ is the asymptotic length of a species, K is the growth co-efficient and T is the habitat temperature. However, fishing mortality rate is the proportion of a fish stock removed by fishing (as opposed to predation or other causes of death). By following formula, we estimated the fishing mortality;

Fishing mortality F = Z − M ……………………....…………..……………….……….(7)

Applied on a fish stock, it is the proportion of the numbers or biomass removed by fishing. A 10% exploitation rate means that 10% of the available stock is being harvested within the time frame considered (per year, per month, etc.). As a measure of fishing pressure, it is proportional to fishing mortality. Exploitation rate (E) were calculated as follows:

Exploitation rate E = F/Z ...………………………....………………………………..(8)

Besides, the relative yield-per-recruit (Y/R) and relative biomass-per-recruit (B/R) was estimated by using the model of Beverton and Holt (1966) as modified by Pauly and Soriano (1986) and incorporated in FiSAT software package, to get E_{max}.

Mortality and exploitation

The total mortality (Z) of White grunter (*P. hasta*), Ramcarat grenadier anchovy (*C. ramcarati*) and Telescope snail (*T. telescopium*) were estimated as 3.00 yr^{-1}, 3.66 yr^{-1}, and 2.84 yr^{-1}, respectively, by using length converted catch curve analysis (Table 6). Fishing mortalities (F) were 1.65 yr^{-1}, 2.18 yr^{-1}, and 1.36 yr^{-1} for White grunter, Ramcarat grenadier anchovy and Telescope snail, respectively.

Table 6. Mortalities and exploitations of selected species from the Sundarbans.

Species	Z	M	F	E	E_{max}
White grunter (*P. hasta*)	3.00	1.35	1.65	0.55	0.61
Ramcarat grenadier anchovy (*C. ramcarati*)	3.66	1.48	2.18	0.60	0.58
Telescope snail (*T. telescopium*)	2.84	1.48	1.36	0.48	0.60

In contrast, natural mortalities (M) of White grunter (*P. hasta*), Ramcarat grenadier anchovy (*C. ramcarati*) and Telescope snail (*T. telescopium*) were calculated as 1.35 yr^{-1}, 1.48 yr^{-1}, and 1.48 yr^{-1}, respectively. Thus, expoitation rate (E) of White grunter, Ramcarat grenadier anchovy and Telescope snail were computed as 0.55, 0.60, and 0.48, respectively (Table 6). The maximum permissible limit of exploitation (E_{max}) values were calculated as 0.61, 0.58, and 0.60 for White grunter (*P. hasta*), Ramcarat grenadier anchovy (*C. ramcarati*) and Telescope snail (*T. telescopium*), respectively. Apparently, ramcarat grenadier anchovy (*C. ramcarati*) was not only exceeded biological reference point but also exceeded the maximum permissible limit of expoitation (E_{max}). Besides, White grunter (*P. hasta*) exceeded biological reference point (E= 0.50).

Experiment/Study-3:

Probability of capture

Probability of capture calculated from the length-converted catch curve routine was used to estimate the final values of L_{25}, L_{50} and L_{75} i.e., lengths at which 25%, 50% and 75% of the fish would be vulnerable to the different gears such as different nets, long lines and traps for a specific species (Pauly, 1984).

Virtual population analysis

Virtual population analysis (VPA) is a cohort modeling technique commonly used in fisheries science for reconstructing historical fish numbers at age using information on death of individuals each year. This death is usually partitioned into catch by fisheries and natural mortality. VPA is the virtual in a sense that the population size is not observed or measured directly but is inferred or back-calculated to have been a certain size in the past in order to support the observed fish catches and an assumed death rate owing to non-fishery related causes.

Virtual population analysis was introduced in fish stock assessment by Gulland in 1965 based on older work. The technique of cohort reconstruction in fish populations has been attributed to several different workers including Professor Baranov from Russia in 1918 for his development of the continuous catch equation, Professor Fry from Canada in 1949 and Drs. Beverton and Holt from the UK in 1957. Because cohort reconstruction is essentially an accounting exercise it was likely independently conceived many times. The virtual population analysis (VPA) was employed to estimate the extent of mortality on various size classes of a species. The fishing pressure on a particular sized fish species was indicated against the number of anticipated population (Figure 5).

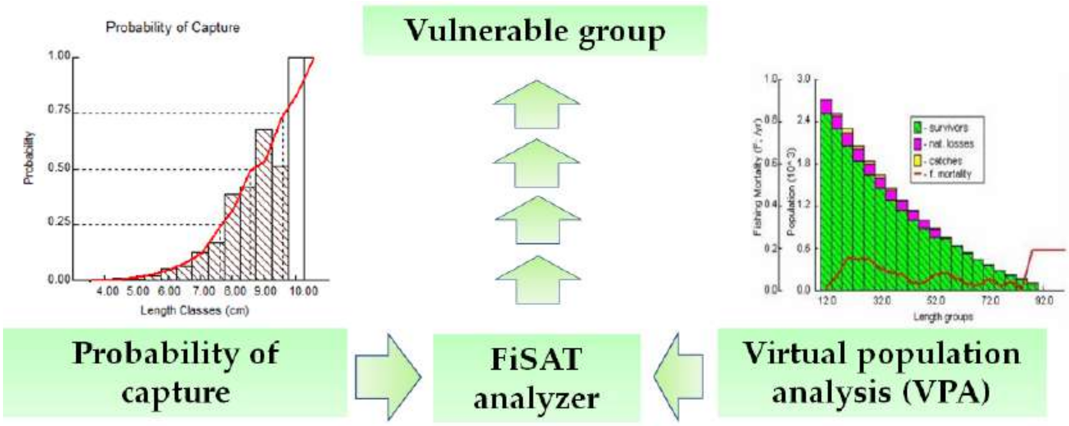

Figure 5. Strategies to calculate vulnerable size group of a species

Vulnerable size groups

Probability of capture is one of the very useful drivers in stock assessment of fisheries science. It shows the vulnerability of different sizes of fin fish and shellfish to different gears in a given location at a given time. The probabilities of capture analysis for white grunter found that 25% of 10.81 cm

TL, 50% of 13.53 cm TL and 75% of 16.25 cm TL were vulnerable to the gears. Therefore, it can be assumed that more than half of the harvested *P. hasta* remained between the total length of 10.81 cm and 16.25 cm. In addition, VPA results reveals that a maximum fishing pressure on *P. hasta* population was found between total length groups of 29.01-33.0 cm.

Similarly, probabilities of capture analysis for *C. ramcarati* depicted that 25% of 7.24 cm TL, 50% of 7.74 cm TL and 75% of 8.25 cm TL were vulnerable to the gears. Therefore, it can be assumed that more than half of the harvested fishes remained between the length of 7.24 cm and 8.25 cm. The high values of F for the species occurred within first length group, ranging from 9.01 cm to 11.00 cm and second length group, ranging from 17.01 cm to 21.00 cm.

Again, the probabilities of capture analysis for *T. Telescopium* showed that 25% of 3.34 cm SL, 50% of 3.82 cm SL and 75% of 4.31 cm SL were vulnerable to the gears. Therefore, it can be assumed that more than half of the harvested Telescope snail remained between the shell length of 3.34 cm and 4.31 cm. According to VPA analysis, the higher values of F than values of M were not found.

Experiment/Study-4:

Assessing ichthyofaunal diversity in the major river of the Sundarbans

Identification of Ichthyofauna

Ichthyofauna was collected from the study area and was identified based on their morphometric and meristic characters.

Calculation of Biodiversity indices of Ichthyofauna

To understand the seasonal diversity of fishes in the study area, month-wise data was collected. Shannon-Weiner Diversity Index (H) (Shannon and Weaver, 1949) was calculated as:

$$H = -\sum P_i \times \ln P_i$$

Richness Index (D) was computed according to formula of Margalef (1968):

$$D = \frac{s-1}{\ln N}$$

In addition, Evenness Index (e) values was obtained by following a formula of Pielou (1966):

$$e = \frac{H}{\ln S}$$

Where, H is the diversity index, Pi is the relative abundance (s/N), s is the number of individuals for each species, N is total number of individuals, D is the richness index, S is the total number of species, e is the similarity or evenness index and ln is the natural logarithm.

Diversity status

As observed, Shannon-Wiener (H) value (2.52 ± 0.31) indicates the moderate status of ichthyofaunal diversity in the wild habitat of the sundarbans. Whereas, Margalef's Richness index (3.34 ± 0.81) revealed the semi distributed and Pielou's evenness index (0.77 ± 0.06) showed the semi balanced species in that community in the study area (Table 7).

Table 7. Diversity indices of Shannon-Wiener (H), Margalef's Richness (D) and Pielou's evenness (e) for ichthyofauna community of the Sundarbans

2023-24	Oct	Nov	Dec	Jan	Feb	Mar	April	May	June	Mean ± SD
H	2.95	2.93	2.56	2.62	1.98	2.23	2.56	2.61	2.26	2.52 ± 0.31
D	4.19	4.75	3.71	3.38	2.68	3.16	3.14	3.15	1.94	3.34 ± 0.81
e	0.83	0.79	0.75	0.80	0.63	0.70	0.79	0.82	0.83	0.77 ± 0.06

Discussion and conclusion

The Sundarbans mangrove forest, known as the largest mangrove forest in the world, is one of the key elements of Bangladesh's coastline. The forests contribute significantly to the nation's economy and give the locals a means of subsistence through fishing, tourism, and the production of wood and non-wood products. The mangroves and nearby tributaries are home to artisanal fisheries, which use a variety of traditional fishing techniques and tools. The coastal regions are where these fisheries activities are most frequently carried out to capture fish. Because so many fish and crustaceans depend on mangroves to complete their life cycles, mangroves contribute to greater fisheries biodiversity.

Despite numerous laws, policies, and management plans, there are now obvious signs of forest degradation. The Sundarbans mangrove regions have recently seen the discovery of numerous new fish species. However, the fisheries stakeholders are completely unaware of the status of the stocks of these identified fish species. In order to better plan how to use the resource in a sustainable way, fisheries stock assessment is a crucial tool. The southern region of Bangladesh relies heavily on the fisheries industry for a living. As a result, people engaged in direct fishing in order to supply the demand for various goods on both domestic and foreign markets. As a result, the fishery for different fish populations may collapse soon. Consequently, a fish stock assessment was done to address the issue of determining the status of the populations in the mangrove regions.

The length-weight and length-frequency data were gathered from various areas of the Sundarban mangrove forest to assess growth parameters, mortality rates, and exploitation levels in order to estimate population parameters for three species (*P. hasta*, *C. ramcarati*, and *T. Telescopium*). For the analysis and estimation of each parameter, we used data spanning a full year. Except for the telescope snail (negative allometric), all species exhibited growth that was almost isometric. For *P. hasta* and *C. ramcarati*, a few particular groups have been named as vulnerable groups. With the exception of Telescope snail, the populations of the two species were described as being overfished in Bangladesh's Sundarbans. In summary, it is assumed that the moderate status of ichthyofaunal diversity is available in the wild habitat of the Sundarbans.


Plate 1: Visit to sampling site


Plate 2: Gears for sampling


Plate 3: Sample landed


Plate 4: Sample purchasing


Plate 5: Data collecting


Plate 6: Record keeping

Potentiality of aquatic weed as alternative feed ingredients for the development of cost-effective fish feed for coastal aquaculture

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Saima Sultana Sonia, SO

Objectives:

- To investigate the status of available aquatic weed in South-west region and make inventory based on morphometry and DNA barcode anY alysis
- To observe the nutritional status (proximate composition, macro & micro elements) of important aquatic weed
- To examine the potentials of explored weed as dietary ingredients in fish feed.

Achievement in the past: (2023-24)

Experiment-1: Formulation of juvenile Nile tilapia diet applying different proportion of aquatic weeds.

Experimental design and methodology

A total of 4 experimental diet containing iso-protein (30% crude protein) was prepared of which 3 diet were formulated with weed at 5%, 10% and 15% inclusion level. Rest one was controlled diet where no weed ingredient was included. Diet was prepared targeting protein replacement by weed (*Chara baltica*). The percentage of different ingredients for diet formulation has been displayed in Table 1. Feed was prepared in the form of pellets (2mm, diameter) by well mixing of ingredients using a laboratory pellet machine. The pellet was air dried and stored in plastic zipper bags at room temperature until feeding trial. Overall experimental feed formulation design has been shown below in Table 1.

Table 1. Formulation of juvenile Nile tilapia diet with different proportion of weed

Ingredients	Control Diet	FM Replaced with 5% Chara	FM Replaced with 10% Chara	FM Replaced with 15% Chara
Fish Meal	20.00	19.00	18.00	17.00
Chara	0.00	1.00	2.00	3.00
Soybean	36.20	37.25	38.35	39.60
Rice Bran	36.90	35.65	34.35	32.80
Flour	5.00	5.00	5.00	5.00
Veg Oil	0.00	0.20	0.40	0.70
Vit	0.50	0.50	0.50	0.50
Min	0.50	0.50	0.50	0.50
Limestone	0.90	0.90	0.90	0.90
Total %	100.00	100.00	100.00	100.00
Protein%	**30.00**	**30.00**	**30.00**	**30.00**

Experiment-2: Characterization of formulated feed with or without aquatic weed inclusion.

Experimental design and methodology:

Evaluation criteria: Characterization was evaluated by following criterion.

Analysis of biochemical composition

a) Proximate composition

b) Amino acid profiling

c) Fatty acid profiling

Proximate composition was analyzed in Fish and nutrition lab, Freshwater station, BFRI. Amino acid fatty acid was estimated at BCSIR, Dhaka.

Results:

Experiment-1: Formulation of juvenile Nile tilapia diet applying different proportion of aquatic weeds.

Feed has been prepared according to selected formula (Table 1). There was some modification with the help of expert nutritionist panel. All diet was formulated fixing 30% protein level. We replaced weed at suggested level of PP. In formulated feed, weed inoculation level was 5%, 10%, 15% and 0% in Diet D(5), D(10), D(15) and control, respectively. Then we prepared and stored feed in room temperature with proper protocol. Feed preparation formula has been shown in Table 1.

Experiment-2: Characterization of formulated feed with or without aquatic weed inclusion.

a) Proximate composition

In the present study, all prepared diet was tested for proximate composition. Surprisingly all diet protein composition was almost near to our targeted protein (30%). Diet D(15) had highest protein percent. Here lipid were 9-11% where highest lipid was 11.16 % in D(10). Ash content varied from 12-15%. Moisture content ranged between 11 to 12%, where highest moisture was 12.56% in D(5), (Table 2).

Table 2. Proximate composition of formulated feed/diet

Code	% moisture	% crude lipid	% crude protein	% Ash
Control	10.52	10.25	30.00	12.72
D(5)	12.56	9.06	29.98	13.18
D(10)	11.85	11.16	29.99	14.19
D(15)	11.48	10.28	30.00	15.01

Experiment-3: Evaluation of formulated feed with or without addition of aquatic weed in culturing juvenile Nile tilapia

To evaluate the performance of formulated feed on cultured fish the following biological criteria was studied:

Analysis of growth performance

The growth assessment variables such as total production, stocking and harvesting weight, net weight gain (NWG), absolute growth rate (AGR), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR) and cost benefit analysis (CBR) was considered for comparative evaluation.

Feed efficiency indicators: Following indicators was calculated chronologically-

Feed efficiency (FE): was calculated using the formula below:

$$\mathbf{FE} = \frac{\textbf{Daily weight gain}}{\textbf{Feed intake}}$$

Feed intake (FI) was calculated on a daily basis as the total amount of feed per tank divided by the number of fish in the tank.

Protein conversion ratio (PCR): It is the ratio of feed protein to net harvest biomass. It is calculated by multiplying FCR by the proportion of crude protein in feed as shown below:

$$\mathbf{PCR} = \mathbf{FCR} \times \frac{\textbf{Feed crude protein content (\%)}}{\mathbf{100}}$$

Protein efficiency (PE): Protein efficiency is estimated by multiplying FCR by the ratio of crude protein percentage in feed to that in the culture species as shown below:

$$\mathbf{P.E.} = \mathbf{FCR} \times \frac{\textbf{Feed crude protein content (\%)}}{\textbf{culture species crude protein content (\%)}}$$

Fishmeal conversion ratio (FMCR): It is the ratio of fishmeal in feed to net harvest biomass. It was calculated as follows:

$$\mathbf{FMCR} = \mathbf{FCR} \times \frac{\textbf{Fishmeal in feed (\%)}}{\mathbf{100}}$$

Apparent protein efficiency ratio (APER):

$$\mathbf{APER} = \frac{\textbf{Live weight gain}}{\textbf{Dry weight of crude protein fed}}$$

Hepatosomatic index (HSI): It was calculated by following formula

$$\mathbf{HIS} = \frac{\textbf{Liver weight}}{\mathbf{FBW}} \times \mathbf{100}$$

Viscerosomatic index (VSI): The net amount of filet produced was also monitored closely.

$$\mathbf{VSI} = \frac{\textbf{weight of animal viscera}}{\textbf{FBWanimal weight}}$$

Analysis of fish body composition: Experimental fish (Juvenile Nile Tilpia) carcass composition (crude protein, crude fat, CHO, ash, etc.) was investigated accordingly before and after culture trials.

Immunological response: Following haemato-biochemical parameters was diagnosed such as THC, RBC, WBC, Haemoglobin (Hb), heterophils, lymphocytes and monocytes was counted following standard procedure (Anderson and Siwicki, 1995).

Results:

Experiment-3: Evaluation of formulated feed with or without addition of aquatic weed in culturing juvenile Nile tilapia

A 30 days feeding trial was applied with formulated diet. In this experiment, there were D(5), D(10), D(15) and control diet was tested with three replications of each diet. Stocking density was 20 pcs/60L. Feeding rate was 3% body weight and feeding frequency was twice in a day. In all treatments, average survival rate was 92%. Highest survival was in Diet D(15). Regular water quality was observed. After ending of trial the final results are shown in the Table 2.

Table 3. Growth performance of juvenile Nile tilapia diet using weed

Parameters	Diet			
	D(5)	D(10)	D(15)	Control
Average Initial wt (g)	17.91±.18	17.33±.34	17.37±.88	17.07±.58
Average Final wt (g)	23.59±2.32	25.01±.67	25.24±3.12	20.59±0.89
Weight gain (g)	5.68	7.67	7.86	3.51
Daily wt gain(g)	0.78	0.83	0.84	0.68
Percent WG	31.72	44.28	45.27	20.60
SGR	0.009	0.0122	0.012	0.006
HSI	3.05	3.05	2.73	5.58
VSI	4.40	4.11	4.11	7.96
FCR	2.3	2.5	**1.8**	2.6
PER	1.44	1.33	1.85	1.28
FMCR	0.00437	0.0045	0.00306	0.0052
FE	0.0144	0.0133	0.0185	0.0128

In this experiment initial body weight was around 17 g. After trial, height final body weight 25.24±3.12 g was found in diet D (15). Weight gain rate, daily weight gain rate, PWG, SGR was also high in diet D(15). Surprisingly, HSI and VSI was high in control where no weed were included. Lowest FCR 1.8 and highest PER 1.85 was in D (15). In control FMCR rate was high where FE was high in D (15).

Immunological response

Haematological report

In this experiment baseline or preliminary haemoglobin was 3.8 g/dl. At the end of experiment, haemoglobin per cent increased in all diet fed. Highest haemoglobin was 9.2 g/dl in D(5). WBC, Lymphocytes, Neutrophils were almost same for all. RBC was high in diet D (5) 1.49 $\times 10^{12}$ /l.

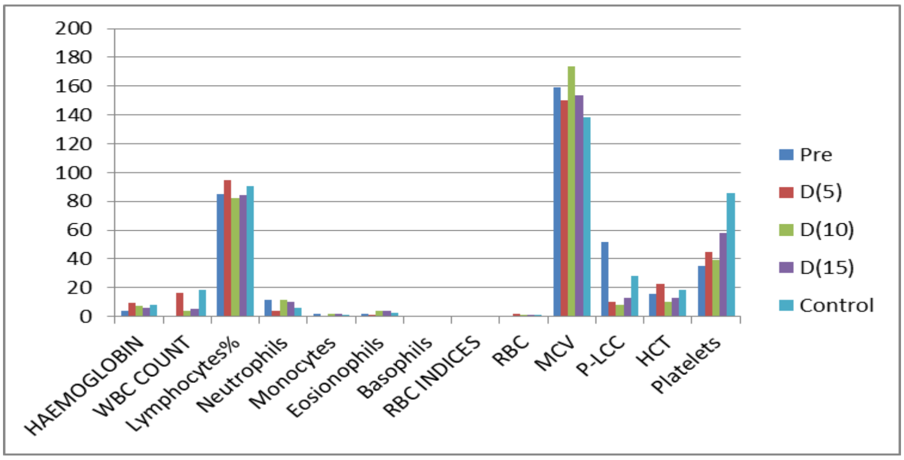


Fig 1: Haematological report of tilapia

Biochemical Test

In biochemical test (Table 5), blood sugar level, S. Creatinine, SGPT, SGOT was comparatively low at baseline or before experiment. Here cholesterol level was almost 0 for all diet except for d(15) which was 0.1 mg/dl. Triglyceride and Albumin level was high in D (15).

Table 4. Blood bio-chemical parameters of juvenile Nile tilapia fed with different diets

NAME OF TEST	Pre	D(5)	D(10)	D(15)	Control	Unit
Blood Sugar	4.3	2.53	2.04	3.61	3.57	mmol/L
S. Creatinine	0.36	0.24	0.16	0.14	0.19	mg/dl
SGPT (ALT)	170.4	49.84	14.06	11.5	15.83	U/L
SGOT (AST)	1.22	.4	0.66	0.99	1.17	U/L
S.Cholesterol	0.0	00	00	0.1	00	mg/dl
S. Triglyceride	1.19	1.38	1.22	1.39	1.6	mg/dl
S. Calcium	0.01	.007	.006	.003	.004	mg/dl
Total protein	0.57	0	0.011	0.7	0.81	g/dl
Albumin	1.15	1.18	1.1	1.18	1.2	g/L

Table 5: Achievement at a glance till reporting date

Sl. No.	Sub. of achievement	Achievement status	Comment
1.	Total collected mangrove weed	32 species	
2.	Morphologically identified	10 species	
3.	Proximate composition	6 species	Mostly important samples
4.	Amino acid profile analysis	7 species	Mostly important samples
5.	Fatty acid profile	7 species	Mostly important samples
6.	Mineral content(Ca, Fe, Mn, Zn, Cu)	7 species	Mostly important samples
7.	Anti-nutritional factor	Sample ready for lab	Need more budget and lab facilities
8.	Anti-oxidant profile	7 species	Mostly important samples
9.	Barcoding	Analysis in progress	
10.	Feed Formulation and Proximate composition	Done	
11.	Trial in culture	Done	
12.	Immunological Study	Done	

Anti-nutritional factors:

Due to short budget and lacking of lab facilities this analysis was not possible in this year. But we will analysis it in next year if the project is extended. Following anti-nutritional factors will be identified using universal protocol suggested by renowned researcher-

- ✓ **Total phenols:** Total phenols will be estimated by following the method of Malik and Singh (1980). A standard curve will be drawn using different concentrations of catechol (0-100 mg/ml) to calculate total phenol content and expressed as mg total phenols/100 g dry flour.
- ✓ **Phytic acid:** Phytic acid content will be estimated using the method of Haug and Lentzsch (1983).
- ✓ **C-glycosylflavone:** It will be estimated by using the method of Akingbala (1991)

Discussion and conclusion

The sustainable growth of aquaculture largely depends on the use of new nutrient sources to partially replace fish meal, without compromising fish growth, health, test and nutritional value of end products.

The present study clearly shows that aquatic weed *Chara baltica* used either single or blended, can be valuable natural ingredients to partially replace FM in diets. This algae have high protein and mineral content, despite it's low lipid level. Seaweeds such as Gracilaria concentrate minerals from seawater, reaching a mineral content 10–20 times higher than terrestrial plants (Moreda-Piñeiro et al. 2012). Among the macro minerals present in the *G. gracilis* biomass used in our study, K was the most abundant element, as corroborated by other studies (Reka et al. 2017; Radha, 2018). The microalga *N. oceanica* also has been shown to be a good source of minerals, namely Na, K, Mg, Ca, and P; when compared with *Chara baltica*, this microalga also had a higher content of essential amino acids, confirming previous observations (Archibeque et al. 2009). Nevertheless, besides the nutritional value of each alga, their nutrient bioavailability is of major importance for nutritional studies. In the present study, the dietary inclusion of 5%, 10% and 15% of *Chara baltica* did not affect nutrient digestibility, or nitrogen and energy retention efficiencies. Previous studies have reported that the inclusion of *G. bursa-pastoris* at 5–10% (Valente et al. 2006) resulted in protein ADC values similar to the control, but defatted *Nannochloropsis sp*. Biomass up to 15% (Valente et al. 2019) impaired energy digestibility. The same defatted *Nannochloropsis sp*. included at 10% in diets for Atlantic salmon significantly reduced protein and energy ADCs (Sørensen et al. 2017). Moreover, a recent study in Atlantic salmon showed that the incorporation of 10% pre-extruded *N. oceanica* in plant-based commercial-like feeds did not affect protein digestibility but reduced the lipid digestibility (Gong et al. 2020). In the present study 15% inoculation level of *chara* showed best growth performance. The significant reduction of growth observed in diets containing *Chara baltica* (D(5) and D(10)) might be explained by the presence of indigestible fibers in the aquatic weed cell wall, which may interfere with the digestion (Angell et al. 2016). Aquatic weeds are multicellular organisms with cell walls composed of complex polysaccharides. A recent study by Zheng et al. (2020) showed that red seaweeds are rich in sulfated polysaccharides of the carrageenan, agar, and agarose types which resist enzymatic degradation in the stomach and small intestine. The presence of such cell wall polysaccharides may limit the access of the digestive enzymes to algal proteins (e.g., phycobiliproteins) and can partially have contributed to the observed reduction of protein and energy ADC values in fish fed *Chara*. Although Aquatic weeds may have even higher levels of total dietary fiber than terrestrial plants, it is mostly soluble (Rajapakse and Kim, 2011). This soluble fiber forms a viscous mass in the gut with binding properties that may trap digestive enzymes and some other nutrients, slowing down the digestibility and impairing nutrient absorption in the intestine. In this study, dietary inoculation level of 15% weed displayed the highest nutrient gain, final body size and whole body composition to all other treatments. According to Valente et al. (2006), macroalgae such as *Gracilaria sp.* have great potential as alternative ingredients in diets for European seabass juveniles at dietary inclusion levels up to 10%. In rainbow trout, the dietary inclusion of 10–12% *G. vermiculophylla* meal or *G. pygmaea* impaired growth (Sotoudeh and Mardani, 2018), but in herbivorous fish such as Nile Tilapia (*Oreochromis niloticus*) inclusion levels could raise up to 20% (Younis et al. 2018). Few studies have evaluated the inclusion of whole *Nannochloropsis sp*. in diets for European seabass, but defatted *Chara sp.* biomass was successfully used up to 15% without affecting feed intake, fish growth, or whole body composition. In Atlantic salmon, incorporation of 10% pre-extruded *N. oceanica* in plant-based commercial-like feeds did not affect the growth, feed utilization, or body proximate composition (Gong et al. 2020). However, reduced feed intake was observed in Atlantic cod *Gadus morhua* fed with 14 and 28% of a *Nannochloropsis sp*. and *Isochrysis sp*. blend (Walker and Berlinsky, 2011). Reduced growth reported in many fish species fed algae rich diets was often associated with decreased intestinal absorption area due to a reduction in villi length or width (Moutinho et al. 2018). Here 15% aquatic weed incorporation showed best result of growth performance while other 5% and 10% inoculation were almost similar to 15%. Though intestinal morphology was not monitored in this study.

In Atlantic salmon, increased cell proliferation (PCNA) was observed in the distal intestine of fish fed 10% *N. oceanica* but without causing any histomorphological changes (Gong et al. 2020). The algae-rich diets resulted in a general increase in circulating glucose level, reaching statistical significance in fish fed diet D(15) relative to the control diet. Also, Belal et al. (2012) observed that Nile Tilapia fingerlings fed with diets containing 1% of Spirulina exhibited higher glucose values in fish serum. However, Vizcaíno et al. (2016) found an inverse relationship of glucose concentration when increasing dietary macroalgae (*G. cornea* and *Ulva rigida*) levels in *S. aurata* diets. Here highest glucose level was found in 15% inoculation level of aquatic weed (*Chara baltica*). Algae inclusion had no effect on blood cholesterol and protein levels which is in accordance with a previous study in *S. aurata* fed with 5% of seaweed biomass (Guerreiro et al. 2019). In the present study there was also zero cholesterol in fish blood except 15% weed inoculation.

However, triglyceride levels were significantly increased in fish fed the algae-based diets and could probably account for the reduced HSI observed in those fish. In red sea bream (*Pagrus major*) fed *Spirulina sp*., reduced total lipids both in serum and liver were associated with elevated activity of carnitine palmitoyl transferase, which is related to fatty acid β-oxidation, activating lipid mobilization (Nakagawa et al. 2000). In humans, DHA supplementation from algal oil reduced serum triglycerides and increased HDL and LDL cholesterol (Bernstein et al. 2012). It seems that algae may modulate lipid metabolism although in the present study fish whole body composition and muscle fat remained unchanged. Moreover, lipid peroxidation (LPO) also remained unaffected by the dietary inclusion of algae.

It seems that the algae blend may be contributing towards the depletion of plasma serum hemolytic activity, perhaps due to the inhibition of the protein cascade that composes the alternative pathway of the complement system. This lack of certain complement proteins may lead to an increased susceptibility to infections, as well as the development of autoimmune diseases in fish. A similar trend was observed in Tilapia fed with 15% *Chara sp* where serum calcium, total protein, almubin level were increased proportionally with inoculation level of *Chara baltica*.

Besides their nutritional properties, algae are also good sources of pigments that may affect the appearance of fish, namely in terms of skin and muscle color. Such attributes are known to influence market value, flavor perception, and acceptability of fish food products. Here we found good body color in 15% weed inoculated feed.

Aquatic weed-based foods have significant nutritional advantages for animal as well as humans such as, omega-3 fatty acid, omega-6 fatty acid, folic acid, iodine, calcium, magnesium, potassium, copper, iron, zinc, and vitamins. This weed may provide direct impact on growth, survival, physical change and immunological changes. The findings of this study will help to make a good formulated feed for fish or crustaceans, especially in hatchery and nursery levels.

Plate 1: *Chara baltica*

Plate 2: Mixing of feed ingredients

Plate 3: Formulated feed

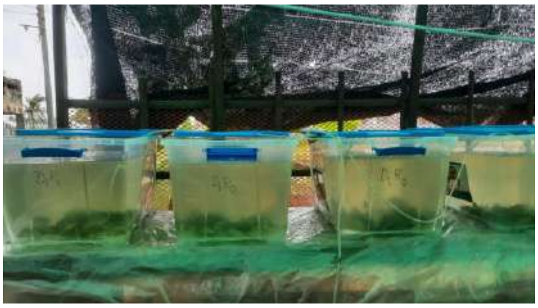


Plate 4: Culture trial

Plate 5: Feeding of fishes

Plate 6: Blood sample collection process

Plate 7: Collected blood

Plate 8: Hematological and biochemical test

Development of Integrated multi-trophic aquaculture systems for south-west coast of Bangladesh

Researcher(s) : A.K.M. Shafiqul Alam Rubel, SSO

: Md. Motiur Rahman

: Md. Masudur Rahman, SO

Objectives:

The aim of the study is to assess the potentials of IMTA systems on the productivity of *P. monodon* and *M. rosenbergii* with GIFT and/or catfish, mullet with aquatic weed and mollusks in brackishwater environment. However the specific objectives are as follows:

i. To develop IMTA based shrimp-fish-aquatic weed-mollusks culture to reduce feed cost;
ii. To optimize species combination and stocking density targeting sustainable production in a IMTA system;
iii. To study environmental and economic benefits from IMTA system in local condition. .

Achievement in the past (2023-24)

Experiment-1: To find out suitable combination and density of different trophic level species for IMTA at Brackishwater Gher

Methodology and experimental design

For developing low environmental impacts with minimum economic inputs of optimum IMTA system of tiger shrimp and freshwater giant prawn with other brackishwater species in coastal ghers or ponds an experiment was conducted during April/2024 in BS station. The experiment had three treatments (T_2, T_3 and T_4) and a set of control (C/T_1). Ponds under T_2, T_3, T_4 and C/T_1 were stocked with *Macrobrachium rosenbergii* at 50000/ha. Ponds under T_2, T_3 and T_4 were stocked with *Planiliza parsia* and *Mystus gulio* at 10000 and 5000/ha, respectively. Ponds under T_2, T_3 and T_4 were also stocked with *Oreochromis niloticus* at 1000/ha and ponds under T_3 and T_4 were stocked with blood cockles at 2000/ha. Whereas, ponds under T_2 and T_4 were grown the common water nymph (*Najas gramenia*) covering an area of 15% each ponds. *M. rosenbergii* was the targeted and fed-species for 180 days of culture period according to research design (Table 1). In this experiment, T_4 was designed for complete IMTA system, besides, T2 and T3 was partial IMTA system where either extractive or filter species was absent.

Table 1. Stocking density of different species in IMTA system in brackishwater ponds

Species (pcs ha−1)	**C/T1**	**T2**	**T3**	**T4**
Tiger shrimp (*P. monodon*)/Prawn (*M. rosenbergii*)	50,000	50,000	50,000	50,000
Gold Spot mullet (*P. parsia*)		10000	10000	10000

Long Whiskers Catfish, (*M. gulio*)		5000	5000	5000
Nile Tilapia (*O. niloticus*)		500	500	500
Common water nymph (*N. gramenia*) (% ha−1)		15	-	15
Blood cockles (*A. granosa*)			2000	2000

Pond Preparation: Five brackishwater having ponds 0.1 ha area of each was selected for this experiment. The ponds were dried through dewatering, bottom sludge's were removed and dykes were repaired. All the pond dykes were encircled with mosquito nylon net for bio-security and partitioned with bamboo fencing net to set replications. Pond bottom was limed with 250 kg/h calcium carbonate. Tidal water was introduced up to the levels and fertilized with Urea 25kg/h and TSP 30kg/h and bio-compost

Weed collection and plantation: Coastal weeds were collected from the Ghers and adjacent rivers, and seedlings were transplanted at (1m×1m) distance in the bottom soil.

Collection of Bivalve, fish Juveniles, PL and stocking: Blood cockle were collected from the Chuna River, Satkhira. Tilapia, mullet and Nona fry was collected from BS hatchery. SPF Shrimp and prawn PL was collected from Desh Bangla Hatchery, Khulna and CP prawn hatchery, Bagerhat, respectively. All the PL and fish fry were acclimatized with pond water, disinfected by a short bath with prophylactic treatment (5% KMnO4) and stocked into the respective ponds as per the design.

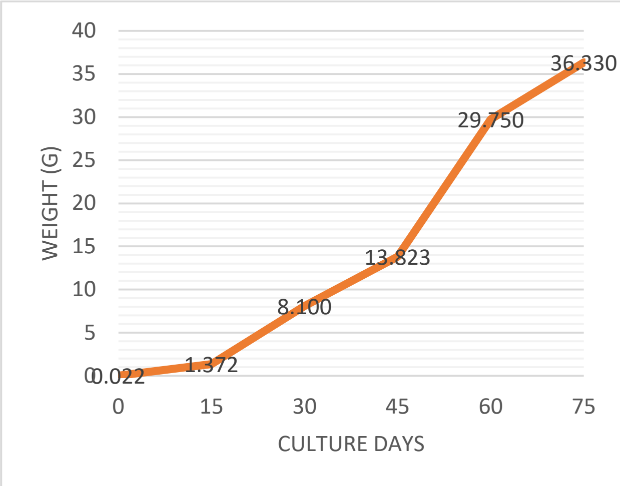
Feeding and management: The fishes were fed to apparent satiation level for the fourth/thrice/twice times per day during the $1^{st}/2^{nd}/3^{rd}$ week of stocking. Later on, the feeding was adjusted to 3% of standing biomass depending on sampling and applied twice in a day at dawn and dust. During feeding time, a close observation was made and a record of supplied feed was kept for determining the feed conversion ratio (FCR).

Sampling and monitoring water quality: A fortnightly sampling of fish was made by using a cast net and the weight of fish was measured by using a digital balance (OHAUS, CT 1200-S, USA) and length by a centimeter scale. Water quality parameters like, temperature (ºC), dissolved oxygen (mg/l), water pH, soil pH, salinity (ppt), transparency (cm), nitrite (mg/l), nitrate (mg/l), phosphate (mg/l), total ammonia (mg/l) and alkalinity (mg/l) was measured and recorded on spot fortnightly throughout the experimental period by using standard procedures and methods.

Sediments quality: Ponds bottom soil sample for the initial and mid of the culture has been collected and total analysis has been done from the SRDI, Khulna to know the concentrations of **nitrogen, carbon** and **phosphorus** compounds in each ponds to study the environmental benefits from IMTA systems. Last sample will be collected at the end of culture.

Results/Achievement or progress

Besides *M. rosenbergii*, *P. monodon* was also cultured in monoculture system in a set of control ponds (C/T1). Growth performance and length-weight relationship was done to compare with previous study (Figure 1 and 2). The initial weight of *P. monodon* was 0.0223±.51 g at stocking and density was 50000 (pcs. ha−1). After culturing 90 days the average final body weight of *P. monodon* reached to 36.33±.84 g (Fig 1). Tiger shrimp exhibited a very strong and positive correlation (R^2= 0.9495) between length and weight (Fig 2).



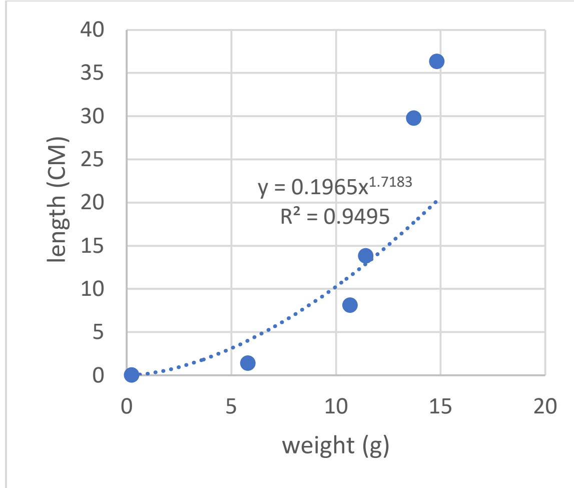


Fig 1. Growth performance of *P. monodon*

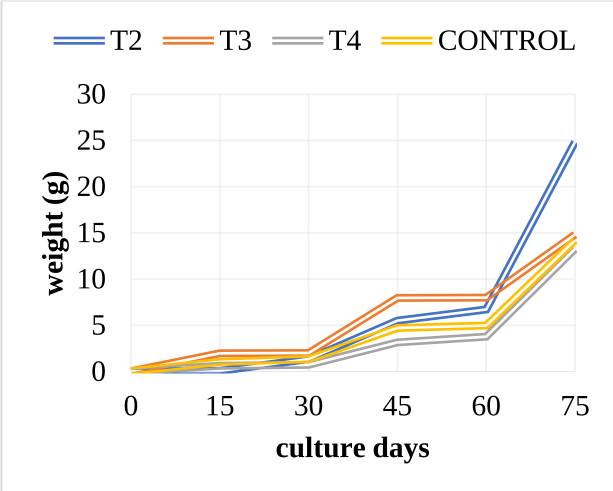
Fig 2. length-weight (g) relationship of *P. monodon*

The initial weight of *M rosenbergii* in all treatments was 0.05±.00 g and stocking density was 50000 (pcs ha−1) (Table 1). In this experiment, final body weight of *M. rosenbergii* was 24.82±.51, 14.19±.3, 14.82±.14 and **13.25**±0.84 g in C/T_1, T_2, T_3 and T_4, respectively. Here highest growth rate was 24.82±.51 g in T_2. The specific growth rate of prawn was 5.78%, 4.21%, 4.15% and 4.29% per day in C/T_1, T_2, T_3 and T_4, respectively with the highest SGR was in C/T_1 (Table 2).

Table 2. Growth performance of prawn and tilapia under IMTA systems in brackishwater ponds.

Treatments	Initial weight (gm)	Final weight (gm)	SGR (%/day)
Prawn (*Macrobrachium rosenbergii*)			
C/T_1	0.05±.00	**24.82±0.51**	**5.78**
T_2	0.05±.00	14.19±0.3	4.21
T_3	0.05±.00	14.82±0.14	4.15
T_4	0.05±.00	13.25±0.84	4.29
Nile Tilapia (*Oreochromis Niloticus*)			
T_2	0.29±0.11	267.9±47.30	
T_3	0.29±0.11	298.6±43.59	
T_4	0.29±0.11	**301.3±67.26**	

Growth pattern of *M. rosenbergii* has been shown in Figure 3, indicated similar growth trends in all the treatments up to 60 days, later on the growth of prawn in T_1 suddenly jumped than than other treatments. Meanwhile the growth of Nile Tilapia was more or less steady up to 60 days then growth trend reduced (Fig.4).



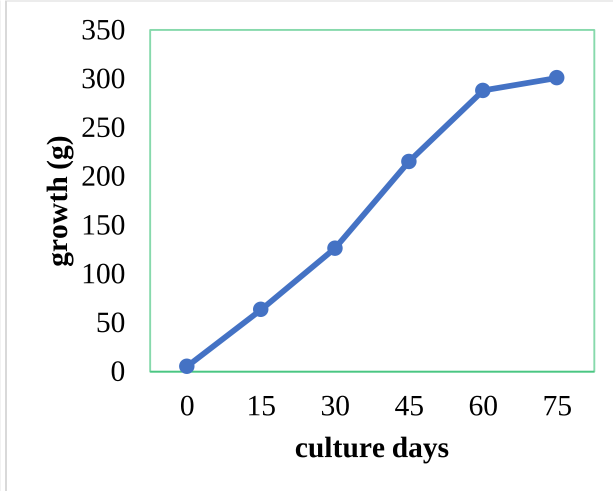


Fig 3. Growth performance of *M. rosenbergii*

Fig 4. Growth performance of Nile Tilapia

After three months of culture, tilapia attained an average growth of 267.9±47.30, 298.6±43.59 and 301.3±68.26 g in T_2 ,T_3 and T_4, respectively (Table 3, Fig. 4). Here highest tilapia growth was recorded in T_4 and lowest growth was in T_1.

Table 3. Growth performance of fed aquaculture species (mullet and tengra) in IMTA systems in BS Station

Treatments	Initial weight (gm)	Final weight (gm)	SGR (%/day)
Long Whiskers Catfish, (*Mystus gulio*)			
T_2	3.2±0.91	**21.9±3.84**	1.62
T_3	3.2±0.96	20.8±4.04	1.58
T_4	3.1±.72	21.3±5.33	**1.60**
Gold Spot mullet (*Planiliza parsia*)			
T_2	1.03±0.55	10.40±1.71	2.10
T_3	1.03±0.43	10.15±1.33	2.07
T_4	0.94±0.21	**10.66±1.22**	**2.20**

The initial weight of *M. gulio* was 3.2±0.91 g and stocking density was 5000 (pcs. ha−1), in T_2, T_3 and T_4 (Table 1 and 3). After culturing 90 days, the average body weight of nona tengra was 21.9±3.84g, 20.8±4.04g, and 21.3±5.33g, with the specific growth rate of 1.62%, 1.58%, and 1.60% in T_2, T_3 and T_4, respectively (Table 3). The highest growth rate and SGR was in T_2 and SGR was in T_4 (Table 3). The initial weight of *P. parsia* was 1.03±0.55g and stocking density was 10000 (pcs. ha−1), in T_2, T_3 and T_4 (Table 1 and 3). After culturing 90 days the recorded average body weight was 10.40±1.71g, 10.15±1.33g, and 10.66±1.22g, with a specific growth rate of 2.10%, 2.07%, and 2.20% in T_2, T_3 and T_4, respectively. Nona tengra (Fig. 5) and that of the Parse (Fig. 6) showed a steady growth trend throughout the culture period.

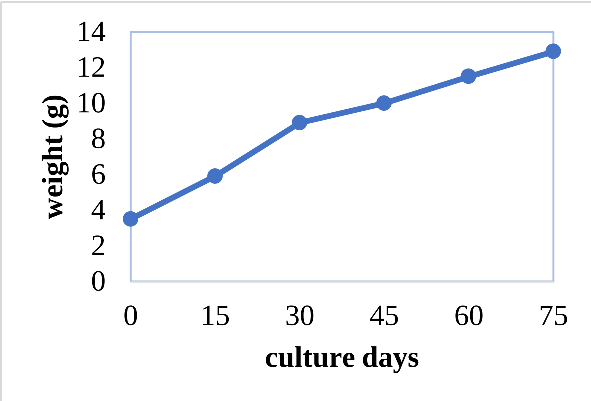


Fig 5. Growth performance of *Mystus gulio*

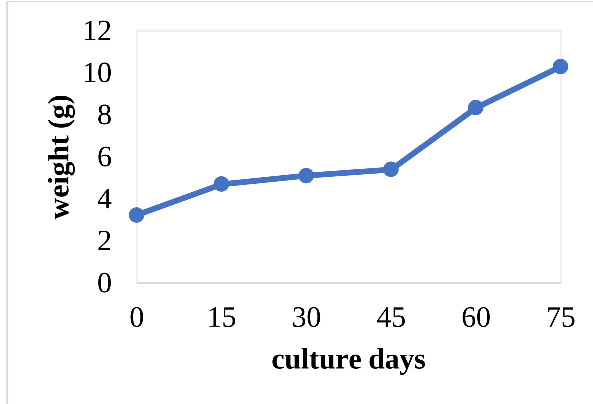


Fig 6. Growth performance of *Planiliza parsia*

Pond bottom soil for the starting time has been analyzed from SRDI and has been shown in Table 4. Samples at the middle and end of culture will be collected and compared with the initial to observe the effect of IMTA on bottom soil. In this experiment amount of sulfur were 219.9±0.1 (µg/g), 240.8±32 (µg/g), 220.54±66.5 (µg/g), 221.31±33.8 (µg/g) in T_1, T_2, T_3 and T_4, respectively. Among other parameters highest pH was in T_1 and T_4, Salinity in T_3, Organic matter in T_3 and T_4, total nitrogen in T_4, phosphorus in T_1, calcium in T_3 and potassium in T_2 was observed (Table 4).

Table 4. Initial sediments quality profile of pond bottom soil under IMTA system

Parameters/ Treatments	Sampling frequencies	pH	Salinity (ds/m)	Organic Matter (%)	(%) N	P (µg/g)	K (meq/ 100g)	S (µg/g)	Zn (µg/g)	B (µg/g)	Ca (meq/ 100g)	Fe (µg/g)
C/T1	starting	7.9±0.01	14.6±1.27	2.05±0.39	0.10±0.05	5.1±0.30	0.68±0.06	219.9±0.1	0.6±0.39	2.91±0.41	20.5±3.99	39.14±7.40
T2	starting	7.05±0.05	19..25±1.20	2.03±0.19	0.14±0.00	4.6±0.30	0.9±0.14	240.8±32	0.79±0.68	2.25±0.74	23.33±3.55	30.2±0.10
T3	starting	7.5±0.07	27.65±0.63	2.17±0.50	0.11±0.02	4.5±1.36	0.7±0.19	220.54±66.5	0.26±0.02	206±0.41	27.31±0.66	20.25±2.9
T4	starting	7.9±0.14	16.5±1.5	2.17±0.50	0.16±0.02	4.80±0.30	0.70±0.11	221.31±33.8	0.205±0.03	2.85±0.17	25.6±3.3	35.93±1.82

The environmental parameters of the ponds water such as temperature, pH, salinity, DO (Dissolved Oxygen), NO_3-N, NO_2-N, NH_3-N, alkalinity, TDS, EC, SSG and chlorophyll-*a* were recorded in fortnightly interval and measured standard method in each pond (Table 5).

Table 5. Recorded environmental quality parameters during IMTA

Parameters	**Control/T_1**	**T_2**	**T_3**	**T_4**
Salinity (ppt)	13.35±3.74	14.36±3.57	**14.52±3.26**	14.38±3.41
Temp (ºC)	**33.84±2.14**	33.52±2.21	33.65±1.97	33.58±1.97
pH	8.66±0.20	8.86±0.17	8.78±0.15	**8.77±0.18**
DO (mg/l)	6.36±0.50	6.69±1.06	**6.66±1.11**	6.22±0.41
Water level (m)	1.06±.13	1.21±0.14	1.23±0.14	1.26±.08
Ammonia(mg/l)	0.05±.04	0.03±0.01	0.02±.03	0.04±.01
Alkalinity(mg/l)	165.5±29.83	162.0±24.46	169.11±25.10	161.04±23.90
TDS (mg/L)	15.49±3.78	15.2±3.74	15.52±3.23	**15.49±3.78**
Chlorophyll-a(ug/l)	0.33±0.40	0.45±0.33	0.70±0.34	0.35±0.38
EC (µmhos/cm)	20.97±8.07	23.66±5.42	**23.88±4.94**	20.97±8.07
SSG	4.95±3.06	5.32± 2.82	**5.7± 2.50**	4.95±3.06

According to APHA (2005) and Boyd C.E (1998) recorded all environmental or physico-chemical variables were found within the acceptable ranges for crustacean aquaculture except higher temperature fluctuation (30-36) **ºC** in all the ponds (Fig. 7) and lower water levels in T_2, T_3 and T_4 treatments (Fig. 8) during the entire culture period. Water level was found more or less similar 1.13±0.14 m (T_2), 1.13±0.14m (T_3) and 1.06±.08 m (T_4), whereas T1 had always a higher water depth of 1.5 to 1.6 cm during the culture period (Fig. 8).

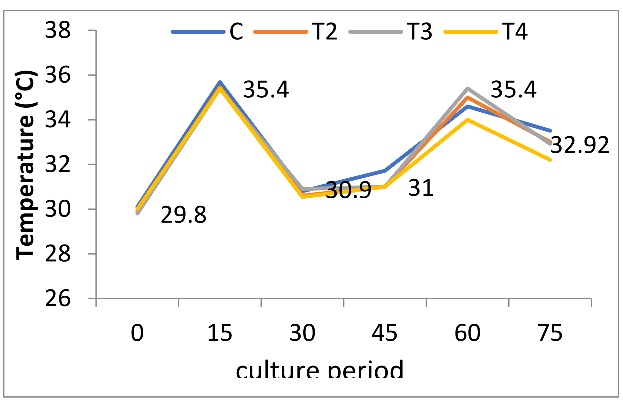


Fig. 7. Temperature variation during culture period

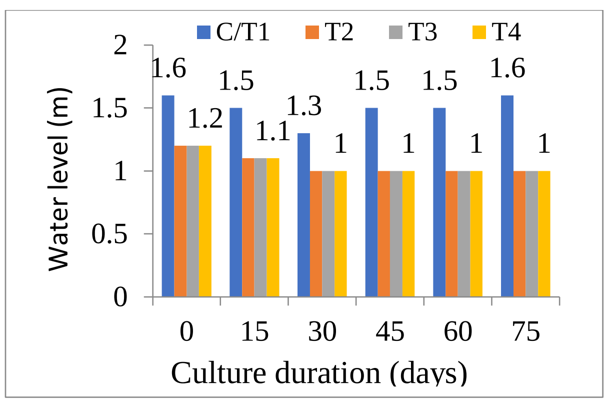


Fig. 8. Water level in ponds during culture period

The economic benefit analysis of IMTA system: As experiment is still ongoing thus economic analysis yet not done.

Discussion and conclusions:

The comparative growth and production performance between three treatments in IMTA systems and monoculture of *M rosenbergii* made in this study indicated the feasibility of farming brackishwater fish with prawn, blood coockle and aquatic weed together in low-saline brackishwater ponds. Two partial and one complete IMTA treatments tested here is a simple improvisation of brackishwater polyculture that does not hold extractive species, such as *Anadora granosa* and common water nymph *Najas spp*. All the three IMTA models were better performers in terms of environmental remediation and better food consumption/utilization, compared to the monoculture system. Thus, our results indicate potential development of IMTA as a low intensive aquaculture system, compared to the IMTA models devised based on intensive culture systems in the western hemisphere (Alexander et al., 2016) and China (Fang et al., 2016). The inorganic nutrient parameters of water improved in all three IMTA systems in comparison to control. Overall, water quality parameters in all the treatment ponds except control remained stable throughout the study period and were suitable for brackishwater shrimp and finfish culture (Biswas et al., 2012). This could have resulted from application of lime at fortnightly intervals during entire culture period. However, in our study, water temperature, pH, salinity, DO and alkalinity did not vary among three treatment ponds, which indicates that differences in cultured species weight gain and total biomass production were possibly due to the variation in species combinations and stocking densities in three IMTA systems and Control (monoculture of shrimp). According to APHA (2005) and Boyd C.E (1998) the recorded mean soil quality parameters were suitable range except Fe and S content in sediments in all treatments. The existence of excessive S indicates presence of acid sulfate soil in these ponds. We tested three IMTA systems, two partial and one complete IMTA. In the partial IMTA systems, one was with inorganic nutrient removing species, common water nymph (T_2) and another system was with organic matter removing species, blood cockle (T_3). The complete IMTA system held both these species (T_4). Compared to polyculture or monoculture system, all the three IMTA treatments were more effective in removal of inorganic nitrogenous (NO_2–N, NO_3–N and TAN) and phosphate-phosphorus (PO_4–P) concentrations. However, level of all these inorganic compounds was reduced at higher magnitude in the complete IMTA system (T_4) where both aquatic weed (*Najas spp*) and blood cockle (*Anadora granora*) were used as extractive species. Among all three treatments T_2 treatment showed higher prawn growth performance for absence of tilapia in this treatment. It means that tilapia was feed competitor with prawn. In T_2 and T_3 treatments in IMTA systems, we used the blood cockle (*Anadora granora* and common water nymph (*Najas spp*) separately as inorganic extractive species. This plant can remove nitrogenous and phosphorus compounds from organically polluted waters and was also used to treat aquaculture wastewater successfully (Zhang et al., 2014). Therefore, we selected this aquatic weed for its effective role in bioremediation and income generation. In T_2 and T_3, this inorganic extractive aquatic weed utilized the available nitrogen and phosphorus compounds, and reduced their levels in water as it can remove nutrients (total nitrogen and total phosphorus) between 41.5 and 75.5% from eutrophic water (Hu et al., 2008) and to a great extent from shrimp farm wastewater (Luo et al., 2012). The growth, survival and production performances of finfishes were better in the complete IMTA treatment except prawn (T_1) than in Control ponds, indicating that the cultured *M rosenbergii* species were facing feed crisis and also failed in feed competition with other three species, especially with tilapia. For getting better growth or economic return from cultured *M rosenbergii* further or more research work is needed for fine tuning of species combination and stocking density in IMTA systems.

Image 1: Water removing

Image 2: Sun drying

Image 3: Excavation

Image 4: Fencing

Image 5: lime transportation

Image 6: Lime application

Image 7: Bamboo splitting

Image 8: Monk preparation

Image 9: Monk sludge removing

Image 10: partitioning

Image 1: .Fencing

Image 12: Embankment repairing

Image 13: Bleaching

Image 14: Screening

Image 15: Application of rice bran juice

Image 16: Stocking

Image 17: Water monitoring

Image 18: Sampling

Image 19.: *Anadora granosa*

Image 20.: *Najas spp*

Image 21: Sediment Sample

Image 22: Netting

Image 23: Harvesting

Image 24: IMTA reaearch site

Domestication, reproductive biology, breeding and culture of indigenous brackishwater prawns under captive conditions

Researcher(s) **:** Dr. Md. Latiful Islam, CSO

: Md. Abu Naser, SO

: Shafiqul Alam Rubel, SSO

: Md. Hashmi Sakib, SSO

Objectives:

The goal or broad objective of the project is to develop captive breeding and seed production technique of indigenous brackishwater prawns (*Macrobrachium villosimanus*, *M. lamarrei* and *M. dayanum*). In achieving the goal, experiments will be conducted with the following specific objectives:

i. Identification of target prawn using taxonomic and barcoding approach,

ii. To domesticate brackishwater prawns under captive condition for broodstock development,

iii. To investigate the reproductive biology (fecundity, GSI, breeding time, embryonic development, etc.) of the prawns,

iv. To develop breeding and larvae rearing protocol of the prawns.

Achievement in the past (2023-24):

Experiment/study-1: Breeding and seed production of Brackishwater Indian whisker shrimp (*Macrobrachium lamarrei*)

The suitable brood of *Macrobrachium lamarrei* was collected from the domesticated ponds and from natural sources. Then, the brooders were fed with earthworms and protein rich pellet feeds at the rate of 5% body weight basis to trigger the reproductive maturation. Once the prawn extrudate the egg and aggregates into the abdominal flaps (turned into berried), the berried females were transferred to the incubation tank after proper disinfection with 20-50 ppm formalin solution for 30 minutes. A little portion of egg sample were collected with sterilized forceps at three days intervals and observed under stereo microscope. Fertilization rate, progress of all embryonic development stages was noted down and sufficient photographs were taken for documentation. As the colour of egg mass turned into dark grey, the berried females were transferred to the hatching tanks filled with 8-12 ppt disinfected water.

Flowchart: Overall breeding activities were done for *M. lamarrei*

After hatching, the larvae were transferred to the prepared larvae rearing tanks and fed with live feed (rotifer and artemia). Powdered shrimp feed and artemia nauplii were provided after 7 days of hatching for larval development and nutrition. Fertilization, hatching and survival rate were calculated using the standard equations given below:

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs in sub-sample}}{\text{Total number of eggs observed in sub-sample}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatchlings}}{\text{Total number of fertilized eggs}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Final population (Pt)}}{\text{Initial population (Po)}} \times 100$$

The embryonic development of *M. lamarrei* is consisted of nine different stages/phases which were characterized by various notable morphological changes (Figure 1).

Stage 1: Fertilization of eggs (00-04 hours): Fertilization of eggs continued up to four hours from beginning of fertilization and finished just before the first cell division. Fertilized eggs were almost globular shape and consisted of a granulous mass uniform dark olive color which wrapped with a lucid chorion.

Stage 2: Cleavage (04-07 hours): Various cleavage furrows arise in the egg mass, pointing up the formation of the first embryonic cells. A translucid region found at one pole of the egg shrinking slightly the eggs inside mass. These changes occurred at the beginning of embryonic development where egg volume increased slightly.

Stage 3: Blastula (07-35 hours): Translucid area of the egg expanded gradually without remarkable changes. Consequently, two parts inside the eggs were observed where the abdominal part of the developing embryo represented by a light region and cephalic area represented by a dark olive region.

Stage 4: Gastrula (35-130 hours): After 35 hours of fertilization, the internal mass of the egg consolidated mainly in the peripheric region. The abdominal region with the presence of some abdominal segments perfectly separated from the "V" form cephalic region. This stage concluded five days after fertilization.

Stage 5: Nauplius (130-168 hours): A spacious black spot developed in the cephalic region of the embryo which continuously shortened due to the propagation of abdominal part after 140 hours of fertilization. The black spot illustrates a diagram of the embryos ocular region turned more evident. Besides, some vitellin reserve vesicles developed in the peripheric part of the cephalic region.

Stage 6: Post-nauplius with a heartbeat (168-194 hours): Previously developed optic region expanded with more pigmentation. The caudal papilla inhered with an elementary telson at the abdominal region and folded to the optic region. At the moment, remaining contents of eggs was mainly vitelline reserve slendered due to the evolution of embryonic structures and turn into dark grey color.

Stage 7: Post-nauplius with eye individualization (194-240 hours): Eyes separated from the optic region, prolonged, turn into an elliptical form and separated from the cephalic region but still stuck on their base.

Stage 8: Final post-nauplius with eye condensation (240-290 hours): Diameter of eye enlarged with color intensification and eyelashes arise above each eye. Well developed and segmented maxillipeds visible in this phase and overlapped the abdomen. Whole egg space was occupied by the embryo. The vitellin vesicles intensified and were more visible in the cephalic region of the embryo.

Stage 9: Pre hatching (290-320 hours): Cephalothoracic dark grey part reduced significantly. The heart was fully evident from the vitellin mass and compression of the heart was significantly active than the previous phases.

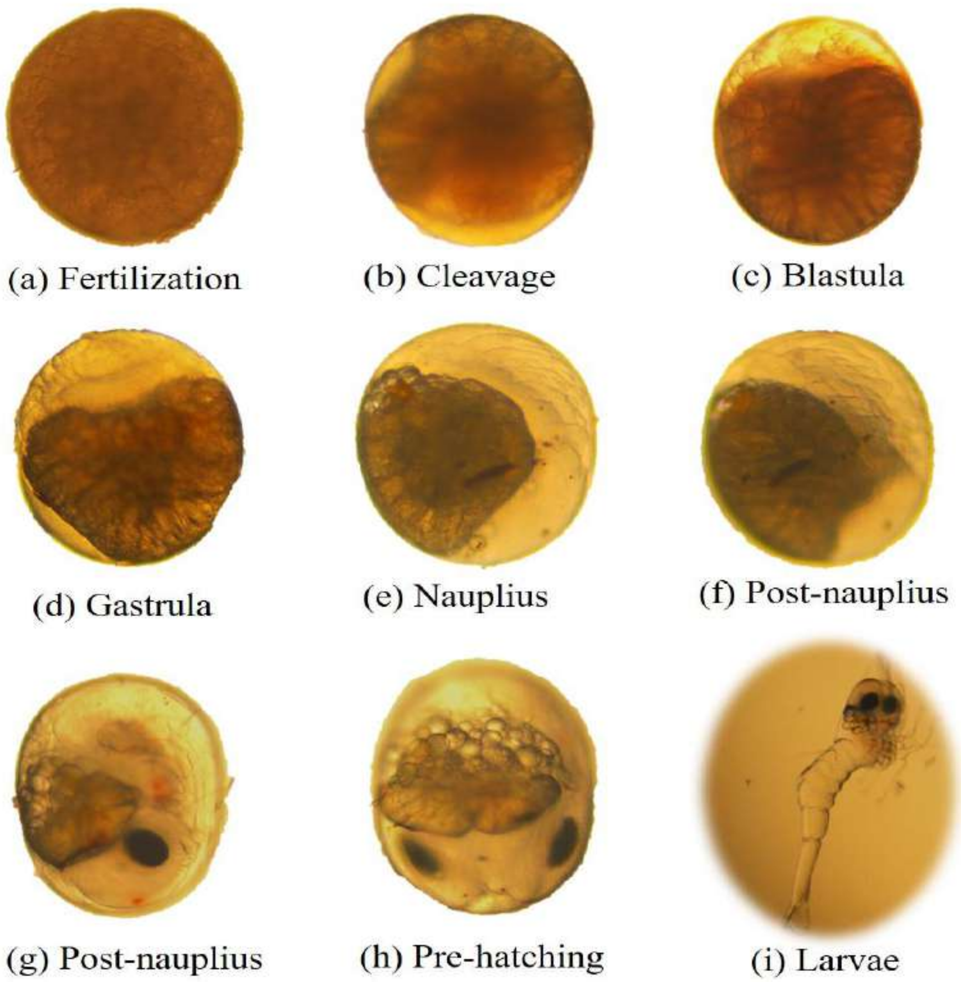


Figure 1: Embryonic developmental stages of *M. lamarrei*.

Experiment/study-2: Effects of salinity on breeding performance and larvae survival of brackishwater Indian whisker shrimp (*M. lamarrei*) under captive conditions

To evaluate the effects of different salinity on breeding performance and larvae survival of brackishwater Goda Chingri, experiment was conducted with four different salinity treatments, viz, T1 (8 ppt) and T2 (10 ppt), T3 (12 ppt) and T4 (14 ppt), respectively (Table 1). The larvae were reared with standard crustacean larvae rearing protocol. The fertilization, hatching and survival rate were calculated and compared among the treatments accordingly.

Table 1: Design of experiment to evaluate different salinity levels on breeding performance and larvae survival of Indian whisker shrimp (*M. lamarrei*).

Treatment	Salinity	Replications
T1	8	3
T2	10	3
T3	12	3
T4	14	3

Three (3) breeding trials were made to produce Goda prawn PL in the hatchery complex of Brackishwater Station in this year. The highest fertilization rate was recorded as 92.0±0.5 % in T2 (10 ppt) which is significantly ($p<0.05$) higher than T1 (80.0±1.0 %), T3 (86.0±2.0 %), and T4 (82.0±2.5 %), respectively. Similarly, the highest hatching rate was estimated as 87.0±2.5 % in T3 which is significantly ($p<0.05$) higher than T1 (63.0±1.5 %), T2 (72.0±2.0 %), and T4 (56.0±1.5 %), respectively. Correspondingly, the maximum survival rate was calculated as 37.0±2.0 % in T3 which is significantly ($p<0.05$) higher than T1 (23.0±2.3 %), T2 (25.0±1.8 %), and T4 (23.0±2.0 %), respectively. The PL were stocked in the nursery pond for nursery rearing. The broodstock inducement with enriched artificial (commercial diet) and natural feed (earthworms) are ongoing.

Table 2: Effects of salinity on breeding performance and larvae survival of Goda

Treatment	Fertilization Rate (%)	Hatching rate (%)	Survival Rate (%) Up to 12 days
T1	80.0±1.0	63.0±1.5	23.0±2.3
T2	**92.0±0.5**	72.0±2.0	25.0±1.8
T3	86.0±2.0	**87.0±2.5**	**37.0±2.0**
T4	82.0±2.5	56.0±1.5	23.0±2.0

Discussion and conclusion:

Shrimp is Bangladesh's one of the largest sources of foreign exchange earnings, contributing significantly to the country's economy and creating employment opportunities. Due to low salinity for most of the year, mass shrimp deaths caused by WSSV and AHPND/EMS invasions have become common, prompting farmers to be extremely cautious when stocking shrimp in their ghers. Many bagda farmers have already planned to shift their culture pattern and search for suitable alternate species. Flavor and affordability of native prawns caused high demand both domestically and internationally. Since commercial prawn farming mostly relies on natural collection, one of the main issues is the lack of necessary number of high-quality seeds. There is limited, erratic, and unscientific seed availability. Existing hatcheries are not generating enough seeds to meet their installed capacity because of a number of issues, including a lack of healthy broodstock, diseases that affect the mid-larval cycle, and huge larval mortality that results in low yield. In the meanwhile, scars are the technology for native shrimp species' sustained seed development. Therefore, in order to successfully breed and nurture the native prawn under study in captivity, the biology, maturation, breeding, life cycle, etc. were researched.

The current study provided baseline data on the maturation, reproduction, and larval development of *M. lamarrei*, and it came to the conclusion that the species would be another viable option for Bangladeshi brackishwater prawn rearing. Since this species has a high market value and is consumed by Bangladeshis, the construction of hatcheries will make it easier to obtain seeds for the inland and low-saline areas that the country's southwest coastal region needs for prawn culture.

Plate 1: Brood Goda Prawn

Plate 2: Broodstock Pond

Plate 3: Hatchery Installation

Plate 4: Reproductive Biology Study

Plate 5: Monitoring PL

Plate 6: Experimental Set-up

Domestication, breeding and seed production of some commercially important brackishwater fishes

Researcher(s) : Dr. Md. Latiful Islam, CSO

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: Saima Sultana Sonia, SO

: Md. Abu Naser, SO

Objectives:

The goal or broad objective of the project is to fine-tuning of larvae rearing of S. argus and P. hasta. Whereas, augmentation of diversified information on broodstock development, breeding season identification, captive breeding and seed production of five important brackishwater finfish species *Mugil cephalus, Datnioides polota, Plotosus canius, Glossogobius giuris and Polynemus paradiseu*s. In achieving the goal, experiments will be conducted with the following specific objectives:

- To enhance the survival rate of Chitra and Datina fish seed,
- To domesticate the commercially important fishes in brackishwater environment,
- To observe the food-feeding habit and reproductive biology of the fishes,
- To develop induced breeding, seed production and nursery technique of the fishes.

Achievement in the past: (2023-24)

Experiment/study 1: Domestication and broodstock development of Bhangon (*Mugil cephalus),* Royna *(Datnioides polota),* Kain magur *(Plotosus canius),* Baila *(Glossogobius giuris)* and Taposi *(Polynemus paradiseu*s).

In continuation of previous year work, domestication and broodstock development of four targeted fishes Bhangon, Royna, Kain magur and Baila are going on. The fishes (Bhangon, Royna and Baila) are being reared under brackishwater pond conditions and properly feeding with commercial brood diets (30-35% protein) fortified with combination of vitamin C and E. The overall status of the brood stocks is given below:

Bhangon (*M. cephalus*)*:*

There are about150 broods weighing 900-1400 g of Bhangon which are being reared in Brackishwater ponds and all are sexually mature enough to hormonal induction for spawning in the running and upcoming season. They are being fed with a commercial brood diet (protein, 35%) fortified with a mixture of vitamin C and E. There are two spawning for Bhangon in a single year based on the histology and GSI study. The major peak is in March and the minor one is in between September to October.

Kain Magur (*P. canius):* Based on gonadal histology and GSI study, Kain magur has single peak spawning annually which is in July-August. There are about 30 individuals of Kain magur weighing 1.5-3 kg which are potentially mature and are being reared with utmost care and feeding (with beheaded shrimp and prawn head, small crabs, blood cockles and small fishes) to induce them with different doses and types of hormones in the current and upcoming season.

Baila *(G. giuris):*

According to our study and the literature reviewed, it is confirmed that Baila has a single peak spawning over a year and that is in November. We are trying to induce them to spawn using different hormones at different primary and secondary dosages. There are abundant numbers (above 250) of brood available here (weight ranged between 30-180 g). We are to try their spawning next season.

Royna *(D. polota):*

Royna has two spawning seasons in a year. The major one is in July and the minor one is in October. We rearing the brood of Royna of about 40-50 pcs and they are being fed with commercial brood diets (35% protein) at 5% of body weights. They are going to under hormone hormone-induced breeding trial in July and the subsequent season for optimum results.

Taposi *(P. paradiseu*s):

A total of 35 Taposi fish was collected (weight ranged between 35-150 g) and released in a separate broodstock pond. Unfortunately, we did not get any fish after a month of sampling. We further collected a few brood fish, but the scenario was the same.

However, the research team has already investivated the food and feeding habit, GSI, fecundity, gonad histology and found out the peak breeding season of all the above fishes. Preliminary success on ovulation has also been achieved on bhangon, royna and kain magur breeding. The hormones that are being used are namely Homogenates of the Pituitary gland of carp (PG), Human Chorionic Gonadotropin (HCG) Hormone, Luteinizing hormone-releasing hormone analog (LHRHa), Gonadotropin-releasing hormone analog (GnRHa) etc either in alone or in various combinations at different feasible dosage for optimum induction of spawning.

Experiment/study 2: Effects of different salinity on breeding performance and larvae survival of *M. cephalus* (Bhangon) and *D. polota* (Rekha)

There was a specific design to fulfil this objective in the reporting year. The design is given below-

Table 1. Experimental design for Bhangon and Royna breeding performance against salinity

Treatment	Salinity (ppt)		Replica
	Bhangon	Royna	
T1	24	15	3
T2	27	18	3
T3	30	21	3
T4	33	24	3

The success that we achieved in 2022-23 in regard of Bhangon and Royna breeding was the reflection of a specific design of the study. The design is given below-

Table 2. Different hormones and dosage for Bhangon and Royna breeding

Treatments	Inducing agent	Hormone doses (µg.kg^{-1} body weight)		
		E1(♀: ♂)	E2 (♀: ♂)	E3 (♀: ♂)
T1	PG	40:20	50:25	60:30
T2	LHRHa	40:20	50:25	60:30
T3	S-GnRHa	40:20	50:25	60:30
T4	0.9% NaCl	40:20	50:25	60:30

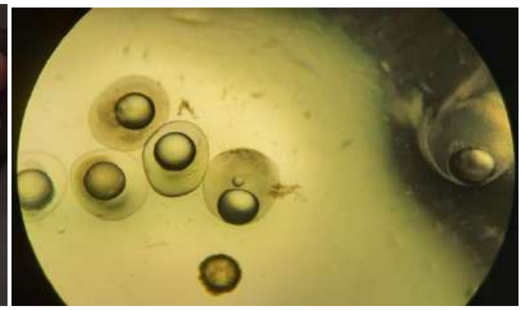
It is a matter of regret that last year's study using different hormone treatments did not show any positive response in the breeding of Bhangon (*M. cephalus*). Royna (*D. polota*) has never been tried with the mentioned dose of last year yet. So, we employed different hormone doses and dosages for Bhangon again this year. The new experiment design is given below-

Table 3. Different dosages of hormones for Bhangon (female)

Treatments	Inducing agent	Dosages	
		1st dose	2nd dose
T1	HCG	5000 IU/kg	-
	LHRHa	-	200 µg/kg
T2	HCG	17000 IU/kg	-
	LHRHa	-	360 µg/kg + 5 mg/kg Domperidone
T3	LHRHa	120 µg/kg	240 µg/kg

Among those three treatments, T2 has only been implemented with successful ovulation. Collected oocytes of Bhangon were mixed with the milt collected by dissecting of potential male for fertilization. Regretfully no fertilization occurred. The team investigated the possible causes behind infertility and found that the oocytes collected were not potentially feasible of fertilization. Successful fertilization required >500 µm oocyte diameter, upon investigation we found that <400 µm was the oocyte diameter which resulted in no fertilization. Other T1 and T3 treatments are yet to be tested on the availability of the potential Bhangon brood in the next spawning season.

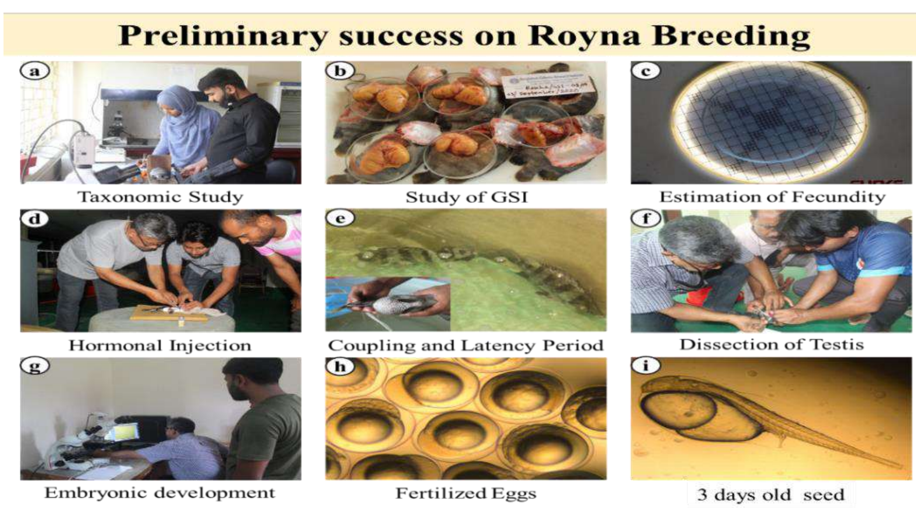
Figures (left to right): Dissection of male for gonad, collected gonad, collected egg of female Bhangon



Figures (left to right): Mixing of milt with eggs, unfertilized eggs under the microscope

Immediately after ovulation, a small amount of milt with good motility was sprinkled over the released eggs to ensure successful fertilization. Immediately after 10 minutes of incubation, only the buoyant eggs from the surface water were collected and shifted to the hatching tank (250 L fiber-reinforced plastic tank) holding seawater (30 ppt salinity) for additional incubation. The fertilized eggs were found floating on the water surface. Microscopic investigation revealed that 13 of the fertilized eggs were translucent with an unbroken nucleus whereas the unfertilized were opaque and broken nucleus. Embryonic and larval developmental stages of fertilized eggs were monitored continuously at every 20–30-minute interval to ensure every stage and documented under LEICA DM1000 LED microscope from zero hour post-fertilization (hpf) to larval stage.

Regarding Royna, an induced spawning attempt was also carried out according to the previous year's hormone treatment design. Ovulation did occur but there was not any potential male available at that time. The experiment regarding Royna will be carried out again when the mature potential brood is found in our brood development ponds.



After that preliminary success in Kain magur breeding, the team is still actively working on the breeding of Kain magur. Shortly An induced breeding trial will be attempted on the breeding of kain magur.

- ➢ The peak breeding season of Kain Magur is June-July
- ➢ Early brood was available in the broodstock pond, thus a trial is ongoing

- Female @ 100µg/kg LHRHa (2 dose, 30% +70%)

-Male @ 50% LHRHa single dose

Table 4: Hormone and dosage for Kain magur breeding

	Inducing agent	**1^{st} dose (µg/kg)**	**Interval (hours)**	**2^{nd} dose (µg/kg)**
Female	LHRHa	30	18	70
Male	LHRHa	-	-	50 (single dose)

Discussion and Conclusion:

Marine and coastal fish breeding is very important as the natural stock of these saline waters declining due to unregulated capture policies. Here in the BS@BFRI is trying to breed coastal fish in the manner of artificial propagation or induced breeding technique. Breeding of coastal fishes is of tire most jobs to carry on due to insufficient information on breeding success. In addition a lot of parameters, like, temperature, salinity, nutrition of brood stocks, etc are to be addressed and mitigated. This year we were again successful on induced ovulation of Bhangon. But the problem is the eggs are not that much mature (>500 µm). As a result, fertilization is not happening after we put that in incubation. We are adopting measures to resolve the problem. Besides regarding Royna and Kain magur breeding, we did have some success on the initial trial. We are trying different types of hormones on induction of spawning and to optimize the dose and dosage of those hormones. Breeding those brackishwater fish will help to prevent the natural habitat f those species and will open a window for mariculture which is a much-needed aquaculture practice to adopt and to combat biodiversity degradation and climate change. However, long term project is needed to be approved to be success on coastal and marine breeding.

Biochemical analysis of bottom soil of ghers in relation to shrimp production

Researcher(s) Dr. Md. Ariful Islam, SSO
Md. Touhidul Islam, SO
Md. Iqramul Haque, SO
Rabina Akther Lima, SO

Objectives:

- ✓ **To assess the biochemical parameters of shrimp ghers bottom sediment**
- ✓ **To determine the residual concentration of biochemicals in shrimp and their toxicological risk**
- ✓ **To estimate the nutrient budget of shrimp ghers**
- ✓ **To evaluate the energy budget of the shrimp ghers in relation to their production**

❑ **Achievements:**

Experiment-1: Assessment of the biochemical parameters of bottom sediment
Study-1 Assessment of the chemical parameters of bottom sediment

❑ **Monitoring of the chemical features of gher bottom sediment**

Most of the adjuvant minerals and nutrient components like phosphorus, sulfur, zinc, boron, manganese along with the total organic matter, total-N, calcium, magnesium, and potassium were available up to the middle layer of the gher sediment column which concentrations were 5 times higher in the sediment from cluster ghers in contrast with the non-cluster ghers. The hazardous chemicals like heavy metal composition was mostly sighted to the bottom layer of the gher sediment column.

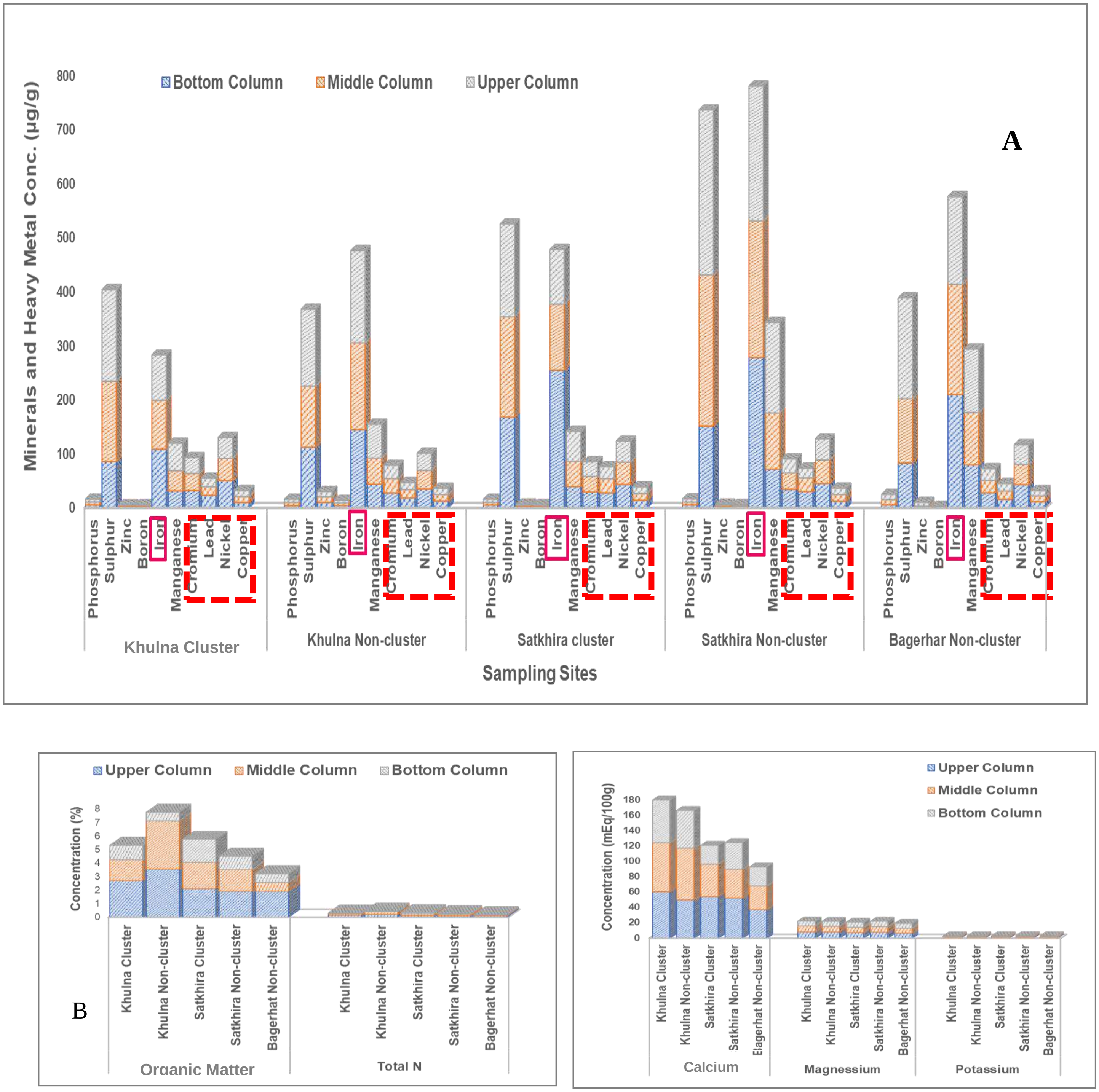


Figure 1. (A) Distribution of minerals and heavy metals composition in gher bottom soils; (B) Organic Matter, and Total Nitrogen composition of soil (C) Availability of Ca, Mg, and K

Study-2: **Microbial profile analysis of bottom sediment in shrimp ghers**

Among the witnessed microbial populations, *Brevibacillus*, *Delftia*, *and Stenotrophomonas* were the major groups in Khulna's middle layer sediment column. In contrast, the dominant genera were *Acinetobacter*, *Chryseobacterium*, *Bacillus*, *Stenotrophomonus*, *Brevibacterium* along with *Delftia* in the bottom layer, and *Acinetobacter*, *Chryseobacterium*, *Bacillus*, *Stenotrophomonus*, *Delftia* where the abundance of *Bacillus* was significantly higher in the middle layer column indicating most congenial environment.

In Bagerhat, the dominant genera were *Delftia*, *Brevibacterium*, *Stenotrophomonus*, *Brachybacterium*, *and Chryseobacterium* in the upper column. On the other hand, the major genera were *Delftia*, *Stenotrophomonus*, *and Bacillus* in the middle column which also indicates the beneficial microbial abundance in this layer.

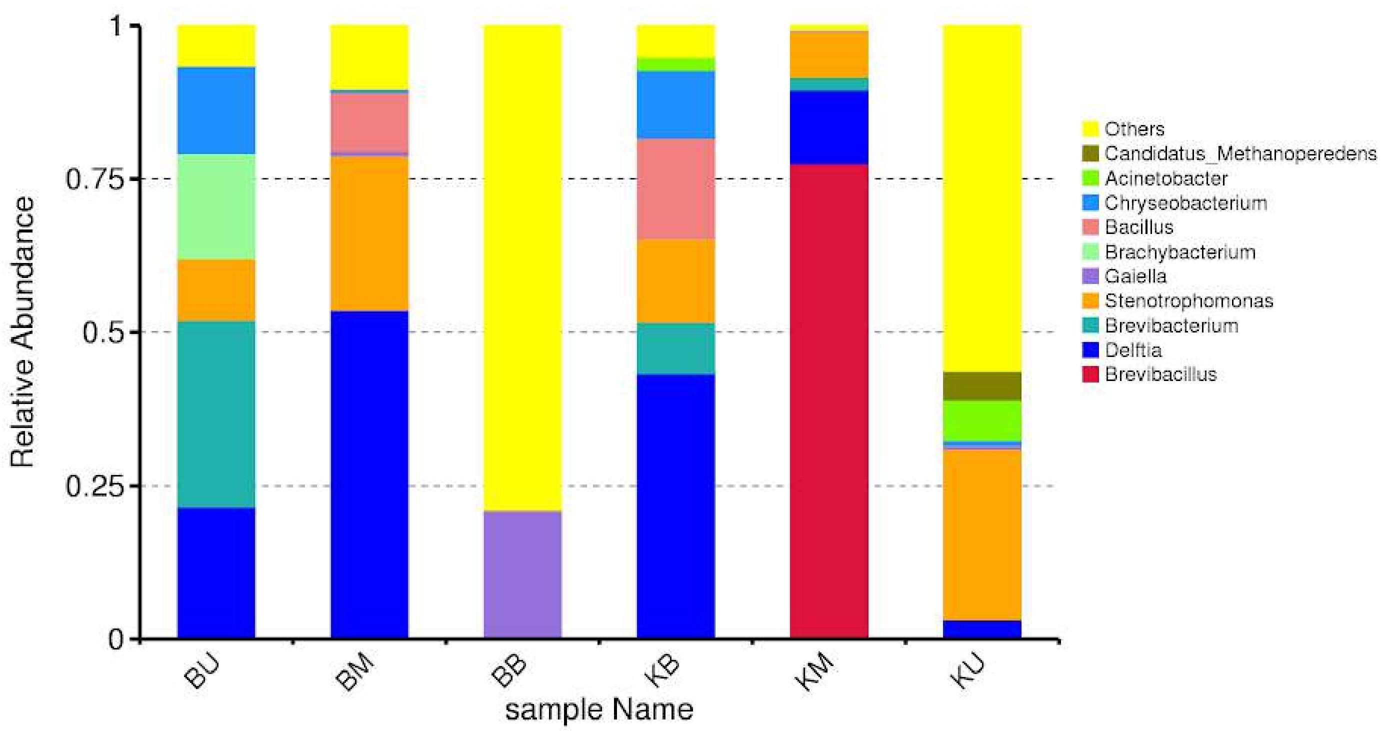


Figure 2. Relative abundance of various microbial genera at different vertical layers of the bottom sediment column in shrimp ghers.

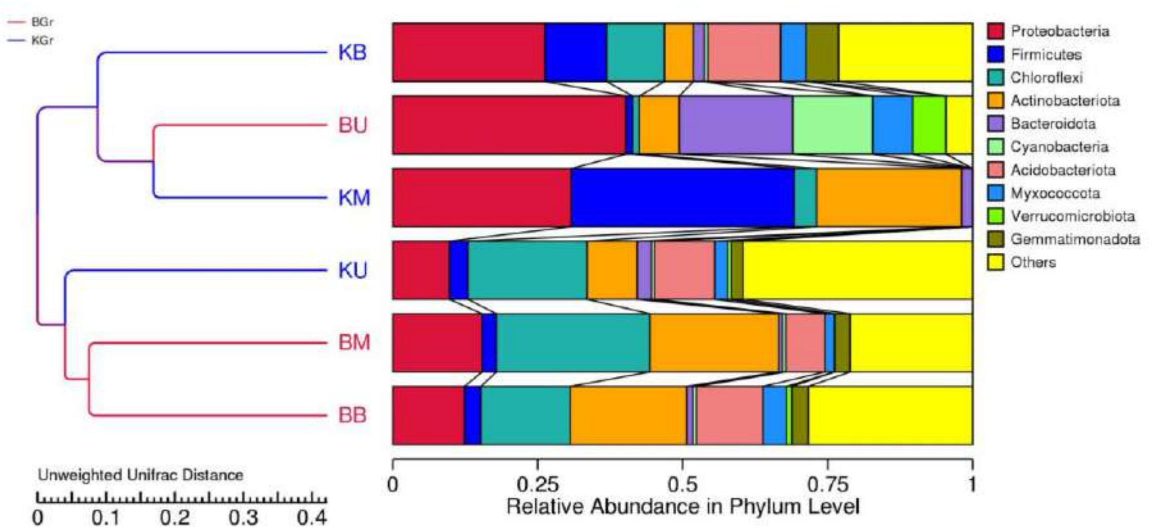


Figure 3. Relative abundance of various microbial phylum at different vertical layers of the bottom sediment column in shrimp ghers.

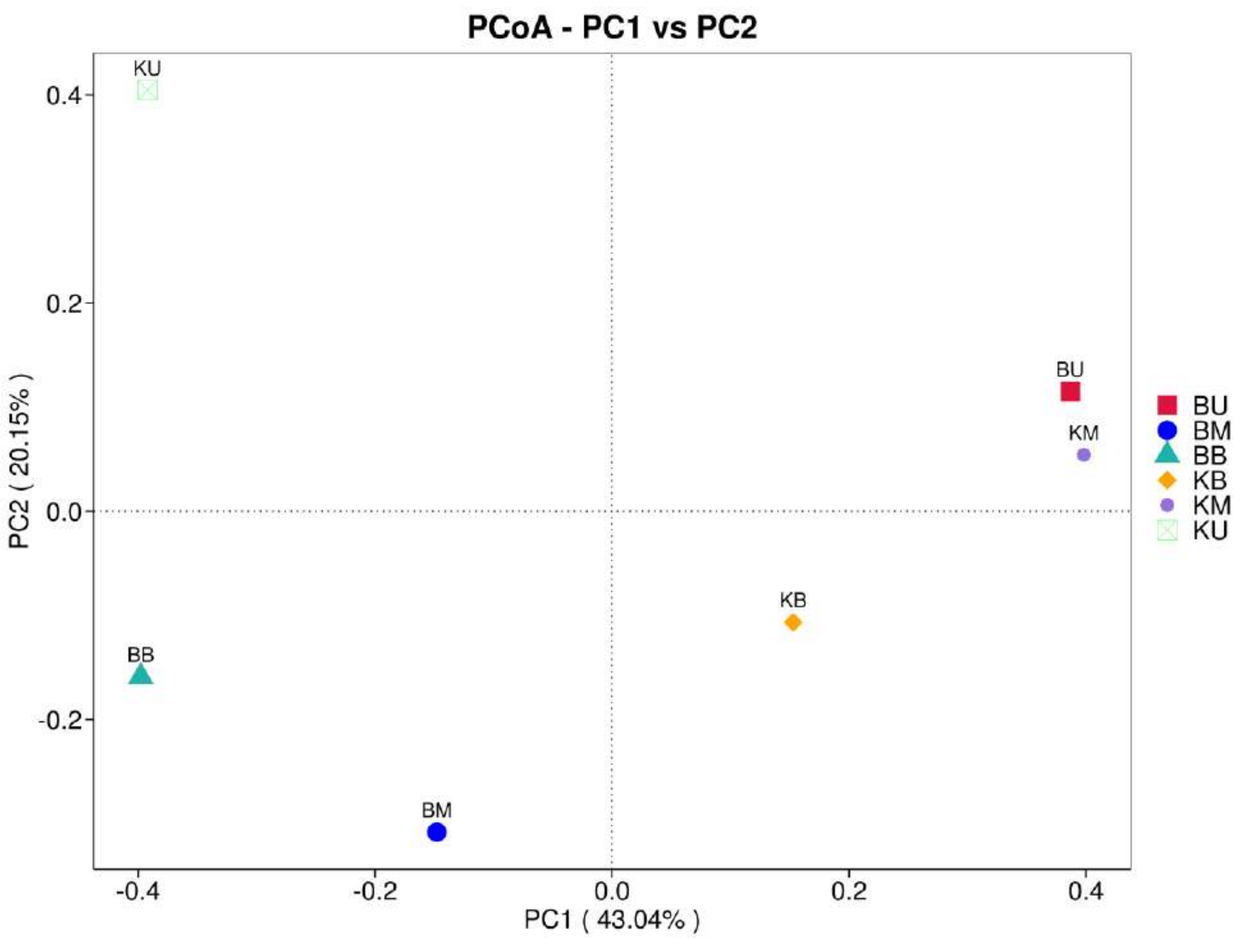
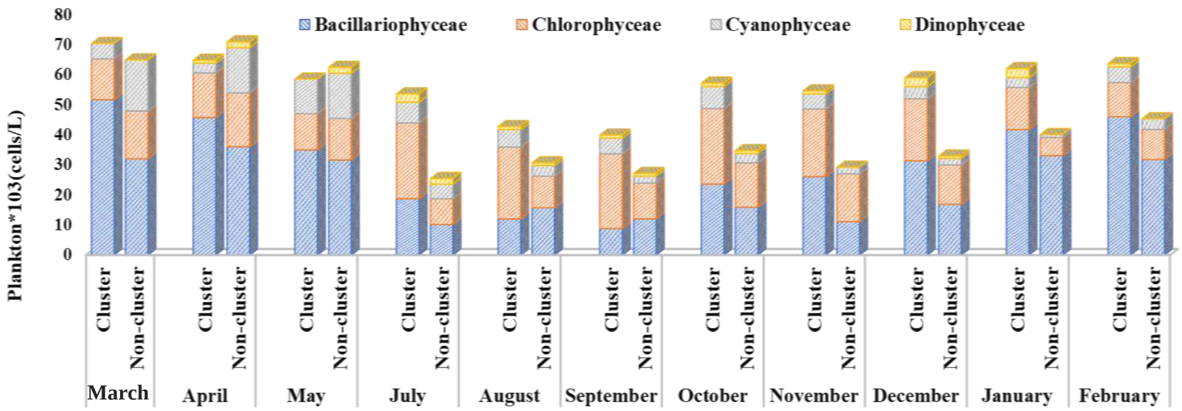


Figure 4. Principal component analysis to relate location-wise abundance of microbial community from the shrimp ghers.

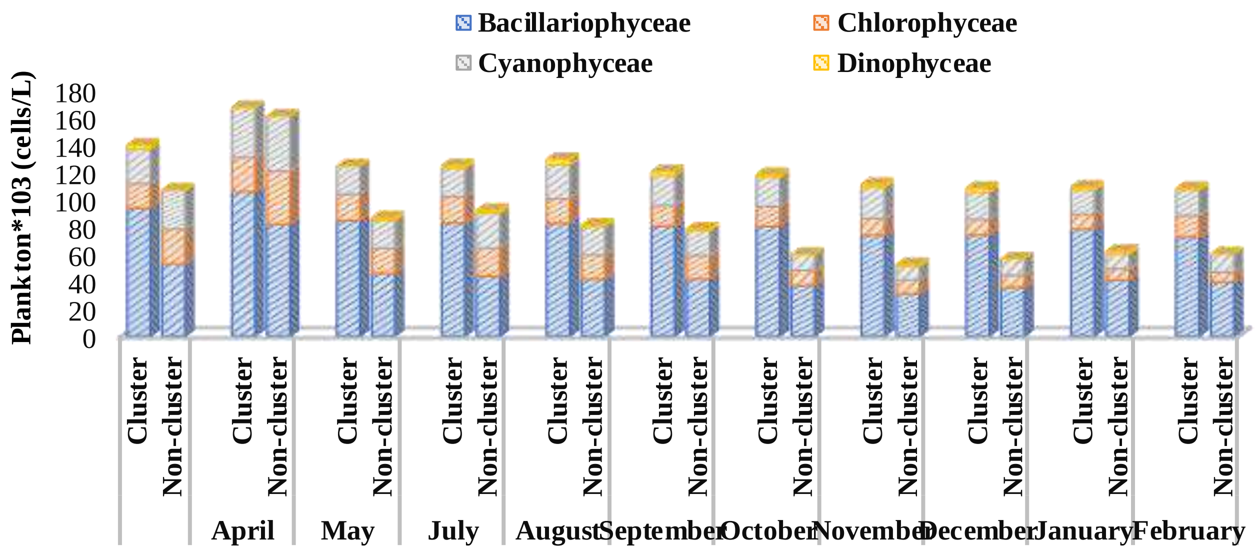
Study-3 Monitoring of the plankton abundance

Among the witnessed phytoplankton population, Bacillariophyceae was the major group where the dominant genus was *Asterionella sp.*, *Cyclotella sp.*, *Navicola sp.* along with *Nitzschia sp.* The phytoplankton abundance was prominently higher (40%) in cluster farming systems rather than in the non-cluster ghers.



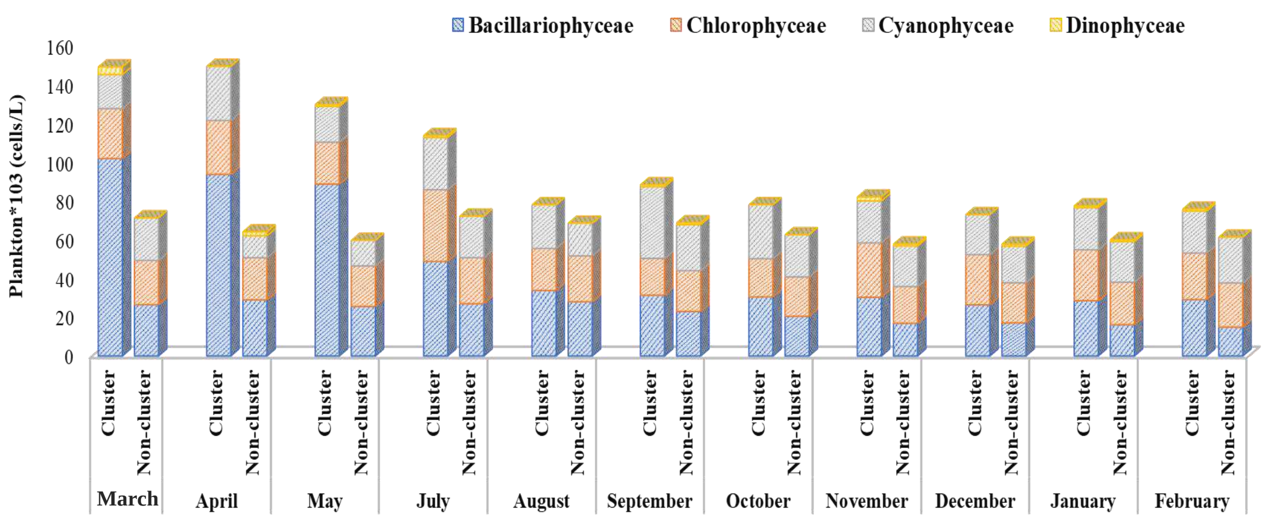
Bacillariophyceae	Cyanophyceae	Dinophyceae	Chlorophyceae
✓ *Asterionella*	✓ *Anabaena*	✓ *Dinophysis*	✓ *Spirogyra*
✓ *Cyclotella*	✓ *Nostoc*	✓ *Ceratium*	✓ *Ulothrix*
✓ *Navicola*	✓ *Spirulina*		
✓ *Nitzschia*			

Figure 5. Month wise distribution of phytoplankton in Bagerhat



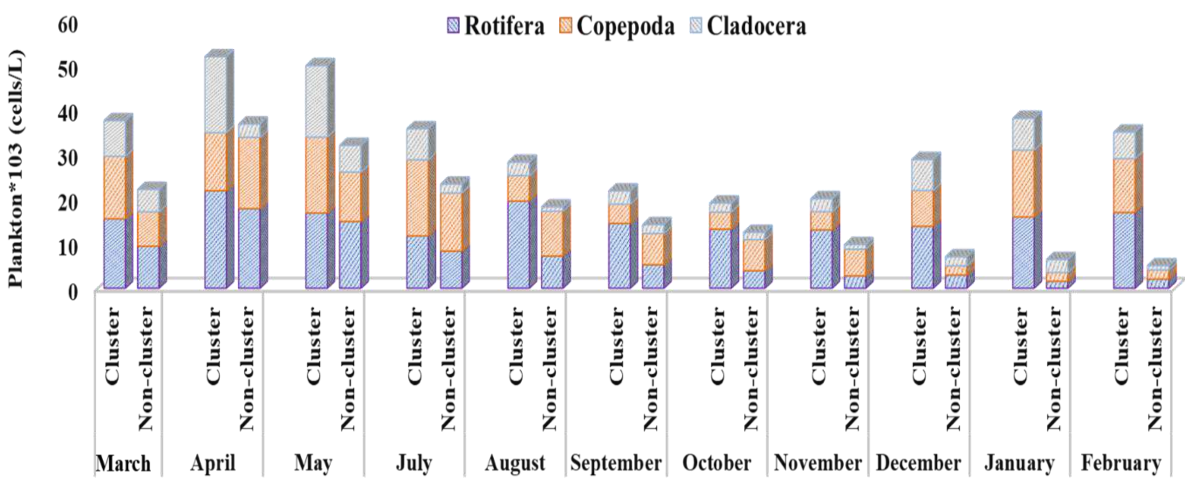
Bacillariophyceae	Cyanophyceae	Dinophyceae	Chlorophyceae
✓ *Asterionella*	✓ *Anabaena*	✓ *Dinophysis*	✓ *Spirogyra*
✓ *Cyclotella*	✓ *Nostoc*	✓ *Ceratium*	✓ *Ulothrix*
✓ *Navicola*	✓ *Spirulina*		
✓ *Nitzschia*			

Figure 6. Monthly distribution of phytoplankton in the shrimp ghers of Satkhira



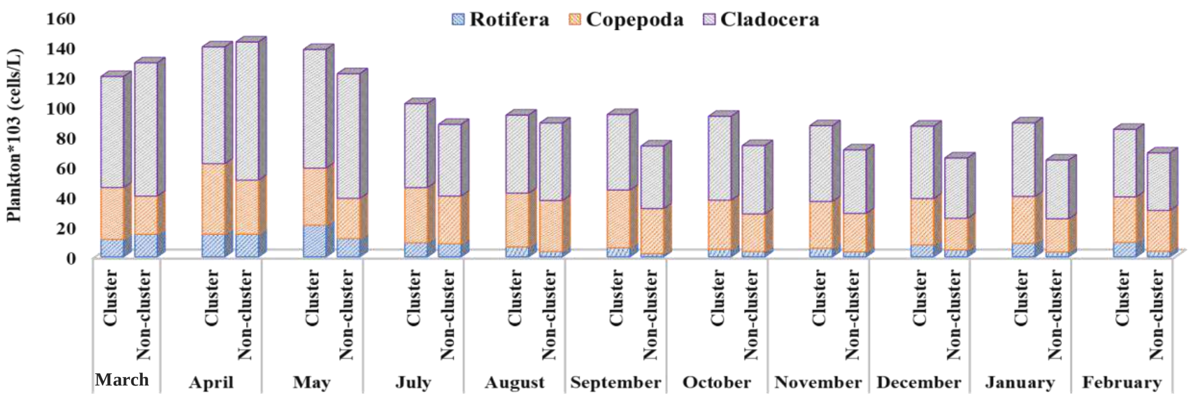
Bacillariophyceae	Cyanophyceae	Dinophyceae	Chlorophyceae
✓ *Asterionella*	✓ *Anabaena*	✓ *Dinophysis*	✓ *Spirogyra*
✓ *Cyclotella*	✓ *Nostoc*	✓ *Ceratium*	✓ *Ulothrix*
✓ *Navicola*	✓ *Spirulina*		
✓ *Nitzschia*			

Figure 7. Monthly abundance of phytoplankton in the shrimp ghers of Khulna



Rotifera	Cladocera	Copepoda
✓ *Brachionus*	✓ *Daphnia*	✓ *Cyclops*
✓*Keratella*	✓ *Moina*	✓ *Diaptomus*
✓ *Navicola*		✓ *Nauplius*

Figure 8. Month wise zooplankton abundance in the shrimp ghers of Bagerhat



Rotifera	Cladocera	Copepoda
✓ *Brachionus*	✓ *Daphnia*	✓ *Cyclops*
✓*Keratella*	✓ *Moina*	✓ *Diaptomus*
✓ *Navicola*		✓ *Nauplius*

Figure 9. Monthly distribution of zooplankton abundance in the shrimp ghers of Satkhira.

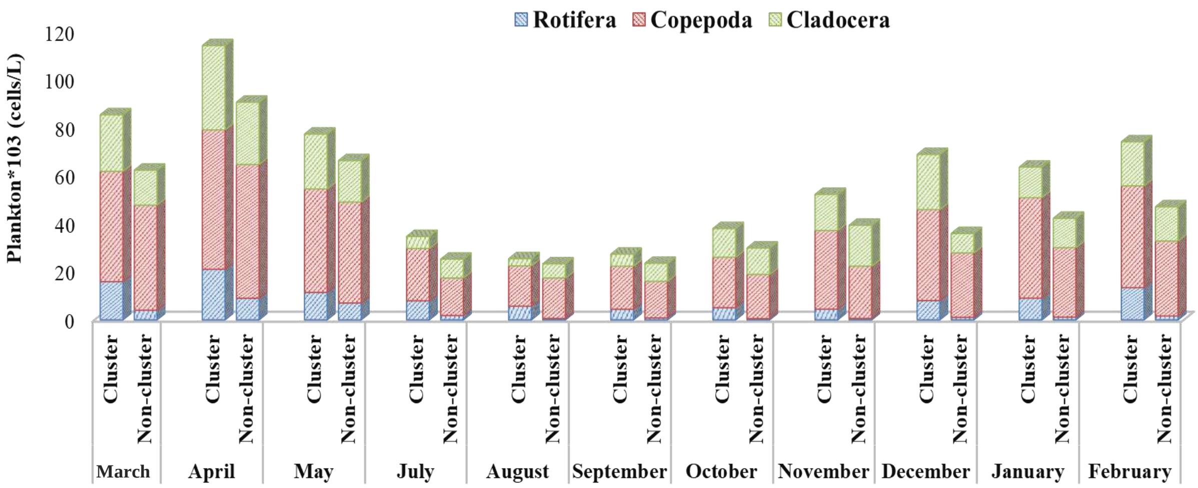


Figure 10. Month wise zooplankton abundance in the shrimp ghers of Khulna

Expt-2: Assessment of hazardous chemicals in shrimp samples

- ✓ In Bagerhat, the concentration level of methoxychlor pesticides was observed as 196.55 ppb, 92.86 ppb, and 84.49 ppb which health risk value was 0.207 denotes no significant health risk to the consumers.

- ✓ In Satkhira, the observed pesticide was methoxychlor whose levels were 116.50 ppb, 73.55 ppb, and 64.67 ppb which was 0.031 denotes no significant health risk to the consumers.

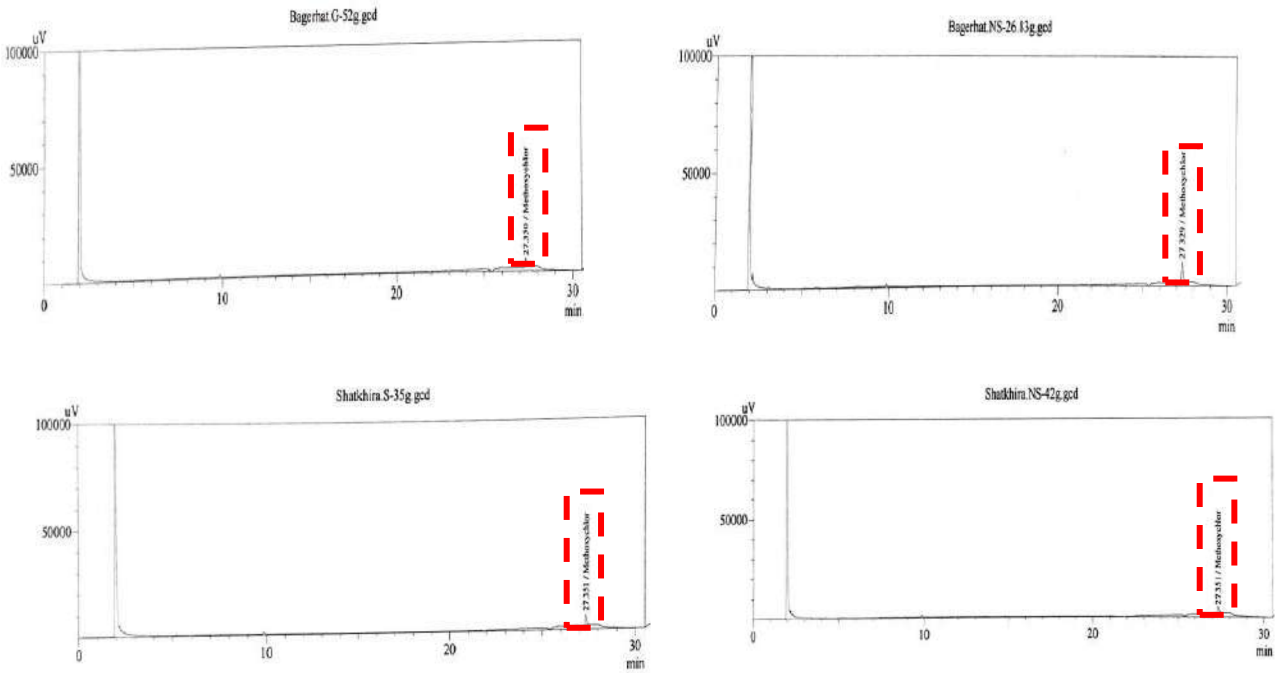


Figure 11. Pesticide residues in shrimp samples collected from Bagerhat and Satkhira

- ❖ ***Sampling and analysis for Expt. 03 is still ongoing as the culture period hasn't been completed yet. After final harvesting, the data will be found at the end of coming December/January.***

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

Researcher(s) Dr. A S M Tanbirul Haque, SSO

Md. Shoebul Islam, SO

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:

Experiment-1: Trial of Shrimp farming with *Najas indica* on farmer level

Table 1. Experimental Design

Treatment	Location	Feeding ratio	*Najas indica* plantation	Stocking density (individual/m^2)	Replications
T_1 (Control)	On station	Based on previous result	20% of pond area	4	3
T_2	On farm				
T_3	On farm				

Two ponds were taken in farmer level and One pond was taken at SRS station as a control based on last year's best results, 20% of the pond space in the treatment ponds were planted with *Najas indica* and 50% commercial feed than general practice was applied.

Table 2. Farmer's address

Farmer Name	NID of Farmer	Adress	Area of pond
Shimul Molla	4176671586	Village: Kundola, Post: Patilakhali Upozila: Bagerhat sadar, Bagerhat	35 decimals
Akash Molla	4222945844	Village: Dokkhin kakarbil, Post: Sholarkola, Upozila: Kochua, Bagerhat	17 decimals

Agreement with farmers

Agreement with farmers were done before starting culture to the farmer ponds. All inputs are supplied from this project. Production will take the farmer and only data will be taken from these ponds fortnightly.

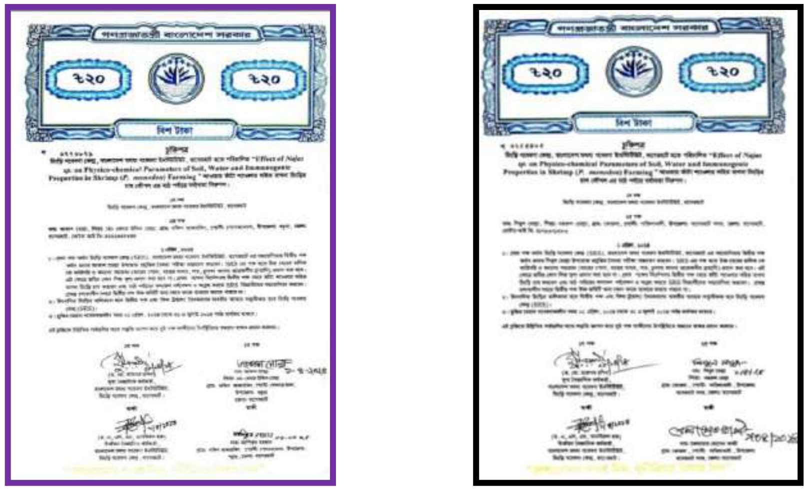


Figure-1: Agreement papers

(a) **(b)**

Figure-2: Farmers (a) Akash Molla (b) Shimul Molla

Materials supply to the farmers

For pond preparation all materials were supplied to the farmers from this project

Figure-3: Materials for pond preparation were handover to the farmers

Pond preparation

The ponds were made ready by drying, liming (quick lime: dolomite 2:1) at a rate of 250 kg/ha of soil, and then filling with tidal water to a depth of 1m. Chlorine was added to the water at a 20ppm concentration to disinfect it and eliminate any microorganisms. urea and TSP fertilization were applied at rates of 25 and 30 kg/ha, respectively, to hasten the development of the water's color and the growth of plankton. 20% of the water area in treatment ponds was planted with *Najas sp.*

Before After Before After

(a) (b)

Figure-3: Pond preparation: (a) Dokkhin Kakarbil, Kochua (b)Kundola, Bagerhat sadar

PL stocking

SPF fry was released on 01 May.

Figure-4: PL distribution to the farmers

The ponds were made ready by drying, liming (quick lime: dolomite 2:1) at a rate of 250 kg/ha of soil, and then filling with tidal water to a depth of 1m. Chlorine was added to the water at a 20ppm concentration to disinfect it and eliminate any microorganisms. urea and TSP fertilization were applied at rates of 25 and 30 kg/ha, respectively, to hasten the development of the water's color and the growth of plankton. 20% of the water area in treatment ponds was planted with *Najas sp.*

Feeding behavior and well-being of shrimp was checked twice daily by setting check tray. After 105 days of culture, shrimps were harvested by complete dewatering.

The water quality variables *viz.*, temperature, depth, transparency, salinity, pH and total alkalinity were monitored at 15 days interval following standard methods. The recorded average water quality variables are shown in Table 2.

Table 3. Water quality parameters in different culture ponds.

Physicochemical Parameter of water	T_1 (Control)	T_2 (17 decimal)	T_3 (35 decimal)
Temperature (ºC)	30.28±1.36	30.36± 0.48	30.14± 0.19
pH	8.1± 0.58	8.27± 0.64	8.39± 0.31
Salinity (ppt)	8±1.88	8.1± 1.25	8.5± 1.4
Alkalinity (mg/l)	102.5±55.05	86.5±12.75	96.5±22.24
Ammonia (mg/l)	0.03±0.01	0.035±0.01	0.025±0.01
Dissolved Oxygen (mg/l)	7.78± 2.54	8.54± 2.13	9.25± 0.91
Conductivity (mS/cm)	7.79± 1.47	6.68± 1.03	6.68± 1.17
Total Dissolved Solids (g/l)	3.76± 0.72	3.54± 0.51	3.43± 0.62

11.1.5 Growth performance:

The average weight of shrimp was 43.38 ± 0.51g, 44.86 ± 2.12 in control, T_1 *and* T_2 pond respectively after 105 days culture period. T_3 pond was over flooded due to Ramel and heavy rainfall.

Table 4. Growth performance of cultured shrimp.

Parameters	Initial	T_1 Control (C)			T_2 (17 decimal)			T_3 (35 decimal)		
Days of culture	1st	30 d	60 d	105 d	30 d	60 d	105 d	30 d	60 d	105 d
Length (cm)	------	10.42 ± 0.39	14.82 ± 0.8	18.54± 01.5	11.32 ± 0.39	15.52 ± 0.8	18.04± 01.5	Data was not found; pond was flooded two times due to Remel and		

Weight (gm)	0.006	10.21± 12.20	21.35 ± 0.41	43.38 ± 0.51	10.01± 11.20	20.28 ± 0.46	44.86 ± 2.12	heavy rain fall

The farmer was satisfied his overall production and growth performance of shrimp. And he was very happy that other ghers next to his gher were affected by virous but our treatment pond was not affected. These productions scenario implies that, production rate has been increased manifolds than the traditional culture practice.

Figure-5: Production of tiger shrimp in farmer gher

Experiment-2: Bioactivity analysis of *Najas indica* and Shrimp health status

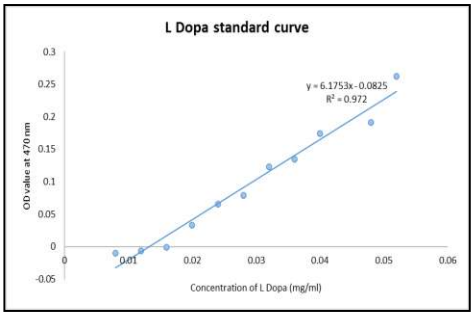
Analysis of Antinutritional factor of *Najas indica*

This study investigated the presence of Tanin, L-Dopa, Tripsin inhibitor and Phytic acid a bioactive compound, in the aquatic plant *Najas indica* which are known as antinutritional factor. In *Najas indica;* tannin was higher (10.77%) among them.

Antinutritional components	Amount (%)
Tanin	10.77
L-Dopa	0.01

Tripsin inhibitor	0.0176
Phytic acid	0.592

Table 5. Antinutritional components in *Najas indica*



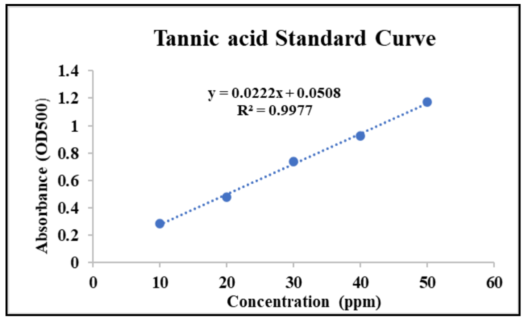


Figure 6. Standard curve for antinutritional analysis

The analysis revealed a concentration of 0.01% (dry weight basis), the concentration found in *Najas indica* falls within the lower range typically reported for plants (0.04% - 10%).

L-Dopa naturally found in various plants. Its concentration varies significantly depending on the species, variety and even the plant part.

The phytic acid concentration (0.592%) in *Najas indica* falls within the range reported for various aquatic plants. However, further research is needed to determine its specific impact on shrimp. While some studies suggest that shrimp can utilize phytate phosphorus to a certain extent others report potential negative effects on growth and mineral absorption.

11.2.2 Histology analysis of shrimp muscle

The muscle of Shrimp from *Najas* sp. pond has much more integrity than the control ponds. That means in *Najas* sp. containing shrimp feels less stress than without *Najas* sp. containing pond that why it accelerates their growth.

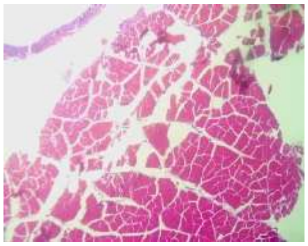
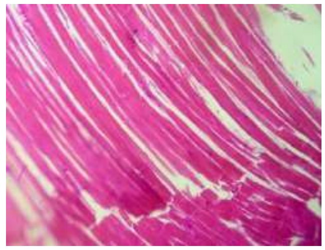
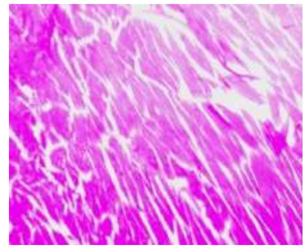
		
Control pond (without Najas + Normal feeding)	20% Najas + 50% feeding	20% Najas + No feeding

Figure 7. Histology of culture pond's shrimp

Trial for pure compound isolation from *Najas indica*

Initially pure compounds are isolating using gel column chromatography. 8 fractions are collected from *Najas indica* extract. From these, 3 compounds are highly sensitive in UV light that means these compounds are double bond compounds. Its need further study to compound structure determination and their activity.

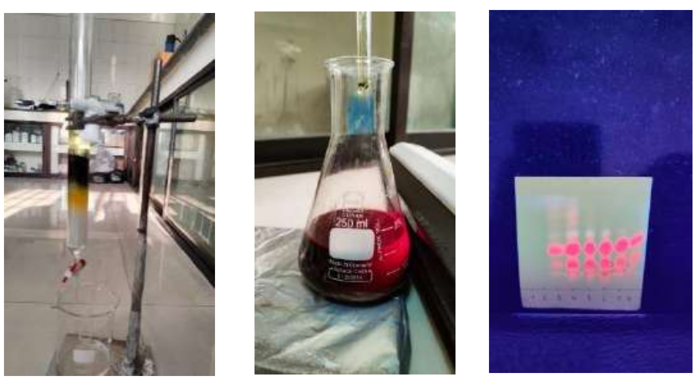


Figure 8. Pure compound isolation of *Najas indica*

Application of Nanoparticles (NPs) as Feed Additives and Efficient Therapeutic Strategy for Shrimp Health Management

Researcher(s)

: Dr. ASM Tanbirul Haque, SSO and PI
: Md. Shoebul Islam, SO

Objectives:

- ✓ To explore antimicrobial capabilities of different multifunctional Nanoparticles (NPs) against infectious pathogens of shrimp.
- ✓ To develop an efficient feed additive for aqua feed industry.
- ✓ To assess the biocompatibility of using NPs.

Achievements for the year 2023-24

Experiment-1: Eco-friendly green synthesis of Nanoparticles (NPs) with different plant extract

Nanoparticle formation:

With repetitive trial and error basis, concentration of $AgNO_3$, pH of extract and Extract: $AgNO_3$ ratio were fixed at 5mM, pH-10 and 1:2 respectively considering the highest peak (OD value) between 300-500 nm. After 6 hours, the synthesis solution of silver nanoparticles using pH variations from *A. ilicifolius* leaves extract showed various colors. The colors formed at pH 4 were yellowish, light brown at pH 6 and dark brown at pH 10 and pH 12. This color is the characteristic from the surface plasmon resonance of AgNPs (Roy et al., 2015; Nurfadhila et al., 2018; Handayani et al., 2020). The UV-Vis spectra were subtracted to show the equalize baseline between treatments. The difference in the value of λ max and the number of λmax can indicate differences related to the size and shape of AgNPs (Aziz et al., 2018). The AgNPs absorbance value increases with the increasing of pH, except pH 12, where at pH 10 there is a high absorption value, and there were 3 peaks that indicate different sizes of AgNPs. A higher absorption value can also indicate a higher number of nanoparticles (Handayani et al., 2020). The formation of Nanoparticle were accomplished following steps: (1) incubation at 60 ^{0}C for 6 hours (2) centrifugation at 10000 rpm for 10 minutes, (3) washing with sterile ddH_2O 2/3 times at 10000 rpm for 15 minutes & (4) drying at room temperature for 2 days

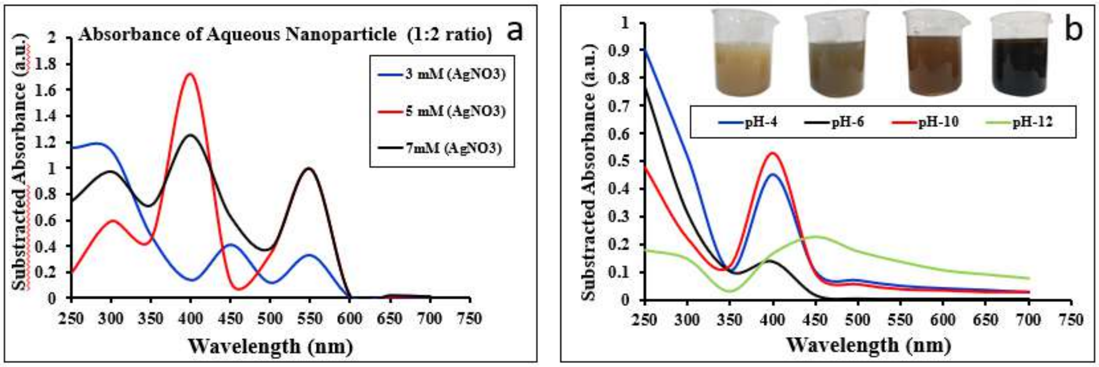


Figure 1 : Formation of nanoparticles through green synthesis

Characterization of (structural and chemical composition) of *A. ilicifolius* and *M. oleifera* leaf extract coated composite AgNPs:

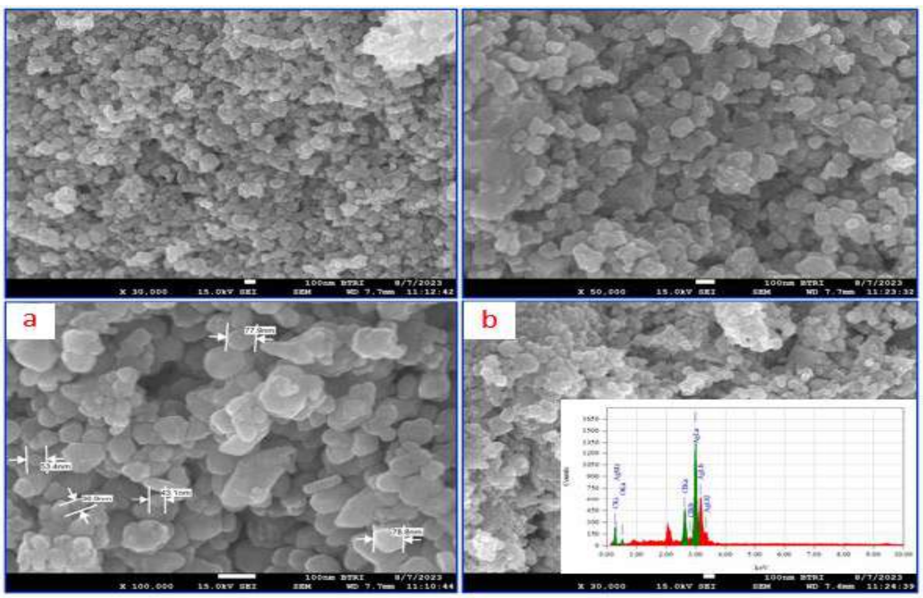


Figure 2: SEM with EDX image of *A. ilicifolius* extract mediated AgNPs.

According to Song et al. (2009), Nanoparticles usually referred as particles with a size up to 100 nm. In the present study, results showed in figure 2 (a), the average particle size was 36.9 nm to 78.8 nm which confirmed that nanoparticle formation. The result of EDX analysis showed in figure 2 (b), where stone signals for silver and nitrate ions were detected. The signal at 3.0 strongly suggested that corresponding binding energies of AgLa was the element of the NPs. Average particle size in sajina plant mediated nanoparticle was 38 nm to 60 nm which confirmed that nanoparticle formation (Figure 3 a). Additionally, C, Ag and Oxygen also detected showed in figs. 3 (d).

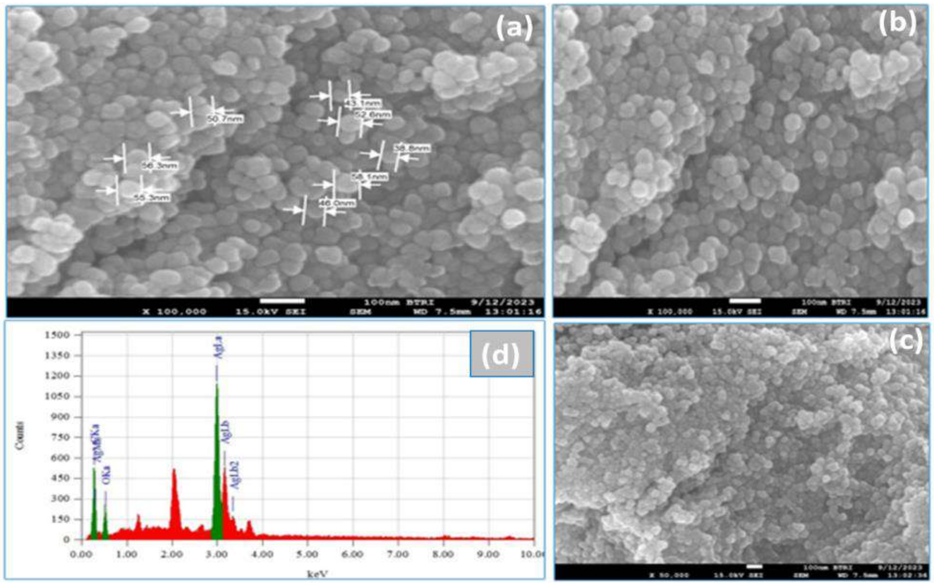


Figure 3: SEM (a, b, c) with EDX(d) images of Sajina extract mediated AgNPs

Total phenolic content (TPC) and Total flavonoid content (TFC)

The total phenolic content and total flavonoid content of the extracts are expressed as mg/L of gallic acid equilavent (GAE), Catechin equivalent (CE), Trolox equivalent respectively (Figure 2). The TPC in the extracts were determined from the gallic acid calibration curve with a regression equation of y=0.001x+0.0028 and R^2 =0.9948 (Figure 4). And the TFC in the extracts were determined from the Catechin calibration curve with a regression equation of y=0.0003x+0.0115 and R^2 =0.9754 (Figure 5). The values clearly indicate and confirm the presence of phenolic compounds in the leaf extracts of *Acanthus ilicifolius*.

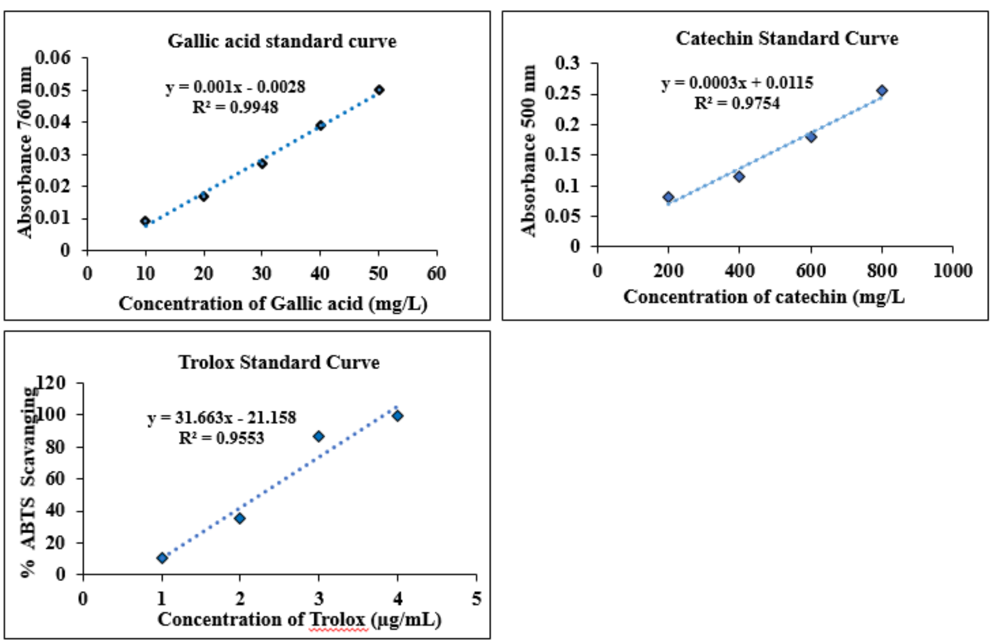


Figure 4. Different standard calibration curves

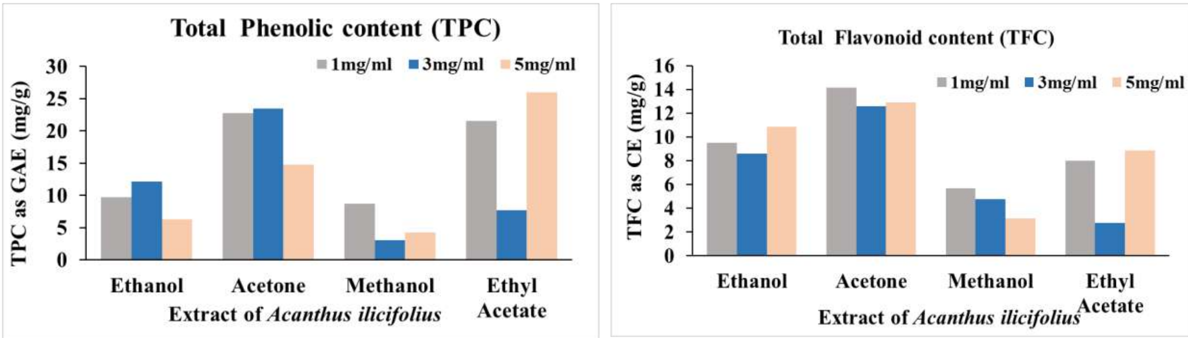
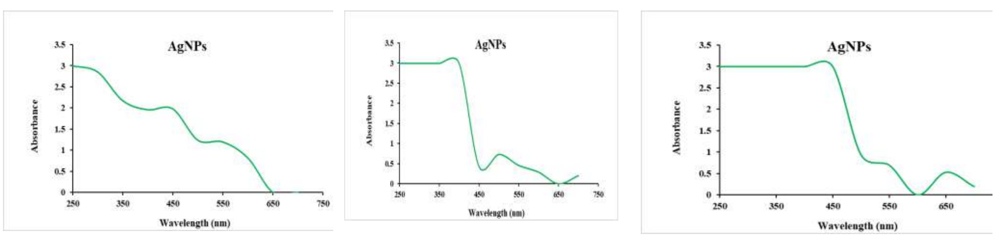


Figure 5: TPC and TFC content of *A. ilicifolius*

Storage Optimization of Nanoparticles:

In our study, we looked at how well nanoparticles can be kept safe over time. We tested two storage methods: keeping them at room temperature and putting them in the fridge. We found that nanoparticles can last for 21 days at room temperature, but they change a bit during that time. On the other hand, when we stored them in the fridge, they didn't change as much, suggesting that refrigeration might be a better option for keeping them safe for longer. This study highlights the importance of storing nanoparticles properly.



Day7 at Room Temperature Day 14 at Room Temperature Day 21 at room Temperature

Figure 6: Storage Optimization of Nanoparticles

18.4. Infection experiment for the determination of NPs efficacy against specific pathogen

We stocked post-larvae (PL) shrimp and started to rear them and then stocked the juvenile in the aquarium of wet lab.

Figure 7 : PL reared in nursery pond and stocked in wet lab for challenge experiment

In this study, *Penaeus monodon* shrimp were used to evaluate the efficacy of nanoparticles (NPs) in enhancing their immune response and survival against a pathogen responsible for Early Mortality Syndrome (EMS). The experiment consisted of five groups, including one control group fed with commercial feed without any nanoparticles, and four treatment groups that were fed diets supplemented with nanoparticles at concentrations of 5 mg/kg, 10 mg/kg, 15 mg/kg, and 20 mg/kg, respectively, for a duration of 15 days. During and after the feeding period, samples were collected to analyze various immune parameters, including total hemocyte count, phenoloxidase activity, and antioxidant enzyme levels such as superoxide dismutase and catalase, in order to evaluate the immune-modulating effects of the nanoparticles.

Figure 8: Some activities during challenge experiment

Following the feeding period, the shrimp were challenged with the pathogen responsible for EMS by injecting each shrimp with 10 µL of a bacterial suspension containing 10^6 colony-forming units (CFU) per milliliter. Mortality was monitored daily over a 7-day period, and mortality rates were recorded for each group. Further samples were collected post-infection for more detailed analysis of immune parameters and pathogen load in the shrimp tissues.

The analysis of the collected data, including immune response parameters and mortality rates, will allow us to conclude on the efficacy of the different nanoparticle concentrations in enhancing the immune response and survival of shrimp against EMS. These findings will provide critical insights into the optimal nanoparticle concentration that could be used to improve shrimp health and disease resistance in aquaculture systems.

Besides, hemolytic test of Nanoparticles at 25 mg/ml, 50 mg/ml and 100 mg/ml in concentration found toxic for blood cells

Figure 9: Hemolytic test of Nanopartices at different concentrations

To further understand the dose-dependent toxicity profile, we plan to extend this study by evaluating the hemolytic effect at lower concentrations. This will help determine the threshold at which nanoparticles remain biocompatible, contributing to the development of safer nanomaterial applications in biomedical settings. Future experiments will focus on systematically reducing the concentrations and analyzing the corresponding hemolytic responses to identify safer exposure limits.

Isolation, Production and Development of Indigenous Microalgae Based Live Feed Supply Chain for Prawn Hatchery

Researcher(s) : Rabina Akther Lima, SO
: Dr. A.S.M. Tanbirul Haque, SSO
: Md. Iqramul Haque, SO

Objectives:

- To identify the commercially important native microalgae from the south-western rivers of Bangladesh
- To utilize live feed as ready primary feed for various shrimp and prawn larvae
- To develop a portable photo-bioreactor model to facilitate pure live feed stock culture at field level

Achievements in 2023-24

Study-1: Identification of the commercially important microalgae from south-western rivers of Bangladesh
Achievements:

Live feed laboratory establishment

In the absence of a pre-existing laboratory dedicated to live feed culture, a new facility was specifically designed and constructed to meet the experimental requirements (Figure 1). This laboratory was outfitted with a comprehensive array of equipment to guarantee optimal environmental conditions that would foster the successful cultivation of live feed throughout the entire experimentation process.

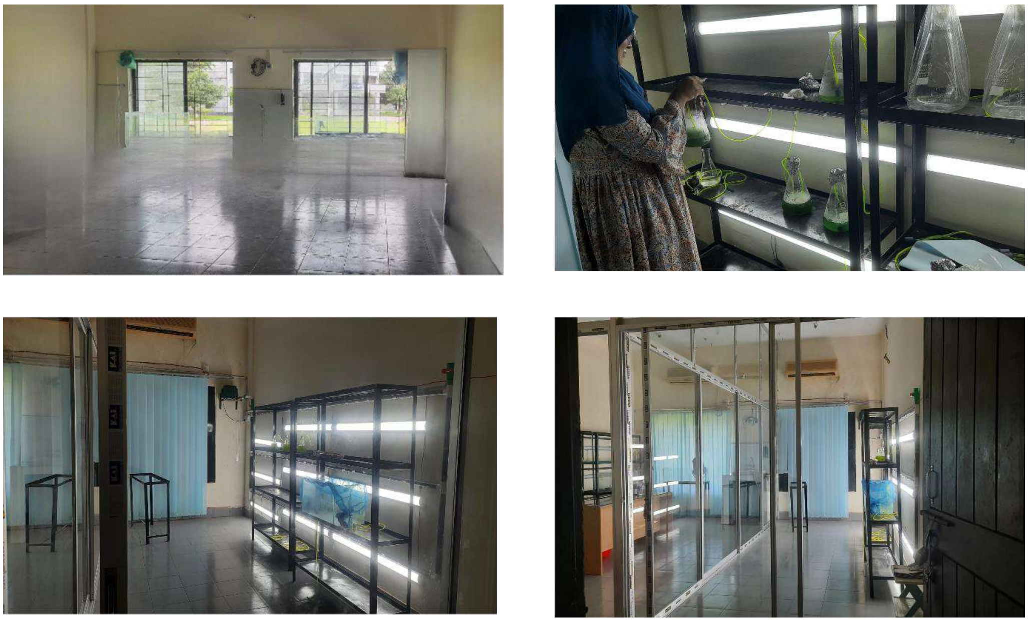


Figure 1. Pictorial view of live feed lab establishment

Collection of Plankton

The plankton sample collection was conducted in Bhairab river and it's connected branches, situated in Bagerhat district (Figure 2). A plankton net of 50 µm mesh size netting material was used to collect the plankton sample and stired in a Styrofoam box with ice to maintain 20-25^{0}C temperature during transportation. The study identified a total of 43 distinct phytoplankton species classified within 8 taxonomic classes. Notably, two genera, *Tetraselmis* and *Nannochloropsis*, were isolated from this diverse community due to their recognized commercial significance.

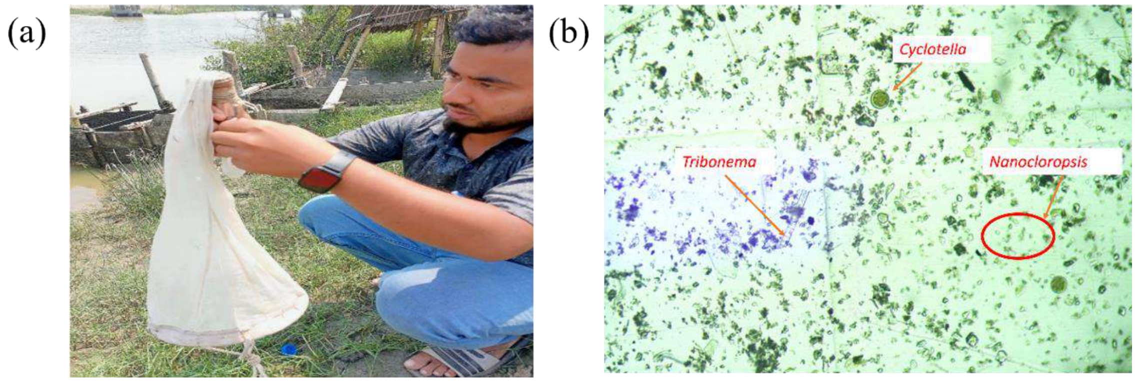


Figure 2: Pictorial view of microalgae identification (a) collection of phytoplankton (b) collected sample under microscope (40x).

Isolation of microalage

The *Tetraselmis* and *Nannochloropsis* specimens obtained from the designated sampling sites were cultured in f/2 medium to facilitate their isolation via the streak plating method (Figure 3). This approach aligns with established laboratory protocols for cultivating microalgae. The f/2 medium, a widely recognized formulation in algal cultivation, provides a balanced nutrient composition conducive to the growth and proliferation of microalgae. The streak plating technique, employed subsequently, involves the sequential streaking of diluted algal suspensions onto solid agar surfaces, enabling the isolation of individual colonies. Recipe of our using Guillard's f/2 medium depicted in Table 1.

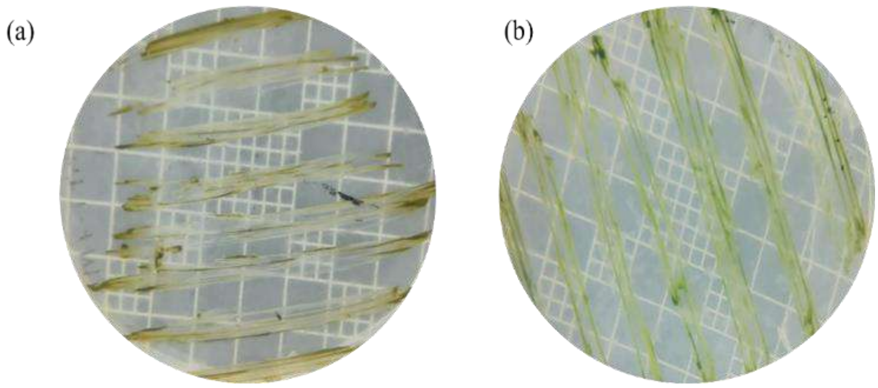


Figure 3. Isolated (a) *Nannochloropsis* and (b) *Tetraselmis* microalgae.

Table 1. Chemical composition of Guillard's f/2 medium.

Group A (Macro nutrients solution)	
Compound name and molecular formula	**Proportions**
Sodium nitrate ($NaNO_3$)	75 g
Monosodium phosphate ($NaH_2PO_4.4H_2O$)	5 g
Group B (Silicate solution)	
Sodium silicate ($Na_2SiO_3.9H_2O$)	30 g
Group C (Trace Metal solution)	
Zinc sulfate ($ZnSO_4.7H_2O$)	22 g
Copper sulfate ($CuSO_4.5H_2O$)	10g
Cobalt cloride ($CoCl_2.6H_2O$)	10 g
Manganese chloride monohydrate ($MnCl_2.4H_2O$)	180 g
Sodium molibodate dihydrate ($Na_2MoO_4.2H_2O$)	6 g
Zinc sulfate ($ZnSO_4.7H_2O$)	22 g
Group D (EDTA solution)	
Disodium EDTA salt (Na_2 EDTA.2 H_2O)	4.36 g
Ferric chloride ($FeCl_3.6H_2O$)	3.15 g
Group E (Vitamin solution)	
Vitamin B_{12}	1 ml
Biotin	10 ml
Thiamine (Vitamin B_1)	200 ml

Algal culture operations

1. Preservation of stock culture: Pure stocks of microalgae (*Nanocloropsis, Tetraselmis,* and *Chlorella*) were collected from the live feed lab of BFRI, Marine Fisheries Technology Station, Cox's Bazar, and Brackishwater Station, Paikgacha, Khulna (Figure 4b, 4c, and 4d). Test tubes (10 ml) filled with enriched sea water were inoculated with 0.1 ml of stock culture and incubated in light for 12 hours (Figure 4a). In this method, the algae was stored and maintained for 15 days. Afterwards, the above procedure was repeated to keep the algae in the active growth phase. The stock culture is stored in the refrigerator with the renewal, as its shelf life is one month.

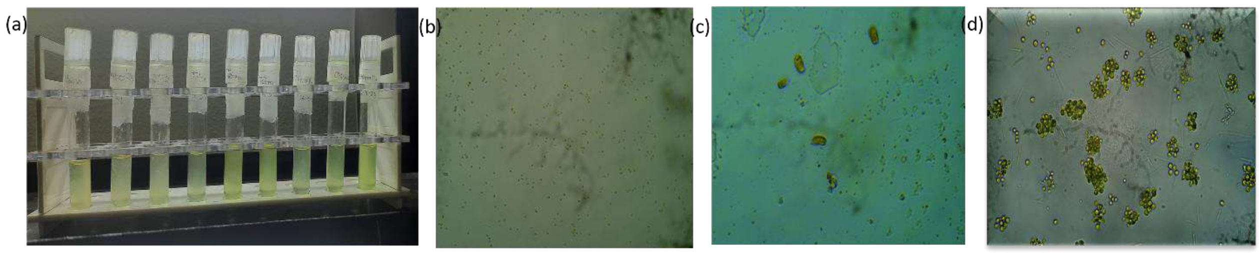


Figure 4. Isolated pure stock of microalgae (a) stored sample (b) *Nanoclioropsis* (c) *Tetraselmis* and (d) *Chlorella* .

2. Flask culture: The stock culture maintained in a 5 ml testube was passed through many progressive culture steps for flask culture (Figure 5). At first, 50 ml of stock culture was inoculated into 250 ml sterile flasks with enriched sea water and incubated in light for two days with continuous aeration to get a density of 5 million cells per ml. These 250-ml flasks were scaled up to 500 mL, then 1000 mL, and 2000 mL, respectively. Around 7-8 days of culture, microalgae reach the highest density. Table 2 depicts the highest density of the studied microalgae.

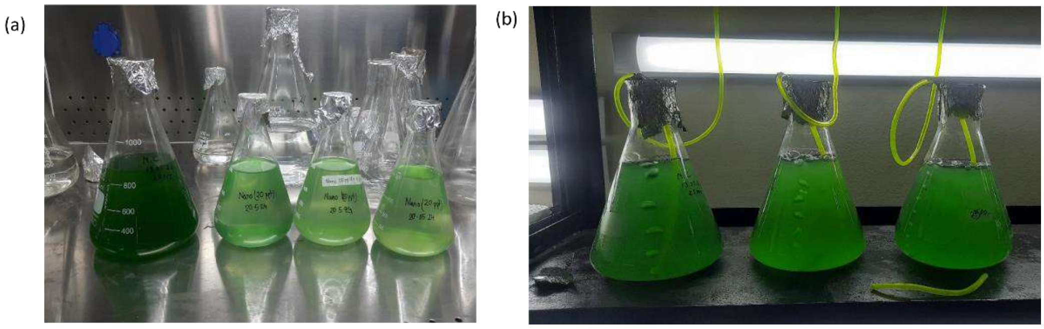


Figure 5. Flask culture of microalgae (a) scale up from previous stock (b) 3 days after inoculation.

Table 2. Highest density after the scale up of isolated microalgae.

Species name	**Cell size (µm)**	**Isolation method**	**Salinity during isolation (ppt)**	**Highest density (cell/ml)**
Nanocloropsis oculata	2-4	Serial dilution	29	8.79×10^{7} at 8^{th} day
Nanocloropsis	4-12	Do	25	6.79×10^{7} at 6^{th} day
Chlorella sp.	4-10	Do	27	4.84×10^{8} at 7^{th} day
Tetraselmis suecica	8-10	Do	27	8.36×10^{7} at 7^{th} day

Refinement of existing organic shrimp *(Penaeus monodon)* farming using eco-friendly management protocol in southwest region of Bangladesh

Researcher(s) : Dr. Md. Harunor Rashid, CSO & PI
: Rabina Akther Lima, SO
: Md. Iqramul Haque, SO

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 in 2023-24

Validation of organic shrimp (*Penaeus monodon*) farming through multi-location trial

Achievements:

Study area:

For Validation of organic shrimp (*Penaeus monodon*) farming through multi-location trial, the following interventions had done. Two farmers were selected in three different locations for the study. Two potential experimental sites were selected in Bagerhat Sadar upazila (Site: Bemorta and Karapara) by physically visiting the farmers ghers. Similarly, one ponds in Shrimp Research Station was used considering the experimental design. During trial, selected farmers got all sorts of inputs such as fish fry, fish feed, fertilizers, lime etc. Production will take the farmer and only data will be taken from these ponds fortnightly.

Treatments	Locations	Farmer Name	Address	Feed	Stocking density (individual/m^2)	Culture period (days)
T1	On station		Shrimp Research Station, Bagerhat	Formulated organic feed	5	120
T2	On farm	Md. Shohag Ali Sheikh	Joygachi, Bagerhat sadar,			
T3	On farm	Aswab Ali Molla	Gomoti, Bagerhat sadar,			

Figure 1: Validation of organic shrimp (*Penaeus monodon*) farming in Bagerhat sadar

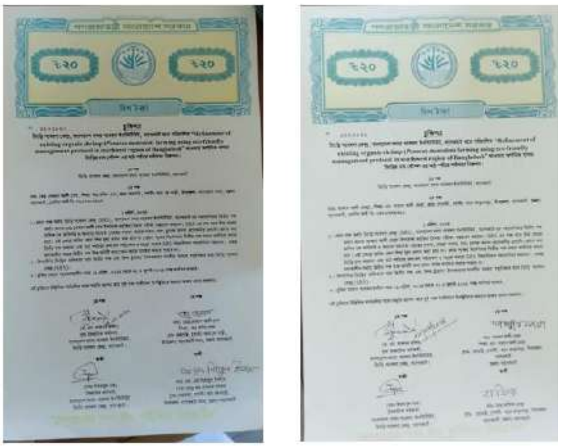


Figure 2: Agreement papers

Pond preparation and pre-stocking management

After being drained out and re-excavated to clear away the polluted layer of bottom mud, all of the chosen ponds were entirely exposed to the sun for 5-10 days to get rid of other unpleasant gases. Ponds were prepared by repairing embankments and clearing weeds of various kinds. To maintain post-larval shrimp in rearing ponds for a short period of time (10-15 days), approximately 10% of the area of each treatment was surrounded by nylon mesh secured with a bamboo frame. The surrounding pond area was biosecurity walled off with blue net to keep out virus-carrying organisms. Prior to the study, ponds were given a 250 kg/ha agricultural lime ($CaCO_3$) treatment based on the pH of the soil. Small mesh filter nets were used to fill the ponds with tidal water till a depth of 1.0 m and the water was disinfected and cleared of all animal life using chlorine at a concentration of 20 ppm. Organic fertilizers such as fermented mixes of molasses, rice bran, and yeast were administrated to the ponds in a 40:25:0.12 kg/ha ratio.

Figure 3: Pond preparation

After applying molasses for two to three days, 60 kg of liquid mustard oil cake was applied. The water's color changed to green after 4-5 days of fertilization

Source of post-larvae and pond stocking

Solely organic tiger shrimp, *P. monodon* (specific pathogen free; SPF) post-larvae (PL-12) collected from organic hatchery was purchased from Cox-bazar. Shrimp post-larvae was transported in plastic bags and upon arrival at the farm, slowly acclimated to the pond conditions for 1 h. After acclimation properly, the PL was released directly into the pond according to the experimental design on 4^{th} week of June 2024 in Bagerhat upazila at the stocking density of 5 individual/m^2.

Fig 4: Stocking of shrimp

Feeds and feeding

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

Table 2: Proximate composition of Formulated organic feed

Parameter	Calculated value (%)
Protein	32.0
Lipid	3.24
Moisture	7
Ash	12.5
Fiber	9.5

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) @ 40 kg/ha; 120 g/ha; 25kg/ha were applied. Post stocking management such as liming, water exchange is being done regularly. Shrimp and water quality sampling are being done at 7 days interval.

Physicochemical characteristics of Soil:

Physicochemical characteristics of soil (salinity, pH, organic carbon, total nitrogen, phosphorus and potassium) was analyzed in the laboratory of SRDI Khulna throughout the culture period. Soil profile indicated that initially the soil was moderate in pH, but pH level in the final sample reduced in all the treatments than the initial. The presence of nutrient (Nitrogen and phosphorus) and organic matter in soil was very low in the initial sample, but increased with culture duration and with the increment of stocking densities

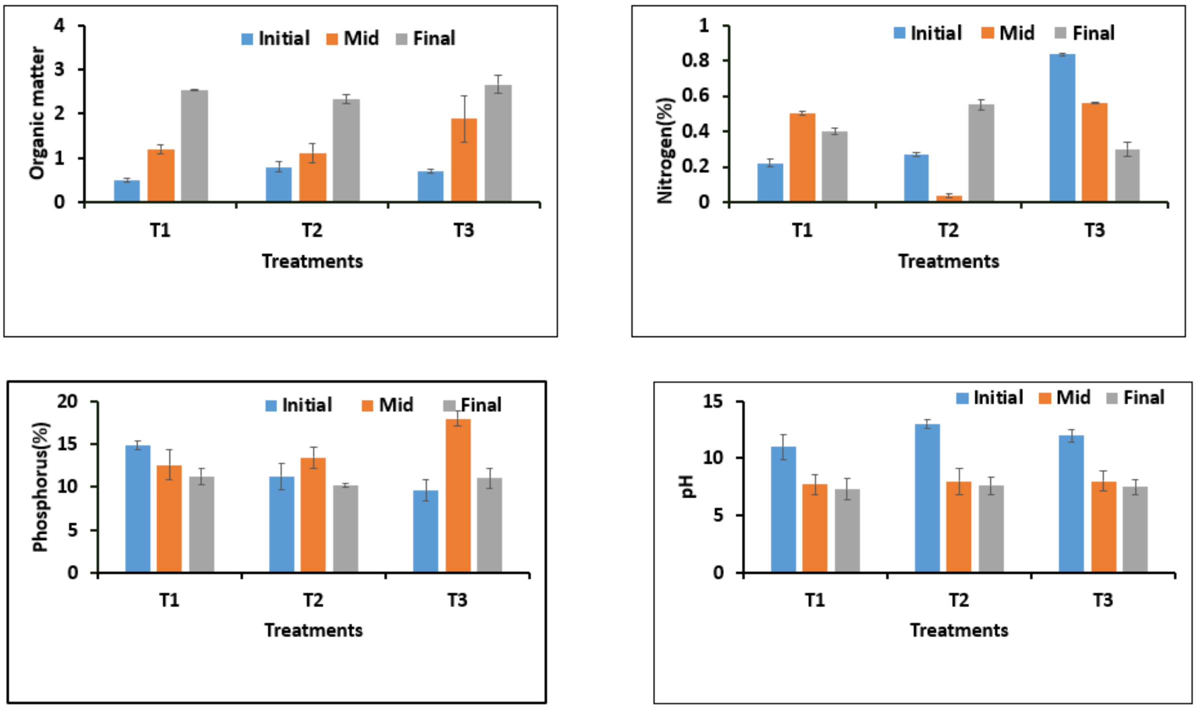


Fig 6. Soil characteristics of organic shrimp ponds a) Organic matter b) Nitrogen (%) c) Phosphorus (%) d) pH under different treatments

Water quality management and measurements

Water quality parameters such as water temperature, pH, Dissolved oxygen (DO), Salinity, Alkalinity and Ammonia were measured regularly at 7 days interval throughout the experimental period. All the parameters were found almost suitable in all treatments in every sampling date without showing considerable difference (Table 3)

Table 3. Water Quality Parameters of organic shrimp ponds

Pond	DO (mg/L) (Mean ± SD)	Temperature (^{o}C) (Mean ± SD)	pH (Mean ± SD)	Salinity (ppt) (Mean ± SD)	Alkalinity (mg/L) (Mean ± SD)	Ammonia (mg/L) (Mean ± SD)
T1	7.43±2.80	31.59±0.69	7.01± 0.14	5.18±2.58	142.30±48.48	0.12±0.08
T2	6.59± 1.16	30.11± 1.03	7.17± 0.93	6.39± 2.00	160.33±40.23	0.19±0.01
T3	7.28±1.06	31.18±1.09	7.19± 0.43	7.14±2.22	145.33±8.86	0.2±0.03
Recommended range	≥ 5	28-32	7.0 – 8.3	5-30	≥ 100	≤ 0.1

Temperature of the experimental ponds varied from 34.56 to 29.92 ^{o}C with slight fluctuation during the culture period. Highest temperature was recorded in the 1st, 2nd week of the culture period which was due to the fluctuations of seasonal variations in sunlight exposure and length of the day. Dissolved oxygen (DO) level in all treatment was always >5.0 mg/L and ranged between 5.25-10.67 mg/L. Lazur (2007) mentioned that the optimum range of dissolved oxygen for shrimp and prawn is >4 ppm which is very similar to finding of the present study. pH is the concentration of hydrogen ions (H^+) present in water is a measure of acidity or alkalinity. The pH value of all experimental ponds ranged between 6.31-9.11. The salinity level in different experimental ponds varied within 4.31 to 10.04 ppt. Transparency is the most crucial factor for shrimp culture ranged between 15 to 28 cm in all experimental ponds. Meanwhile, that of total alkalinity ranged between 142-180 mg/L. However, all the monitored water quality variables were within acceptable ranges and favorable for the growth, survival and culture of organic shrimp in earthen ponds.

Qualitative and Quantative Plankton analysis:

A number of Phytoplankton and Zooplankton groups were found dominated in T2 compared to others. Among the zooplankton groups, Euglenophyceae, Rotifers, Copepods, Crustaceans and Phytoplankton groups Bacillariophyceae, Cyanophyceae, Chlorophyceae were available. Higher quantities of zooplankton in T2 compared to other treatments were recorded which might be due to availability of nutrients and favorable water quality parameters.

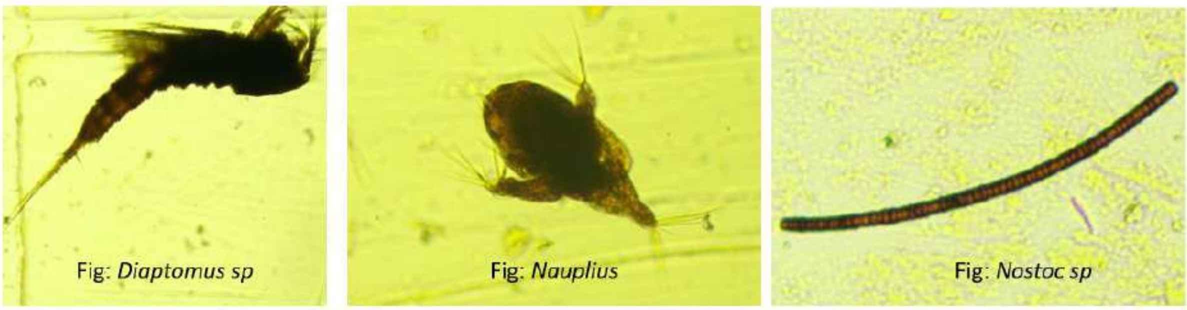


Fig 6. Isolated plankton species during the experimental period

Lowest Phytoplankton counts were found in T3 (3.86×10^3 cells/L) and highest in T2 (11×10^3 cells/L). Similarly, Zooplankton counts were found in T3(2.8×10^3 cells/L) and highest in T2 (5.8×10^3 cells/L).

Chlorophyll-a measurement:

Chlorophyll-a was calculated following the equation,

Chlorophyll-a (µg/l) = v/(Vxd) x [11.60(E665-E750) -1.31(E645-E750) - 0.14(E630-E750)

Where, v = vol. of extract (ml)

V = vol. of water sample (1)

d = Path length of the cuvette (cm)

Chlorophyll- a was found higher in T2 than the other ponds similarly with phytoplankton and zooplankton abundance.

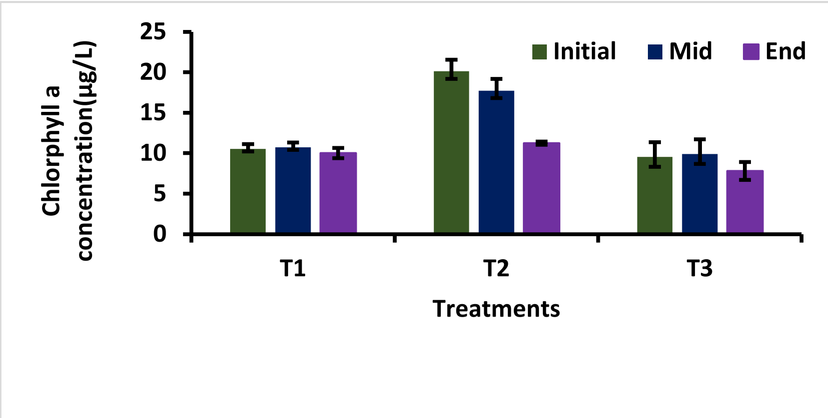


Fig 7. Chlorophyll-a concentration in experimental pond

Microbial load count:

Bacterial load in bottom sediment was found low at initial sample and decreased to almost zero after application of liming, but increased further with the increase in culture duration. The concentration of bacterial load was higher in the end sample and it was highest in higher stocking density at the end of culture period. However, increasing of culture of shrimp might lead excessive influx of uneaten feed, faeces and molted shell on to the bottom sediment, which may have increased the bacterial load and activity onto the sediment-water interface.

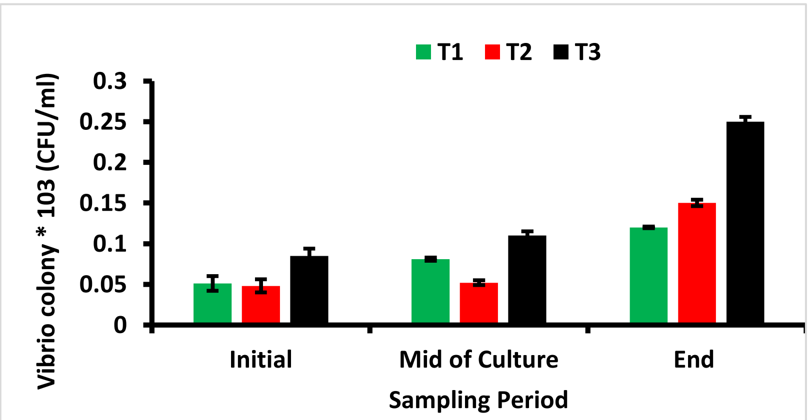


Fig 8: Microbial load count

Growth Performance of Organic shrimp

After 120 days of culture, all shrimps will be harvested first by seine netting and then by draining out of the ponds and production will be estimated. Higher growth was observed in T1 in comparison to others after 90 days culture period, in T1, T2 and T3 were 21.56±0.41g, 20.53±0.48g and 17.87±0.73g respectively. Compare to other treatments, T1 shows good result. After 90 days of culture average length in T1, T2 and T3 12.45± 1.01, 10.5±0.42 cm and 9.99±1.12 cm respectively. Growth performance of organic shrimp under different treatments which was shown in the following figure:

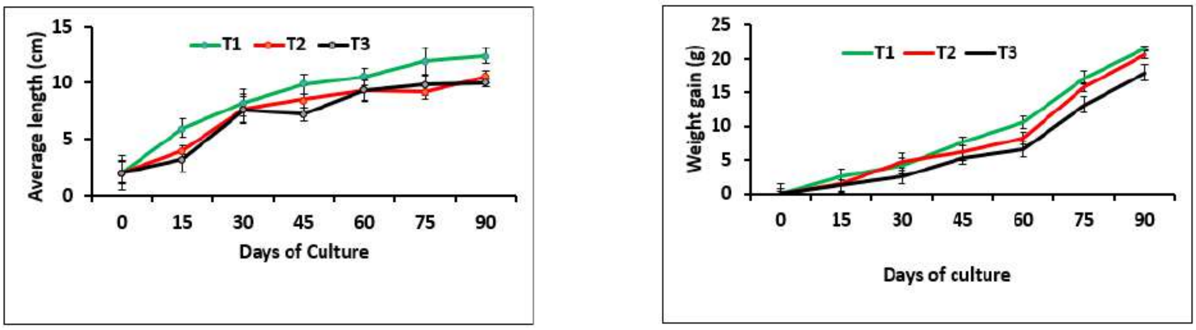


Fig 9: Growth Performance of Organic shrimp

Prophylactic and Nutritional Properties of Algal Supplements as Larval Molting Promoter and Immune Modulator in Shrimp and Prawn Aquaculture

Researcher(s) : Md. Shoebul Islam, SO & PI
: Dr. A.S.M. Tanbirul Haque, SSO
: Md. Ariful Islam, SSO
: Md. Iqramul Haque, SO

Objectives:

- To develop pathogen checklist for prawn hatchery
- To infer prophylactic activity of different natural sources
- To develop improved larvae rearing technique using enriched artemia, higher nutrient rich compounds and probiotics
- To develop medicated feed for shrimp and prawn culture

Achievements in 2023-24

Study-1: Investigation of causative agents for larval mortality of prawn

Under this experiment, Samples were collected from the rivers Kocha , Baleshwar and Meghna rivers of Bangladesh in fresh condition during breeding season and were transported to Shrimp Health Management Laboratory, SRS Bagerhat. Prawn pleopods from each prawn were finely chopped and then 20~30 mg was used for the DNA and RNA extraction for PCR-based diagnosis of OIE-listed and non-listed pathogens. Along with the brood, all probable source of pathogen viz., artemia, brine, fresh water, ingredients of egg custard, etc. were screened for pathogens (Figure 1 and 2).

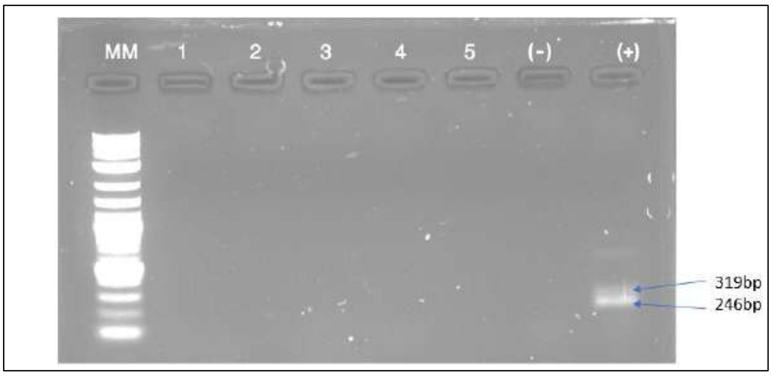


Figure 1: PCR assay for MrGV. UV illumination of PCR product after gel electrophoresis; MM: molecular marker, Lane 1~3: brood from Kocha river, Lane 4: Larvae from control tank of hatchery (with no microalgae), Lane 5: Larvae from treatment tank (with algae), Lane (-): Negative Control, Lane (+): Positive Control.

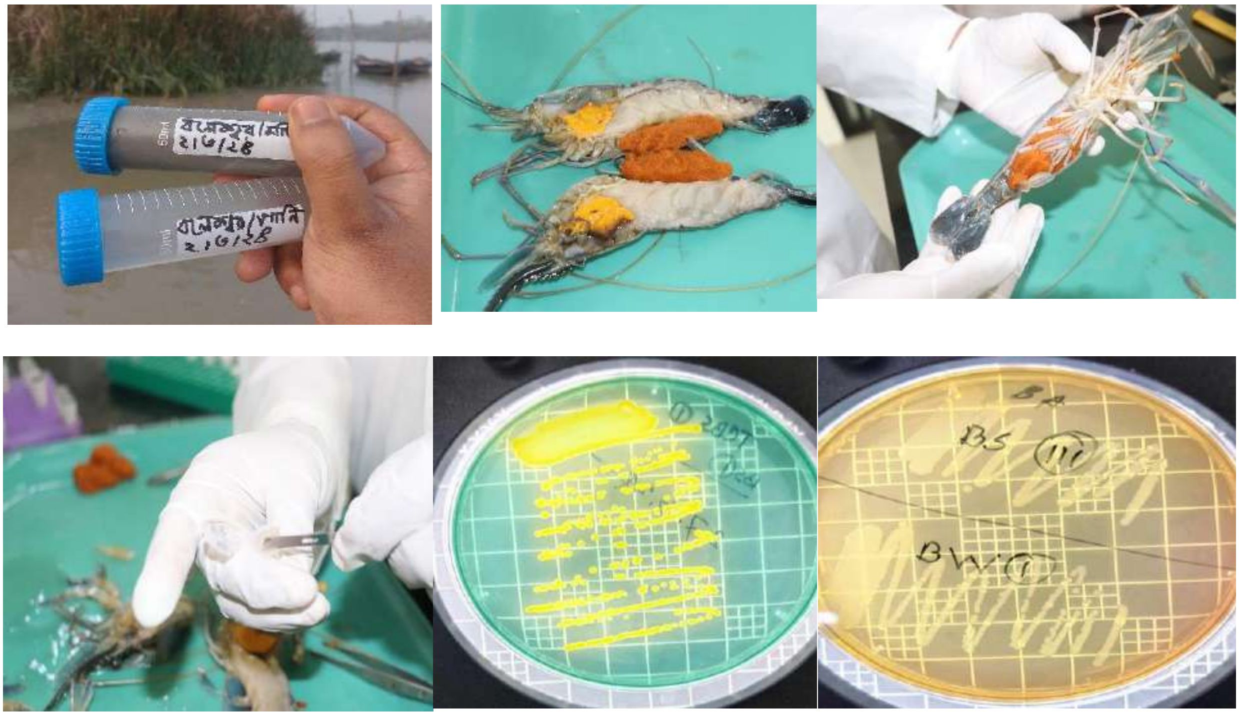


Figure 2: Sample collection and ongoing microbial analysis

Study-2: Assessment of larval molting efficacy using different plants & seaweeds

Table 1: Experimental Design

Treatments	Sources of the natural Extract	Dose of microalgae (Black algae powder + spirulina powder)	Stocking Density
T_0 (Control)	Black Algae	(2+2) 4 ppm	2000 larvae/litter
T1	*Ulva intestinalis*		
T2	*Hypnea musciformes*		
T3	Microalgae		

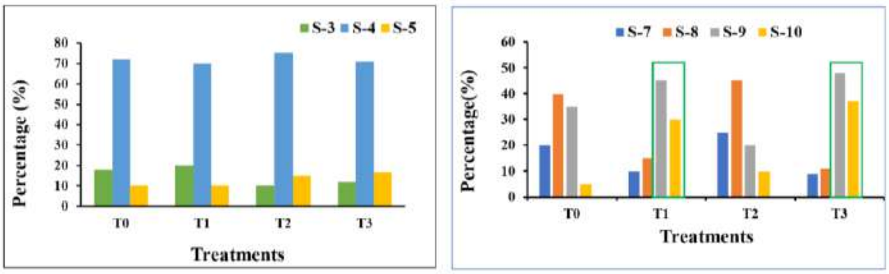
To make wet-lab experiment, Three treatment and one control group of prawn larvae (stage-3:72%, Stage-4:27%, Stage-5: 1%) with 2000 individuals were transferred in to the each 300 L fiber glass tank containing UV treated water. Black Algae, *Ulva intestinalis, Hypnea musciformes* and Microalgae was provided at a dose of 4.0 ppm with 4 and 6 times per day in the fiberglass tank of T0, T1, T2 & T3 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.

Figure 3: Some activities during Wet Lab Experiment

During the larval rearing process, continuous aeration was maintained to ensure a sufficient supply of oxygen, which is essential for the optimal growth and development of the larvae. Aeration helps maintain dissolved oxygen levels in the rearing environment, supporting metabolic processes and promoting healthy development across the larval stages.

Figure 4: Observation of Larval stages of Prawn PL

The rearing period extended over 15 days in the controlled conditions of the wet lab. The rate of metamorphosis was determined by categorizing larvae into different stages. Following this, histological analysis was performed to examine tissue development, allowing researchers to observe cellular changes

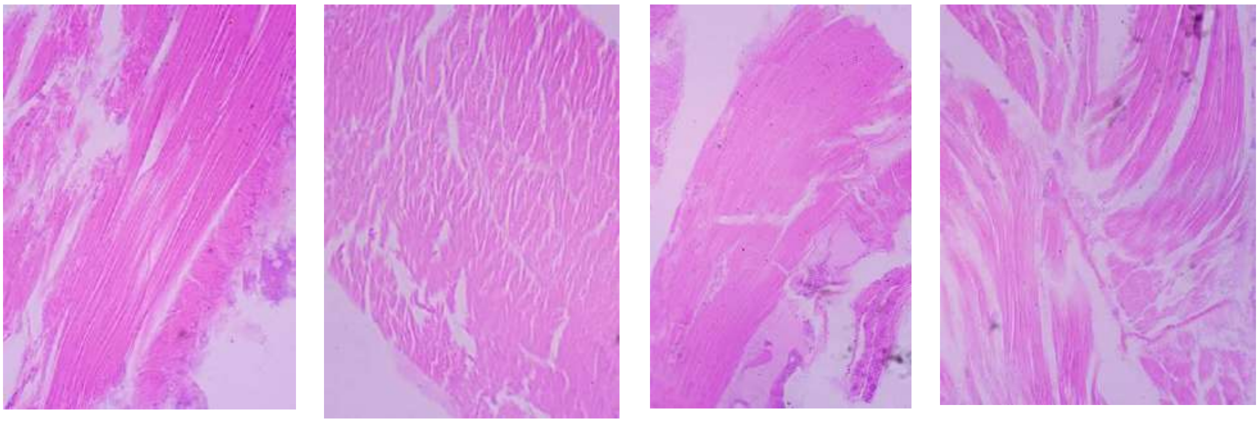


Stage Variations Before experiment

Stage Variations After experiment

Figure 5: Stage Variations of Prawn PL

Among all Treatments, T_1(*Ulva*) and T_3 (Microalgae) showed higher stage variation (stage-6: 10%, stage-7: 30%, stage-8:40% and stage-9:20% than other treatments. Histological analysis also showed similar results (Figure 4) where larvae of T_3 showed much more integrity of muscle than the others.



T_1 (*Ulva* sp) T_2 (*Hypnea* sp) T_3 (Microalgae) T_0 (Control)

Figure 6: Histological analysis

Digestive Enzyme Activities

Amylase, Protease and Lipase activity

Amylase activity was assayed by starch hydrolysis method in which the increase in reducing power of buffered starch solutions was measured. (Bhavan et al., 2010). The protease activity was estimated by using the casein-hydrolysis method by the method of Furne *et al.* (2005). 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).

Among all Treatments, T3 and T1 showed higher protease, amylase and lipase enzyme activities than other treatments. So,this could increase protease, amylase and lipase enzyme activity in shrimp.

Impact of higher nutrient content, LPS and probiotics on shrimp culture

In this experiment, the impact of the probiotics, higher nutrient content and LPS will be assessed during shrimp culture (Experimental Design described in Table 2).

Table 2. Impact of higher nutrient content, LPS and probiotics on shrimp culture.

Treatments	Description	Replication	Stocking Density
T1	Commercial Feed + LPS/ attenuated bacterial vaccine	3	$4m^{-2}$
T2	Commercial Feed+ LPS+ Probiotics		
T3	Commercial Feed + Probiotics		
T4 (control)	Commercial Feed		

Pl were stocked in the pond of the station. The growth performance is observed and shrimp sample and haemocyte is collected in every week for further analysis.

Figure 7: PL stocked in the growout pond for further analysis

Till now, 9 weeks have passed and the graph (**Figure 7**) represents the growth perform ance of shrimp across four treatments[(T1, T2, T3, and T4 (control)] over a 9-week period.

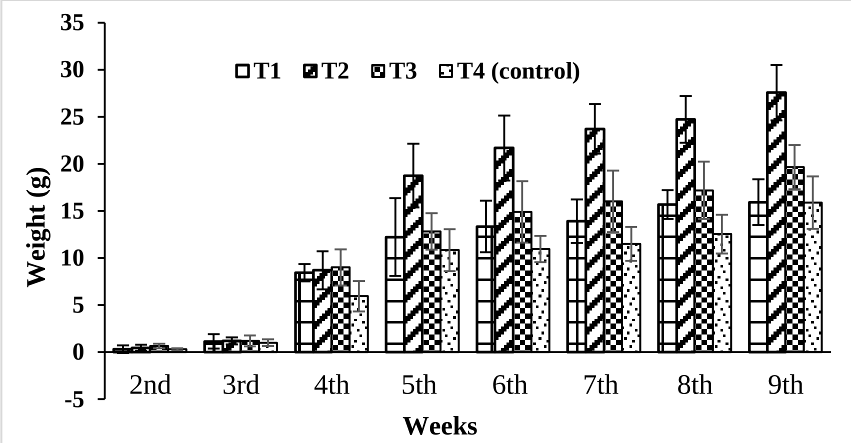


Figure 8: Growth Performance of shrimp after 9 weeks

The shrimp weight has shown a progressive increase starting from the 4th week, with notable differences observed among the treatments. As the experiment is ongoing, the assessment of growth performance, microbial load of the ponds, survival rate, and immunity is still in progress. A comprehensive analysis will be conducted after the 90-day experimental period to provide a complete overview.

Figure 9: Some Pictorial views of sampling and sample collection from experimental pond

Additionally, important immune parameters such as total haemocyte count, differential haemocyte count, phagocytic activity, and phenoloxidase activity will also be evaluated at the end of the study.

Identification and Culture Practice of Commercially Important Seaweeds in Bangladesh Coast

Researchers : Md. Mohidul Islam, SSO
: Mohammad Khairul Alam Sobuj, SO

Objectives of the project (2023-24)

i. To make a detailed inventory of available seaweed species in Bangladesh coast
ii. To develop culture technique (indoor to field) of selected seaweed in suitable areas
iii. To develop *in-vitro* tissue culture technique of some selected seaweed species
iv. To develop integrated seaweed-fish culture technique
v. Analysis of bioactive compounds from red seaweed

Achievements (2023-2024)

Study-1: Inventory of available seaweed

Surveys were conducted in and around Cox's Bazar (St. Martin Island, Teknaf, Inani, Bakkhali, Patowartek, Shaplapur, and Moheshkhali) from October 2023 to April 2024. Different seaweed samples were collected, photographed, preserved, and identified during the survey. Different species of seaweed i.e., *Cryptonemia seminervis, Cryptonemia crenulata, Jania cultrate, Valonia ventricosa, Chaetomorpha aerea, Gracilaria tenuistipitata, Ulva australis, U. intestinalis, U. torta, Catenella impudica, C. nipae, Colpomenia sinuosa, Bostrychia radicans, Caloglossa beccarii* and *U. compressa* were collected randomly by hand-picking from the study area at the time of low-tide. The collected samples were placed in plastic bags and afterward stored in an icebox for laboratory analysis. The samples were subjected to a gentle brushing procedure while being exposed to a continuous flow of seawater in the laboratory. Subsequently, they were rinsed with distilled water. The seaweed sample was subjected to drying using herbarium pressing frames as an alternative to the conventional method of direct sun drying. Over a period of three consecutive days, wet papers were switched daily, resulting in the preservation of the dry seaweed samples within the laboratory. Till now a total of one hundred and fifty-eight (158) seaweed species have been identified from our coast. This year we preliminary identified four (04) seaweed samples as a new species. After having the DNA Barcoding results, we will enlist these four species as a new one.

Gracilaria salicornia

Ulva compressa

Ulva intestinalis

Bostrychia kelanensis *Catenella impudica* *Catenella caespitosa*

Figure 1. Seaweed species collected during inventory study.

Table 1. Availability and distribution of seaweed.

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

Figure 2. Possible newly identified seaweed species

Study-2: Seaweed culture

Experimental culture sites of seaweeds were set up in sheltered intertidal zones of Chowfoldondi (N21°30'13.1076", E91°59'38.8392"). A culture experiment was set up in early January at the

Chowfoldondi site (Figure 3). The floating raft method was applied for the culture experiment which 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. Micronutrient-enriched seaweed species *Hypnea musciformis* was selected for culture experiments. Seeds were collected from the Saint Martin Island. 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 16 seeds/m^2. The culture period was 90 days and partial harvesting was performed at 15 days intervals.

Figure 3. Seaweed cultivation at the floating raft.

Between January 2024 to March 2024, a total of 06 partial harvests of *H. musciformis* were made in the culture site. Growth and water quality parameters were measured fortnight. Harvesting at the end of the 90-day duration of the culture period resulted in the absolute biomass yield production of 16.8 ± 1.44 kg fw/m^2 with a daily growth rate 4.94 ± 0.09 %/day for *H. musciformis*. Water quality parameters recorded during the experimental period of the study area are presented in Table 2.

Table 2. Water quality parameters of the study area.

Experimental site	Range values of hydrological data					
	Temperature (°C)	Salinity (‰)	DO (mg/l)	pH	Alkalinity (ppm)	Transparency (cm)
Chowfoldondi	25 – 31	31 – 36	6.0 – 8.5	7.0 – 8.5	120 – 145	40 – 55

For the pond/gher experiment:

Experimental culture sites of seaweed (*Gracilaria tenuistipitata*) were set up at a gher of Chowfoldondi. A culture experiment was set up in early January (Figure 4). The floating raft method was applied for the culture experiment which was made of bamboo poles and recycled plastic water bottles. Growth and water quality parameters were measured fortnight. Harvesting at the end of the 60-day duration of the culture period resulted in the absolute biomass yield production of 5.45 ± 0.58 kg fw/m^2 with a daily growth rate 6.03 ± 0.16 %/day for *G. tenuistipitata*. Water quality parameters recorded during the experimental period of the study area are presented in Table 3.

Figure 4. Seaweed cultivation at gher of Chowfoldondi.

Table 3. Water quality parameters of the study area.

Experimental site	**Range values of hydrological data**			
	Temperature (°C)	Salinity (‰)	DO (mg/l)	pH
Chowfoldondi	25 – 30	22 – 25	6.5 – 8.0	7.0 – 8.0

Indoor seaweed culture:

Seaweed species (*H. musciformis*) were stocked in laboratory conditions of MFTS, Cox's Bazar in the tray (0.45m x 0.30m x 0.08m) method (Figure 5). Growth and water quality parameters were measured every twenty days' intervals. Harvesting at the end of the 80th day of the culture period resulted in the absolute biomass yield production of 117.2 ± 3.88 kg fresh weight, where initial stocking was 40.0 ± 2.48 kg fresh weight. Weight increases of *H. musciformis* in indoor conditions are shown in Table 4. Water quality parameters recorded during the experimental period are presented in Table 5.

Table 4. Weight increase of *H. musciformis* on different culture methods.

Culture method	*H. musciformis* weight (Mean ± SD)				
	Initial	20th day	40th day	60th day	80th day
Tray	40.0 ± 2.48	65.8 ± 4.16	92.1 ± 3.70	108.5 ± 5.49	117.2 ± 3.88

Table 5. Hydrological data recorded in different culture methods at the laboratory.

Experimental method	**Range values of hydrological data**			
	Temperature (°C)	Salinity (‰)	DO (mg/l)	pH
Indoor	28 – 32	30 – 32	7.5 – 8.5	7.5 – 8.5

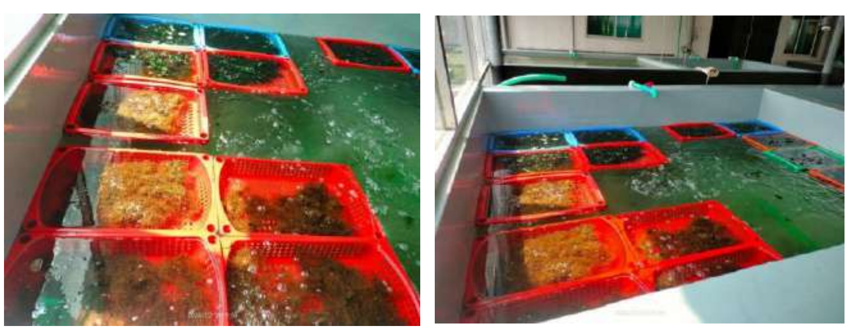


Figure 5. Indoor culture of seaweed.

Study-3: In-vitro tissue culture of seaweed

Seaweed seeds of (*U. lactuca*) 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 for the regeneration of 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.

Tissue isolation and purification: Fresh samples of *U. lactuca*, 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 on the ice. Selected vegetative thallus was once again cleaned thoroughly in autoclaved seawater using a soft painter brush to remove the epiphytic contaminants including dirt and biofilms if any.

Optimize environmental conditions for seaweed tissue culture: The thalli (0.25 mg) were chopped into 2 ± 1 mm in size pieces (Figure 6) 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 ± 1 ºC. The media was added every 2 days interval and the medium was changed every 15 days interval.

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

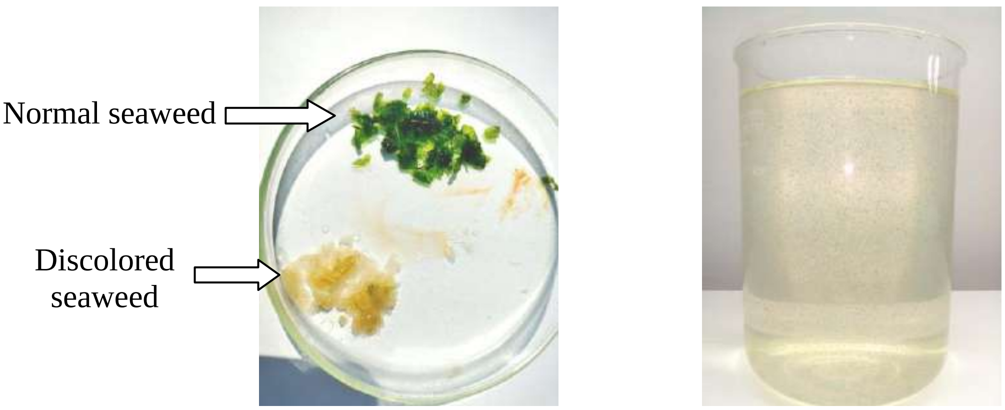
Figure 6. Tissue culture of seaweed

Growth performance of seaweed (*Ulva lactuca*) at different salinity:

Finely chopped seaweed (4 gm) thalli were cultured in flat-bottomed round aerated flasks at different salinity (0 − 40). We observed their growth performance at different salinity for 60 days at laboratory condition. Highest final weight was found in the case of 25 ppt (Table 6). At lower or higher salinity seaweed become stressed and discoloration or defragmentation of seaweed occurred (Figure 7).

Table 6. Growth performance of seaweed (*Ulva lactuca*) at different salinity.

Salinity (ppt)	**0**	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**
Final weight (gm)	1.83 ± 0.14	5.00 ± 0.28	4.60 ± 0.31	9.24 ± 0.46	18.80 ± 2.13	49.25 ± 2.74	32.37 ± 3.53	19.64 ± 2.09	12.88 ± 0.98
Culture period	15 days	27 days	27 days	27 days	60 days	60 days	60 days	60 days	60 days



Discoloration Defragmentation

Figure 7. Discolored and defragmented seaweed due to salinity stress.

Study-4: Integrated seaweed-fish culture technique

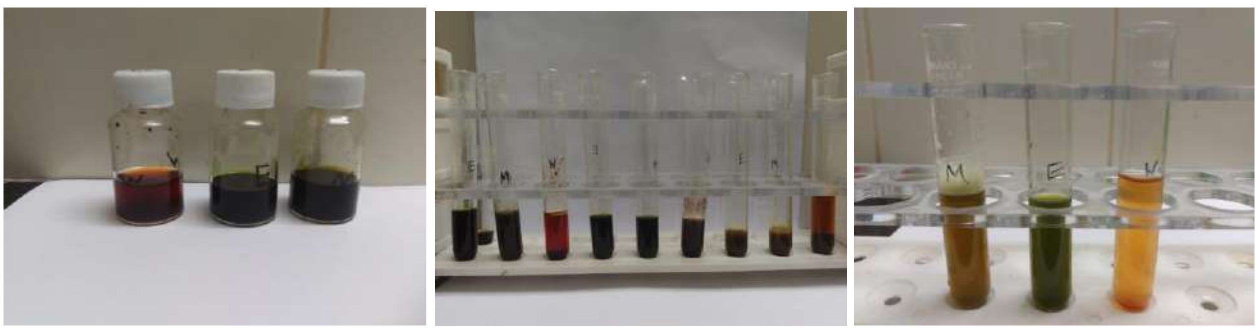
For the integrated experiment the seaweed species *U. lactuca* and fish species *Mystus gulio* were selected. We have prepared an integrated seaweed-fish culture unit at MFTS for the experiment (Figure 8). We have collected the fish from natural sources at late May. Due to fish handling error all the fishes were died and thus we cannot start our experiment on time. Later we collected some fish from natural sources at early July. After salinity adjustment we just started our experiment but haven't get any data.

Figure 8. Integrated seaweed-fish culture unit at MFTS.

Study-5: Bioactive compounds analysis

For the bioactivity analysis commercially important red seaweed i.e., *Hypnea musciformis* was selected. These species are mainly found on the rocky shore of St. Martin island. We have collected the mature *H. musciformis* from the St. Martin island. The samples collected were washed using clean salt water to eliminate debris and other extraneous substances and transported to the laboratory. The specimens washed again using distilled water to eliminate any presence of salt, sand, debris, or other forms of contamination. Then, the seaweed underwent a shed drying process for two days to eliminate moisture. The dried seaweed was powdered and stored in a refrigerator at a temperature of 4 ºC to facilitate further analysis.

An extract was produced by immersing four grams of fine seaweed powder in 100 milliliters (ml) of solvents using the maceration method for extraction. Water, methanol, and ethanol were used as solvents (Figure 9). The specimen was kept in darkness for 24 h and was periodically stirred to improve the extraction process. Following incubation, filtration of the solution was performed through a Whatman filter paper No 4 (20 − 25 μm) to ensure its cleanliness.



Stock solution Working solution Saponin test

Figure 9. Bioactive compounds analysis process.

The newly generated crude extracts of seaweed underwent qualitative evaluations to identify different classes of active phytochemical ingredients, including phlobatannin, terpenoid, saponin, alkaloids, amino acids, and cardiac glycosides, using proper methodologies. Out of 18 tests, 12 were positive and the left over six were negative (Table 7). Among them phlobatannin were showed positive result in water extract and negative result in ethanol and methanol extracts. Phlobatannin is a tannin derivative and tannin generally found in higher plants. Again, saponin were showed positive result in methanol extract and negative result in other extracts. This is an indication that this compound can only be derived in selective extract.

Table 7. Preliminary phytochemical analysis of *H. musciformis* extracts.

Name of the extracts	Terpenoids	Saponin	Phlobatannins	Cardiac Glycosides	Alkaloids	Amino acids
Methanol	+	+	−	+	+	+
Ethanol	+	−	−	+	+	+
Water	+	−	+	+	−	−

Stock solution **Working solution**

Conclusion

Seaweed cultivation at our coast shows better biomass yield and daily growth rate in the floating raft method. However, satisfactory growth performances were found in *H. musciformis* which indicates that this seaweed could be cultured at floating raft method in our coast. However, the effective development of seaweed farming necessitates not only suitable natural environmental conditions and viable technical approaches but also responsive and supporting social and economic situations. Further investigation is required to surplus our understanding of the cultivation of seaweed. The potential for a new era in seaweed culture inside our country may be realized with the successful use of indoor culture and tissue culture techniques for commercially significant seaweed species.

Isolation, production and development of indigenous microalgae based live feed supply chain for marine hatchery

Researchers Zahidul Islam, SO
Dr. Shafiqur Rahman, PSO
Turabur Rahman, SO

Objectives of the project

- To isolate commercially important live feed from the Bay of Bengal
- To utilize live feed as ready primary feed for various marine larvae
- To develop a portable photo-bioreactor model to facilitate pure live feed stock culture at field level

Achievements (2023-24)

Highlighting the progress of major activities

No.	Planned Activities	July/2023-June/2024
1	Isolation and scale up of microalgae in laboratory condition	Previous isolated are being maintained and 1 genera have been isolated and cultured in laboratory condition
2	Utilization of live feed as ready primary feed for various marine larvae	Feeding trials on Rotifer had done
3	Development of a portable photo-bioreactor model to facilitate pure live feed stock culture at field level	The physico-chemical parameters had been optimized for *Skeletonema* sp. 2nd trial using the same species is going on

Technical progress

Isolation and scale up of microalgae in laboratory condition

Methodology

For isolation, samples were collected (using plankton net) and 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

During this experimental period, 19 species of 15 different genera and 7 classes have been identified. Besides, One phytoplankton species have been successfully isolated during the experimental time period. The isolated species is *Chlamydomonas* sp. (Figure 1). Presently, with this new species, eight (08) commercially important microalgae species are available in

Live Feed Laboratory, Marine Fisheries and Technology Station, Bangladesh Fisheries Research Institute, Cox's Bazar.

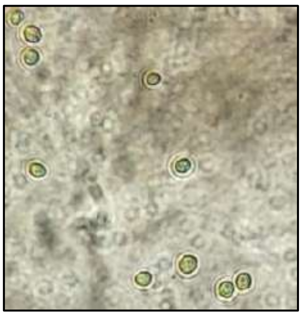


Figure 1: *Chlamydomonas* sp.

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 are trying to isolate and adapt 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 the isolated species.

9.2.2 Utilization of live feed as ready primary feed for various marine larvae

Methodology

Experimental setup

In this experiment, the performance of live feed was evaluated on *Rotifer (Brachionus rotundiformis)* growth. The *Rotifers* were reared in 9 L water tank with treated seawater (SW) at 200 inds/mL (Figure 2). Feeding with *Nannochloropsis* sp., *Chlorella* sp., and mix of each (1:1) was maintained at the rate of 10^4 cells/mL for 4 days.

Figure 2: Experimental Unit

Before transferring the *Rotifer* into the experimental tank, gross body length (µm) of each individual was measured. Body length, *Rotifer* production and the length of *Rotifer* were recorded daily. The experiment was performed for four (4) days. Regardless of algal diet, feeding was done twice a day (morning and afternoon) based on the ingestion rate. Microalgal food and water were changed everyday until the end of the experiment.

Water quality parameters

The water quality parameters *pH*, ammonia, salinity, dissolve oxygen, temperature were maintained and monitored regularly using HANNA HI98129 pH meter, Sera ammonia test kit, HANNA Digital Refractometer HI96822, Lutron 5509 DO meter and thermometer respectively.

Statistical analysis

Mean and standard deviation of mean were calculated using MS excel. When assumptions were met, ANOVA was applied to test the significance of the difference among the different diet treatments.

Results

The nutritional profile of the used diet

In the experimental diet *Chlorella* sp. had the highest protein (42.69%) profile than *Nannochloropsis* sp. (39.37%) (Figure 3). However, the carbohydrate and lipid content also varies among the experimental microalgae diet.

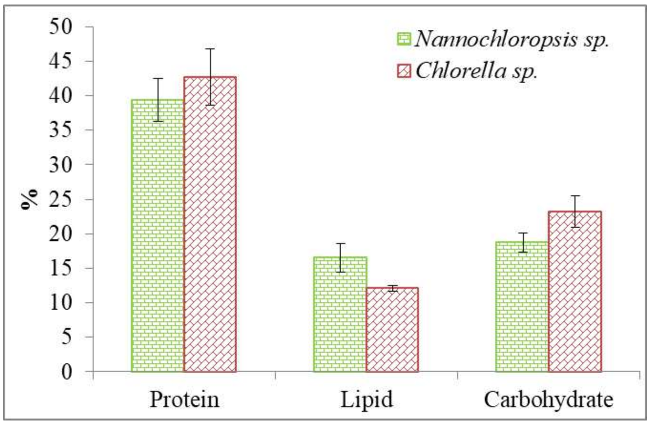


Figure 3: Nutritional profile of the used diet

Growth parameters

Size of the Rotifers

The highest experimental *Rotifer* size was found 226.33 ± 38.17 µm at the final day of the experiment. No significance difference was observed among the species size during this experimental period (Table 1).

Table 1: Size of *Rotifer* during the experimental period

Experimental Species	Day	Size (µm)
Brachionus rotundiformis	1	220.25 ± 34.12
	2	222.27± 40.17
	3	218.86 ± 35.81
	4	226.33 ± 38.17

Cell density and growth rate

All the species started to multiply immediately after stocking days in case of all three diets. There was uniform increasing growth pattern were observed for all the treatments where the highest cell count was observed 306 ± 12.50 Cells/mL in case of mixed diet (Figure 4). In addition, the highest growth rate was observed 0.086 ± 0.081 in case of the same diet.

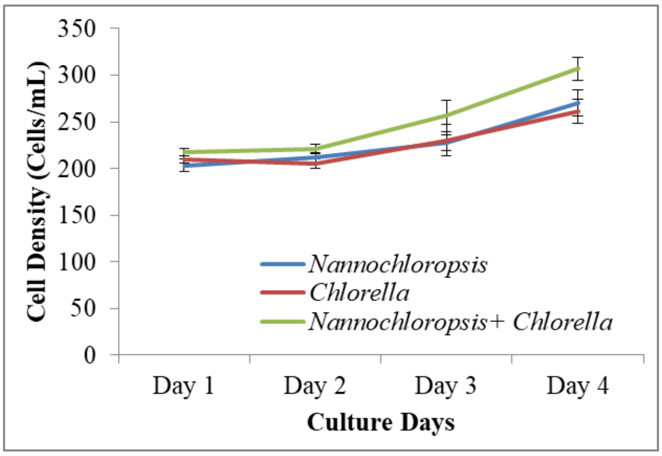


Figure 4: Cell density of *Rotifer* during experimental period

Water quality parameter

All the water *pH* (7.99 - 8.36), ammonia (0 mg/L), salinity (24-26 ppt), dissolve oxygen (4.82 - 5.73 mg/L), temperature (24-26 °C) were within the range. No significance difference was observed among the treatment during this experimental period (Figure 5).

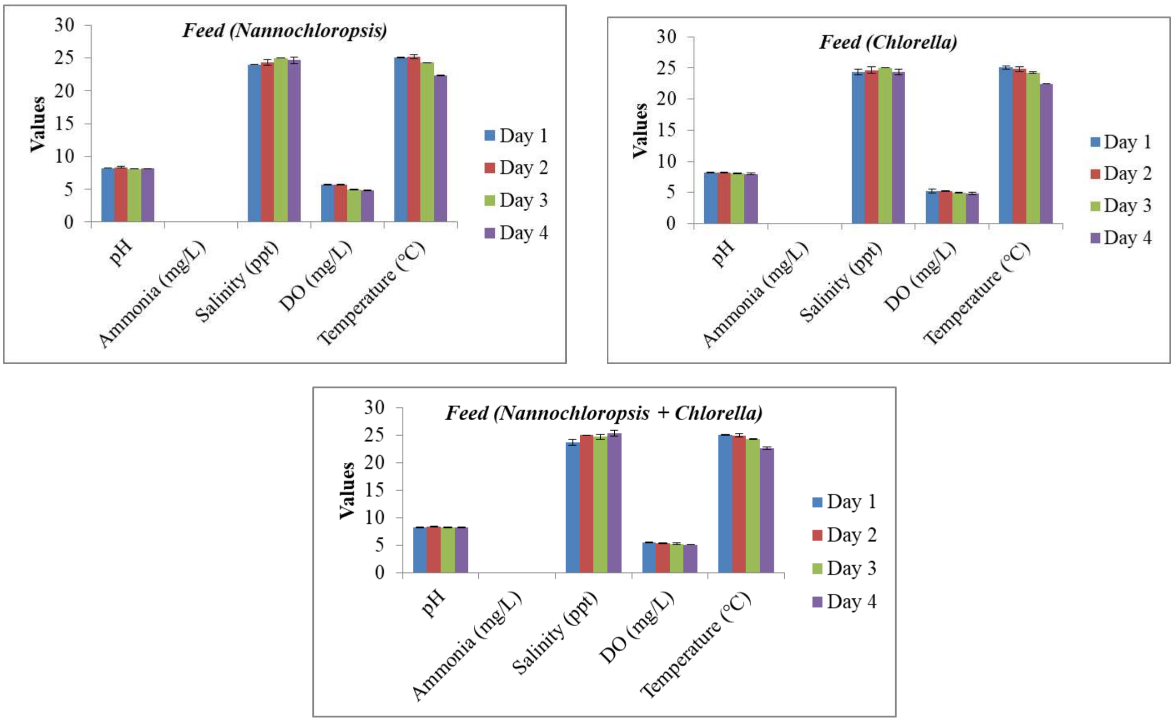


Figure 5: Water quality parameters of different diet treatments

Nutritional profile of the experimental species

All the species were harvested immediately after final day the experimental using plankton net (120 µm). After collection the sample were dried using hot air oven at 60 °C for 12 hours. Finally, the dried powder samples were collected and preserved for nutritional analysis (Figure 6).

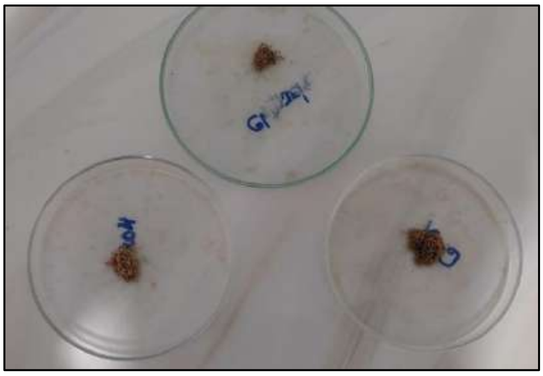


Figure 6: Dried Rotifer sample

Discussion

The results showed that, different microalga diets can significantly affect the growth performance of the *Rotifer* where the mixed diet indicated an enhanced growth among the three treatments. However, after a thorough comparison of nutritional profiles, the most suitable diets can be precisely selected.

9.2.3 Development of a portable photo-bioreactor model to facilitate pure live feed stock culture at field level

Trial 1

Methodology

A portable photo-bioreactor model has been developed. In this system, the physical parameters e.g. light, temperature and aeration can easily be maintained with automatic control system. Figure 7 shows the developed portable photobireactor system.

Figure 7: Developed BFRI Photo-bioreactor

Initially, commercially the most important species *Skeletonema* sp. was selected for culture and optimization in the photo bioreactor system. The experiment was performed using regression models as per response surface methodology of Box Behnken design. The coded and actual values for trial are shown in table 2.

Table 2. The coded and actual values of independent variables

Independent Variable	Units	Symbol	Code Levels		
			-1	0	1
Temperature	ºC	A1	20	25	30
Salinity	ppt	A2	20	25	30
*p*H		A3	7	7.75	8.5

Results

Three independent variables temperature, salinity and *p*H were altered to attain theoretically possible maximal output responses. Numerical solution to get an optimized result for all three output results as per Response Surface Methodology based on Box Behnken design with a desirability of 83.46% is stated in Table 3. Theoretical maximum yield of cell density, biomass and optical density is 0.967×10^6 cells/mL, 1.985 g/L and 0.101 respectively and when temperature, salinity and *p*H were described at 27.76 ºC, 29.81 ppt and 7.04, respectively. Maximum yield of cell density, biomass and optical density were 0.953×10^6 cells/mL, 1.001 g/L and 0.0.09, respectively.

Table 3. Desirability of the optimized model according to Box- Behenken Design

Variable	Values
Temperature (ºC)	27.76
Salinity (ppt)	29.81
pH	7.04
Responses	**Values**
Cell density (Cells/mL)	0.967×10^6
Biomass (g/L)	1.985
Optical Density	0.101

Theoretical optimized medium was validated experimentally. Theoretical optimized culture conditions when performed experimentally exhibited 4.45% increase in cell density, and 10.9% increase in optical density, respectively. On the other hand, 49% decrease was observed in case of biomass production Table 4.

Table 4. The actual values according to desirability of the optimized model

Variable	Values
Temperature (ºC)	27
Salinity (ppt)	29
pH	7.05
Responses	**Values**
Cell density (Cells/mL)	1.01×10^6
Biomass (g/L)	0.993
Optical Density	0.126

The results of theoretical optimized medium were almost similar to the predictive optimized medium (0.953×10^6 cells/mL cell desity, 1.001 g/L biomass and 0.0.09 optical density) yields as exhibited in Run 16 of Table 4.

Discussion

It is difficult to optimize media through autotrophic mode. Mixo-trophic cultivation mode has higher advantages than other modes to optimize media. The study proves to deliver promising results of growth biomass through the optimized media in PBR system. In addition, the findings can also be utilized in large scale production system. However, the successful development of microalgae species management technique will add a new dimension for stock management.

Trial 2:

A modified portable photo-bioreactor has been developed with solving the previous issues (Figure 8).

Figure 8: Developed BFRI Photo-bioreactor

New experimental design has been prepared (Figure 9) using the latest version of Design Expert software (V13) for the same species for validation.

Std	Run	Factor 1 A:Salinity ppt	Factor 2 B:Temperature °C	Factor 3 C:pH	Response 1 Cell Density Cells/mL	Response 2 Culture Days Days
4	1	30	30	8		
9	2	25	15	7		
12	3	25	30	9		
13	4	25	22.5	8		
8	5	30	22.5	9		
7	6	20	22.5	9		
2	7	30	15	8		
16	8	25	22.5	8		
1	9	20	15	8		
6	10	30	22.5	7		
3	11	20	30	8		
5	12	20	22.5	7		
15	13	25	22.5	8		
11	14	25	15	9		
10	15	25	30	7		
14	16	25	22.5	8		
17	17	25	22.5	8		

Figure 9: Experimental design

Discussion

Data will be validated with the previous findings after completing the all experimental run and software analysis.

10. Problems/constraints encountered if any

The photo bioreactor system is a newly designed and developed portable system by Bangladesh Fisheries Research Institute, Marine Fisheries and Technology Station, Cox's Bazar. As it is new mechanical system, there are some difficulties and system errors are being observed during the experimental trial. So some of the experimental trial are being hampered and delayed.

11. Proposed work during the next period

- Isolation of commercially important marine live feed species
- Utilization of live feed as ready primary feed for various marine larvae
- Run and optimization of portable photo-bioreactor model to facilitate pure live feed stock culture

Development of value-added product from marine fishes

Researchers:

Abu Bakker Siddique Khan, SO
Mousumi Akhter, SO
Md. Aktaruzzaman, SO

Objectives of the project (2023-24)

i) To identify the marine fishes and their by-products used in value-added product development.
ii) To develop various value-added products i.e., fish ball, fish sausage etc. from marine fishes
iii) To develop ready-to-eat fish i.e., canned fish from potential marine fishes.

Achievement (2023-24):

Study-1: Identification of marine fishes used in value-added product development

Methodology:

Identification and collection of suitable fish species

Low-valued marine fish were evaluated and collected from different places i.e. BFDC landing centre and local markets of Cox's Bazar. At first, tuna species were selected for making various value-added product developments.

Fig 1. *Euthynnus affinis* (Little tuna)

Study-2: Development of the various value-added products i.e., fish ball, fish burger etc. from marine fishes

Methodology:

I) Preparation of mince

Fresh tuna (*Euthynnus affinis)* was purchased from the BFDC, Fish landing center, Cox's Bazar. Immediately after purchasing, the fish was preserved properly with crushed ice in an insulated box and transported to the laboratory. The average size of the fish was 42 ± 2.50 cm, 1.00 ± 0.20 Kg. Then fish was beheaded in clean water, eviscerated, and peeled. The peeled fish was filleted and deboned manually in iced condition. The mince was then have prepared through a 1 mm inlet diameter mesh plate with mechanical mincer to remove all bone and connective tissue from the muscle (Fig. 2). All utensils used in the experiment were sterilized and stored in a cool place (4°C).

Fig 2. Fresh fish fillet and minced fish (*Euthynnus affinis)*

II) Preparation of fish ball

First, Different ingredients (Table-1) were dried in a 4-layer hanging net dryer (Fig. 3). Then dried spices were mixed properly with minced meat. Then minced fish with ingredients was coated with egg followed by biscuit crumbs. Finally, the ball shape was given. Lastly the fish ball was prepared for frying in fresh soybean oil. Then prepared fish balls will be kept in the refrigerator (4 ℃) and deep freezing (-18 ℃).

Fig 3. Four layer hanging dryer for drying the spices

Table 1: Different type of ingredients with percentage used in making of fish ball (*Euthynnus affinis)*

Ingredients	Percentage
Table salt	1-2 %

Sugar	1.5 %
All spices (Onion, Garlic, Ginger, Red pepper, Black pepper)	2 %
MSG (Mono Sodium Glutamate)	0.15 %
Starch	8 %
Vegetable oil	2 %

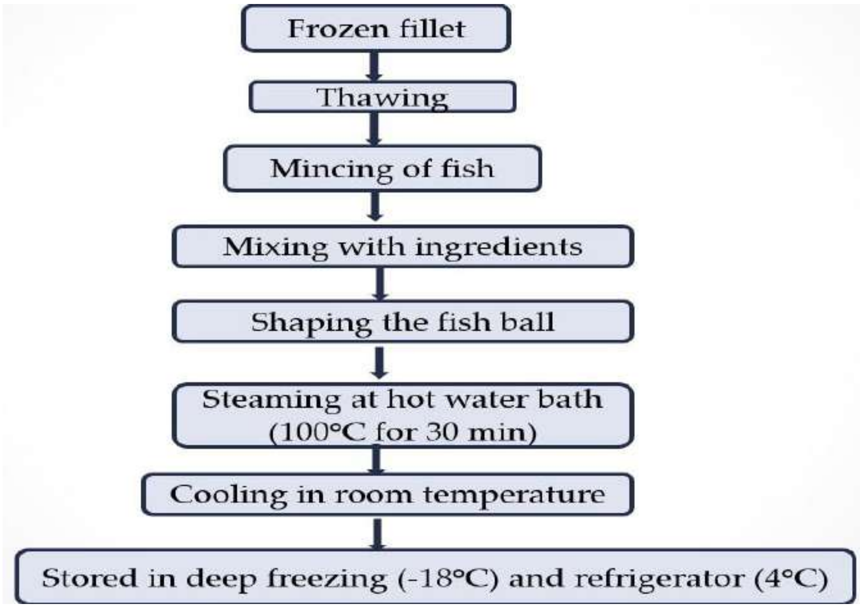


Fig 4: Flow chart of preparation of fish ball

III) Sensory/organoleptic test of fish ball (*Euthynnus affinis)*

The developed fish balls were evaluated by an 8- member trained sensory test panel fortnightly using a 5-point hedonic scales for taste, flavor, color, odor and texture. A panel test score sheet with hedonic characteristics and scores for sensory evaluation was developed considering all critical elements of sensory attributes and their hedonic characteristics, particularly suitable for tuna products.

Table 2. Sensory evaluation sheet for value added product development

Attributes	Characteristics	Scores	Give tick mark
Taste	**Excellent taste**	**5**	
	Very good taste	**4**	
	Good taste	**3**	
	Low taste	**2**	
	No taste	**1**	
Flavor	**Excellent flavor**	**5**	
	Very good flavor	**4**	
	Good flavor	**3**	

	Low flavor, slightly rancid	2	
	No flavor, rancid flavor	1	
Color	Natural color and shiny	5	
	Brownish color	4	
	Slightly to brownish shining	3	
	Moderately brownish shining	2	
	Blackish inner and dark brownish	1	
Odor	Natural odor	5	
	Slight off sour	4	
	Slightly to moderate fishy	3	
	Faint sour odor	2	
	Extremely sour and become	1	
Texture	Firm and flexible	5	
	Slightly moderate soft	4	
	Moderately soft	3	
	Soft and slightly juicy	2	
	Very soft or rigid surface	1	
Overall acceptability	Highly acceptable	5	
	Slight to moderate acceptable	4	
	Slightly unacceptable	3	
	Very unacceptable	2	
	Rejected	1	
Total point gain			
Grand average point			

Results

Earlier, an 10-member test panel was formed from the Marine Fisheries and Technology Station, Cox's Bazar. Most importantly, the test panelists were made available on each 15 days at the fixed date and time of sensory analysis and thus, full-house participation was ensured in each session.

Table 3: Sensory test for different attributes of fish ball in two different preservation methods.

Days	Deep freezing (-18˚C)					Refrigerator (4 ˚C)				
	Taste	Flavor	Color	odor	Texture	Taste	Flavor	Color	odor	Texture
01	4	3	4	5	4	3	3	4	3	3
15	3	4	3	4	3	3	2	3	3	3
30	3	3	3	3	3	3	2	3	2	2
45	3	2	3	3	3	2	2	2	2	2

60	3	2	3	3	2	2	2	2	2	2
Total score	16	14	16	18	15	13	11	14	12	12
Average defect score	**3.2**	**2.8**	**3.2**	**3.6**	**3.0**	**2.6**	**2.2**	**2.8**	**2.4**	**2.4**

On the day of panel test, the assessors were informed at least 30 min before the panel session started that they would not consume any meals or drinks, smoke or use gums or mints and instructed not to talk to other panelists during testing.

Table 4: Grading and defecting scores of value-added products

Grade	Defect scores	Level of acceptance
A	<2	Excellent and highly acceptable
B	2- <3	Good and acceptable
C	>3- <5	Poor and slightly unacceptable
D	>5	Rejected

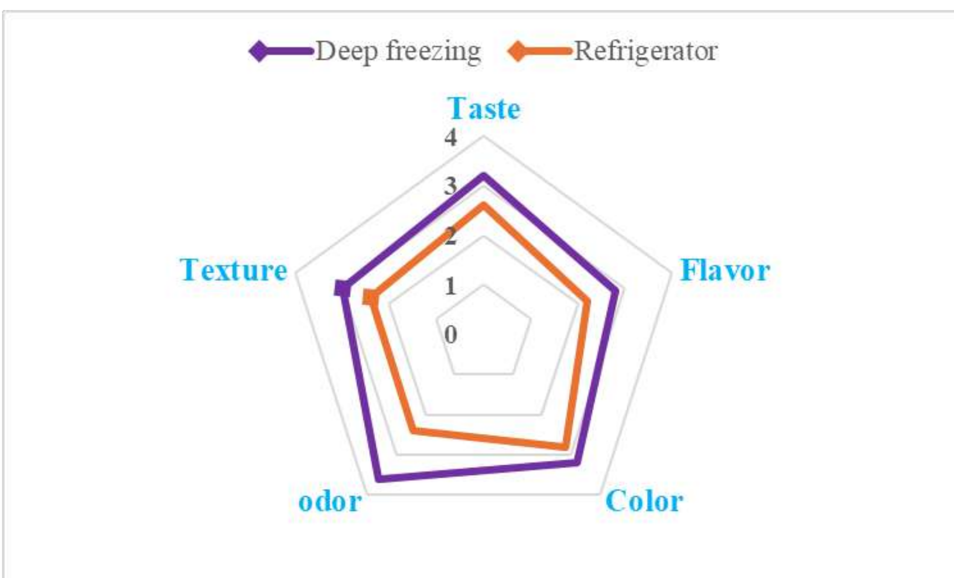


Fig 5. Changes in sensory quality attributes of two different preservation techniques

A piece of score sheet was given to each of the individual panelists for evaluation of the products separately. The panelists put numerical values in respect to each sensory attribute assessed (Table 3). In accordance with table 4, fish ball in both preservation methods got grade "B" (Table 4).

iv) Proximate composition analysis

Fresh fillet of *Euthynnus affinis* (Little tuna) and fish ball of two different preservation methods (deep freezing and refrigerator) was sent to the Fish Nutrition Laboratory, Freshwater Station, Mymensingh.

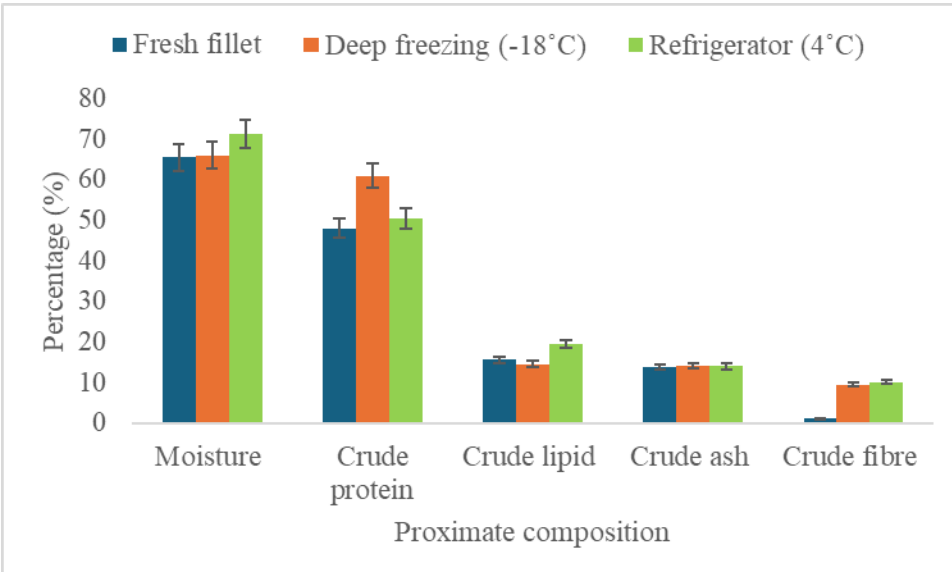


Fig 6. Proximate composition of fresh fillet of tuna and fish ball in two different preservation methods after 60 days.

Results

The proximate composition of the fish fillet, fish balls in deep freezing (-18˚C) and refrigerator (4 ˚C) was portrayed in Fig 4. The results showed that moisture content in wet weight basis was similar in fresh fillet (65.44 ± 1.44 %) and fish balls preserved for 60 days in deep freeze (65.97 ± 2.54 %) but slightly superior in fish ball in refrigerator (71.27 ± 2.12 %). This result indicated that tuna fish ball preserved in deep freeze (60.87 ± 3.21 %) contained significantly more protein than fish fillet and refrigerator storage ($p < .05$). On one hand, fresh fillet had 15.61 ± 1.02 % crude lipid which plunged significantly to deep freezing (14.51 ± 1.74 %), on the other hand, increased sharply (19.57 ± 1.11 %) in refrigerator's fish ball in 60 days ($p < .05$). Crude ash levelled off in three different stages, whereas fiber significantly soared up in deep freeze fish ball (10.05 ± 1.61 %).

v) Bio-chemical test analysis

pH Determination

The pH was measured using a pH meter (ADWA pH Meter, Model 250, Romania) after homogenizing 5g of fish ball samples with 10 ml distilled water in an electric blender (WBL-15GC40, Vision, Bangladesh).

Results

Table 5: Determination of pH of fish ball in two different preservation method for 60 days

Days	pH	
	Deep freezing (-18˚C)	Refrigerator (4 ˚C)
01	6.2 ± 0.56	5.9 ± 0.89
15	6.5 ± 0.45	6.1 ± 0.78
30	6.9 ± 0.23	6.3 ± 0.22
45	6.4 ± 0.45	7.45 ± 0.51
60	7.1 ± 0.63	8.12 ± 0.39

The pH value initially climbed up but remained within the neutral range all along with the storage. However, a slight increase (8.12 ± 0.39) showed up in 60 days of refrigeration, but this slight increment could not show any negative quality implications in the stored products.

TVB-N, TMA and Histamine Content

Initial lower TVB-N values in the fish and products indicated their acceptable quality characteristics. Many authors observed that low TVBN values of the products are the indications of fresh and premium quality fish and increased TVB-N with the increment of storage time were the indications of quality degradation.

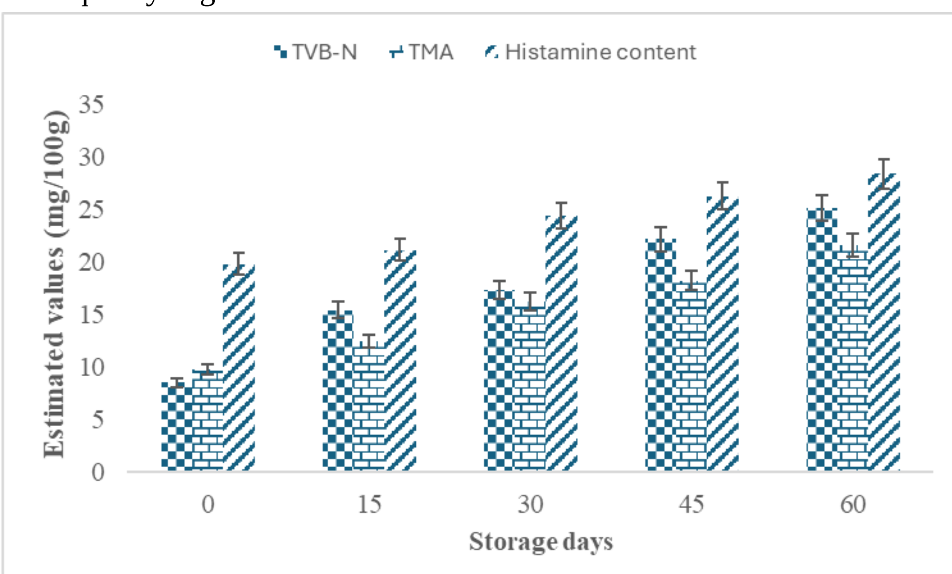


Fig 7. Changes of bio-chemical properties of deep freezing (-18˚C) preservation of fish ball

Results

According to Nowsad (2007), fresh fish contains a TVB-N content of 35–40 mg/100g of fish muscle, while 50–70 mg/100g is considered as the maximum safe level, based on species. The present study also showed similar trends where the TVB-N values were increased in both fish balls (Fig 6,7) with the progress of storage time, but the values remained within the acceptable limit. The degradation of proteins and non-protein nitrogenous compounds might be linked with this rapid increment of TVB-N during storage. Like TVB-N, TMA values were also increased with the increment of storage time in both respective storage conditions. Fish and products with TMA-N value up to 40 mg/100 g is acceptable (Kaya and Basturk, 2015). Both the fish balls remained in acceptable condition up to 60 days of storage with the lower or acceptable range of TMA-N values. This might be due to the use of premium quality fresh fish, removal of gut and skin during the pre-processing of mince blend. Histamine is a sign of quality deterioration of wet fish and products produced from histidine of the peptide chain. Histamine could be a good indicator to understand the product qualities. Chong et al. (2011) reported a value of 50 mg/100 g could be a sign of histamine poisoning. Histamine contents in the present study were found to be much lower than the values reported as poisonous threshold limit (Fig 7).

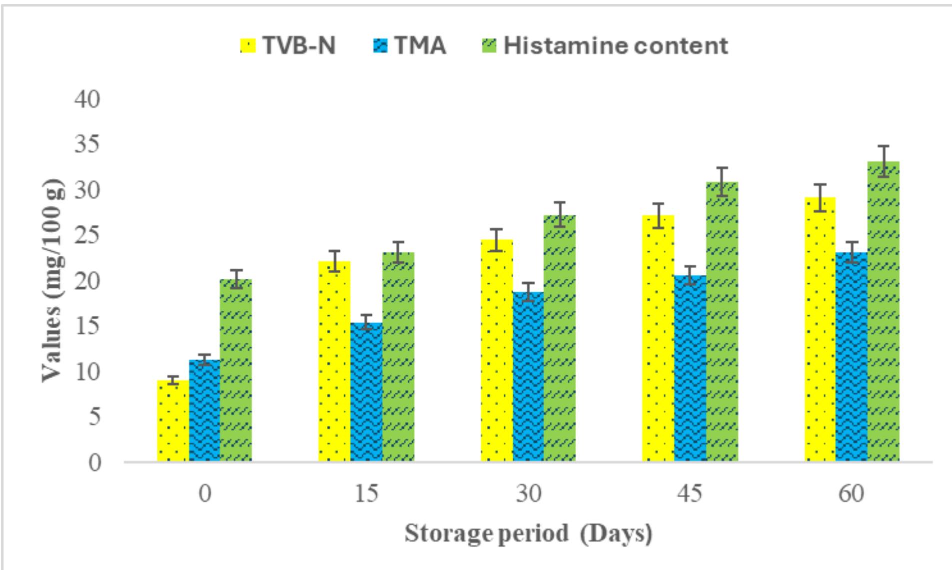


Fig 8. Changes of bio-chemical properties of refrigerator (4˚C) preservation of fish ball

Therefore, the present study could conclude that fish ball in deep freezing (-18˚C) remained acceptable over fish ball in refrigerator (4˚C) up to 2 months, where degradation of proteins did not so occur.

Microbial load assessment

Three distinguished agar media were used for microbial load assessment i.e. *Salmonella-shigella* (SS) agar for the detection of *Salmonella* and *shigella*, Eosin methylene blue (EMB) agar to detect the *Escherichia coli*, and Mannitol salt agar (MSA) for *Staphylococci*. This test occurred in the laboratory of Fish Processing, MFTS, Cox's Bazar and no colony was found for 60 days (Fig 9).

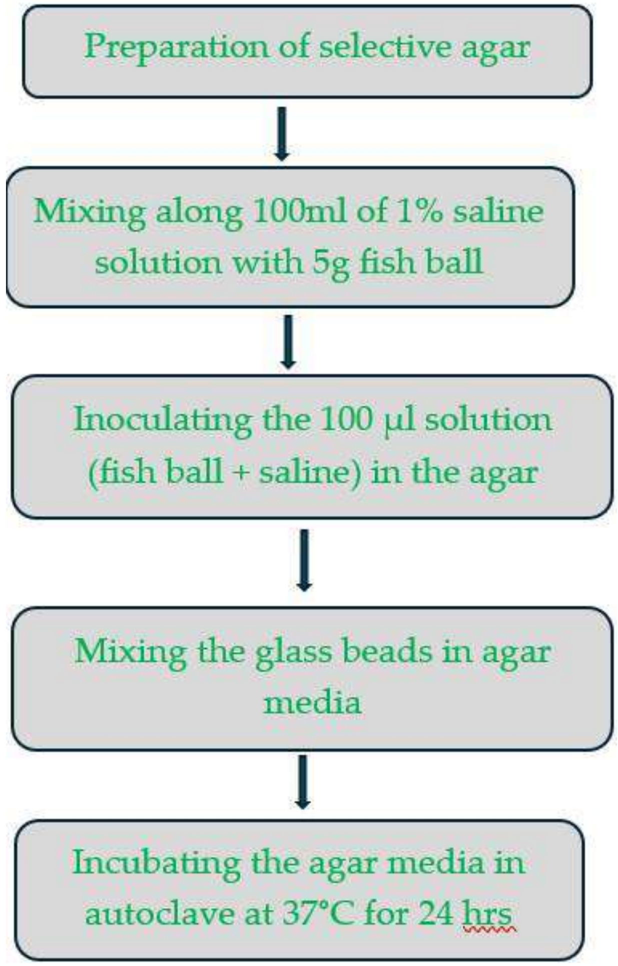


Fig 9. Flow chart for preparation of media for microbial load assessment

Results

Table 6: Microbial load assessment in the two different preservation methods

Days	**Agar media**	**Detection**	**No. of colony counted (cfu/g)**
01	Salmonella-Shigella agar	*Salmonella-Shigella*	
15	Mannitol salt agar	*Staphyloccocus aureus*	**Not found**
30	Eosin Methylene Blue agar		

45		*Eshrechia coli*	
60			

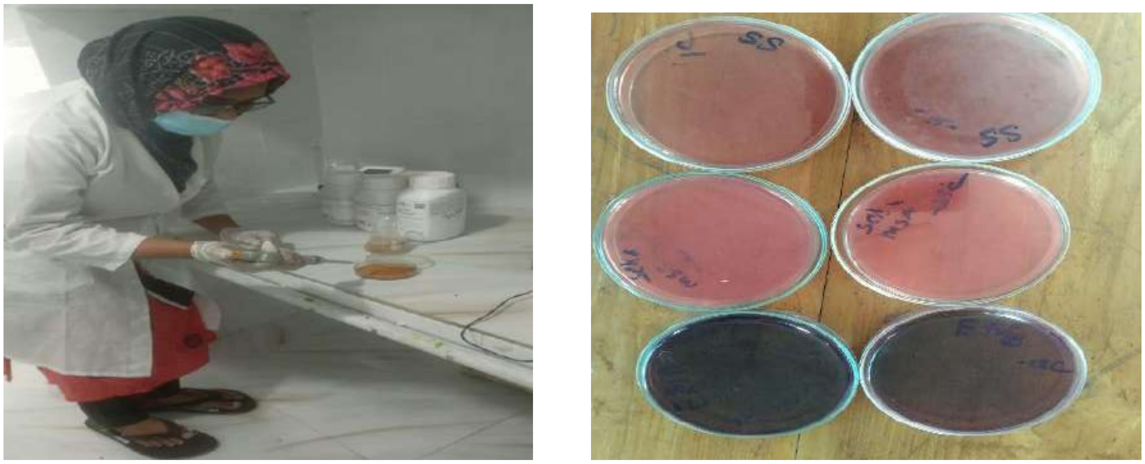


Fig 10. Microbial load assessment for bacteria detection in MFTS Laboratory

Study-3: Development of the ready-to-eat fish i.e., canned fish from potential marine fishes. The experimental set up was ready for the development of canned fish product at MFTS, BFRI Cox's Bazar; however, we were unable to begin the experiment because no proper size of can and nitrogen gas was available. Proper size and galvanized can to prevent from rust and corrosion was ordered from China. As soon as the appropriate can and nitrogen gas is first reported as being available, this system will be in full operation.

10. Problems/constraints encountered if any:

a) Unavailability of nitrogen gas for canning
b) Unavailability of suitable can for canning
c) Limitation of different nutritional and bio-chemical tests in the laboratory.

11. Proposed work during the next period

a) Determination of proximate and different bio-chemical test.
b) Development of canned fish products.
c) Preparation and marketing of fish balls, fish sausages etc.

)

Breeding and Culture Potential of Marine Oyster and Green mussel in the Bay of Bengal Bangladesh coast

Researchers

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Objectives

- To estimate CD (Condition Index) and MI (Maturity Index) of green mussel (*Perna viridis*)
- To study gonadal histology of green mussel (*Perna viridis*) .
- To develop culture techniques of oyster and green mussel in Cox's Bazar and other suitable areas.
- To develop breeding techniques of oyster and green mussel in captivity.
- To develop larval rearing and nursery management techniques of oyster and green mussel.

Achievements (2023-24)

Study-1. Estimation of CI (Condition Index) and MI (Maturity Index) of green mussel

Methodology

Biometric characteristics

Green mussel (*P. viridis*) was collected monthly at Kutubdia, Sonadia and Moheshkhali,Cox'sBazar. Biometric characteristics such as shell length, shell height, shell width, soft body tissue weight were measured. Slide calipers was used to measure the dimension of green mussel; namely, shell lengh, shell height, shell width in millimeter (mm).Weight machine was used to weigh the soft body tissue weight (g).

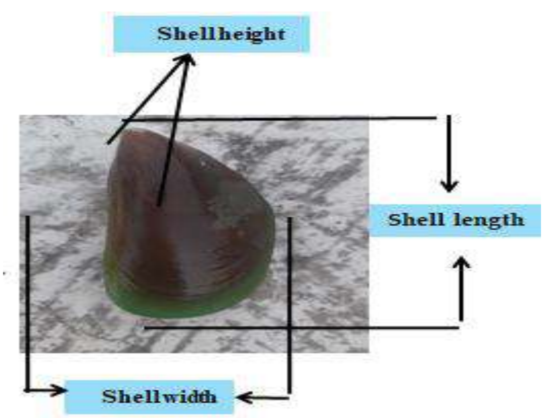

Fig 01: Morphological measurements of the shell of *P. viridis*

Fig 02. Taking biometric data.

Condition Index (CI)

The condition index was applied to bivalve mollusks to indicate the overall condition of the animal. It often followed the gametogenic cycle of mollusks. CI was measured by using the following formula (Uddin *et al*., 2007).

$$\text{Condition Index (CI)} = \frac{\text{Wet tissue weight (g)}}{\text{Shell length (cm)}}$$

Highest CI (Condition Index) indicated peak time of spawning.

Maturity index (MI)

Maturity index calculate for identifying potential breeding month of biological species. Maturity index (MI) was calculated by following equation (Niogee *et al.*, 2019). MI = the total of all of the numerical scores for a month/number of monthly samples analyzed. By dividing the total number of samples dissected in a given month by the sum of the numerical scores for each of the monthly gonadal maturity stages, the maturity index (MI) was calculated. Early developing stage scores 3, late developing stage scores 4, ripe stage scores 5, spawning stage scores 2, spent stage scores 1, and resting stage scores 0 was used to rate the gonadal maturity stages of the oyster. The sum was computed by multiplying the total number of sample in each maturity stage by the category's score. The total number of sample was splited by the outcome.

Results

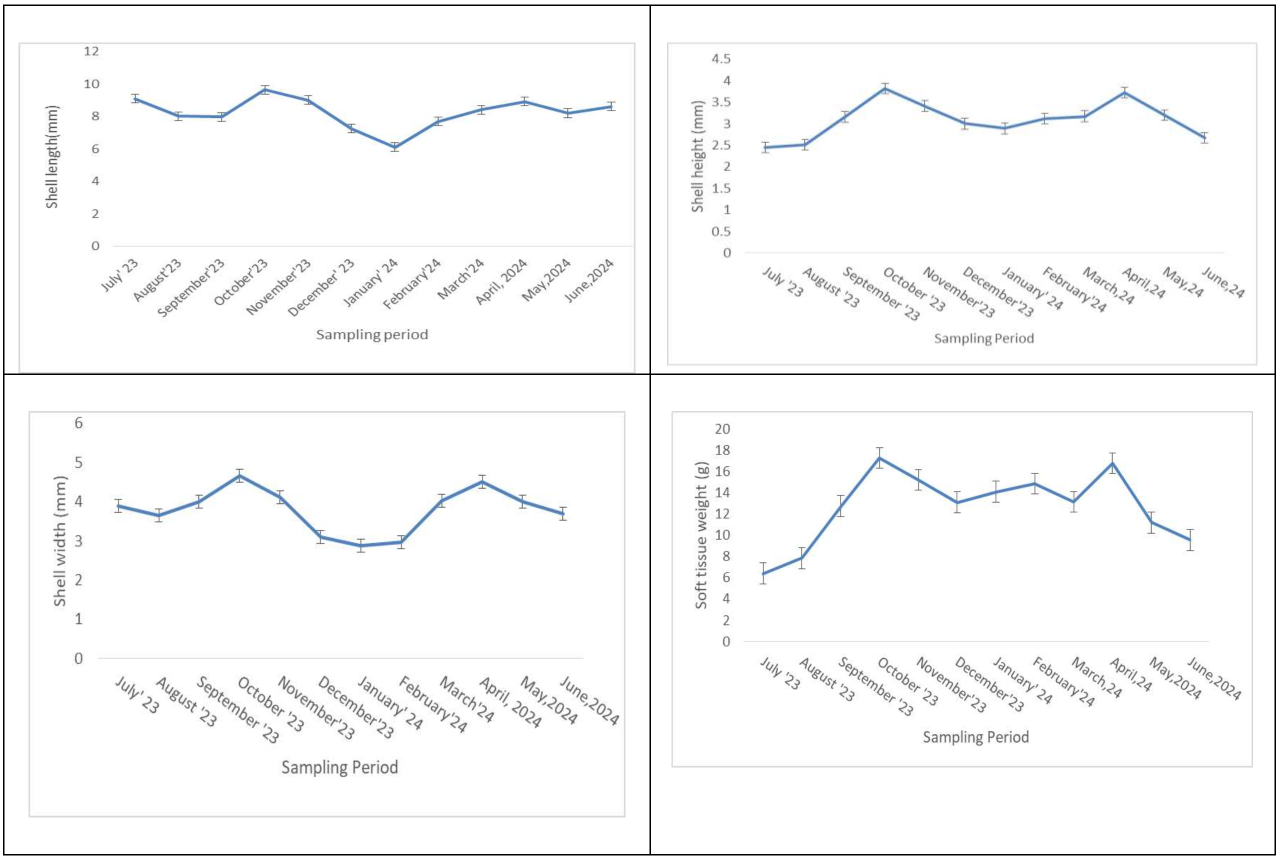

Fig 03. Biometric indices of green mussel over the year

Condition index (CI) is the most widely used quantitative method for estimating the reproductive activity of marine bivalves. CI was calculated individually as the ratio between wet tissue weight (g) and shell length (cm). CI was calculated monthly and expressed as (mean± SD). Average monthly variations in the mean condition index (CI) of *Perna viridis* are presented in Table 01.

Table 1: Condition index (mean± SD) of analyzed samples of *Perna viridis* recorded during study period.

Month	Condition Index (mean ± SD)
July, 2023	1.2± 0.36
August, 2023	0.85± 0.21
September, 2023	1.57± 0.17
October, 2023	2.56± 0.18
November, 2023	2.11± 0.32
December, 2023	1.71± 0.22
January, 2024	1.52± 0.11
February,2024	1.8± 0.22
March,2024	2.12± 0.31
April, 2024	2.4± 0.32
May, 2024	2.1± 0.28
June, 2024	1.9± 0.17

The maximum values of CI indicates major peak season. From the study maximum value was recorded in October (2.56 ± 0.18). So, peaks of *Perna viridis* species was observed in October. As well as a sharp decline in CI of *Perna viridis* was evident in August (0.85± 0.21) Table -01.

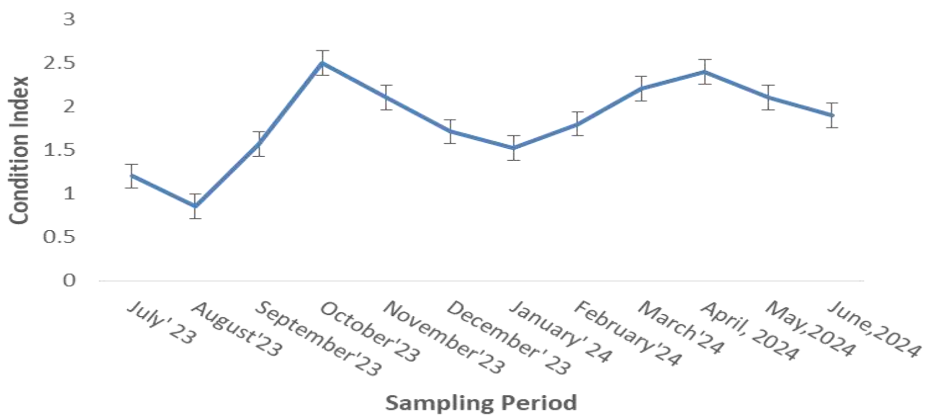

Figure 04. Monthly condition index (mean± SD) of *Perna viridis* recorded during study period.

Maturity index (MI) was calculated by following equation (Niogee et al., 2019). MI = the total of all of the numerical scores for a month/number of monthly samples analyzed. By dividing the total number of samples dissected in a given month by the sum of the numerical scores for each of the monthly gonadal maturity stages, the maturity index (MI)was calculated.Average monthly variations in the mean maturity index (MI) of *Perna viridis* are presented in Table-02.

Table 02: Maturity index of analyzed samples of *Perna viridis* recorded during study period.

Month	Maturity Index (mean ± SD)
July, 2023	3.1± 0.36
August, 2023	3.5± 0.13
September, 2023	3.3± 0.17
October, 2023	2.2± 0.42
November, 2023	1.7± 0.32
December, 2023	1.1± 0.11
January, 2024	2.89± 0.25
February,2024	3.15± 0.45
March,2024	3.89± 0.12
April, 2024	2.30± 0.36
May, 2024	2.0± 0.22
June, 2024	1.4± 0.7

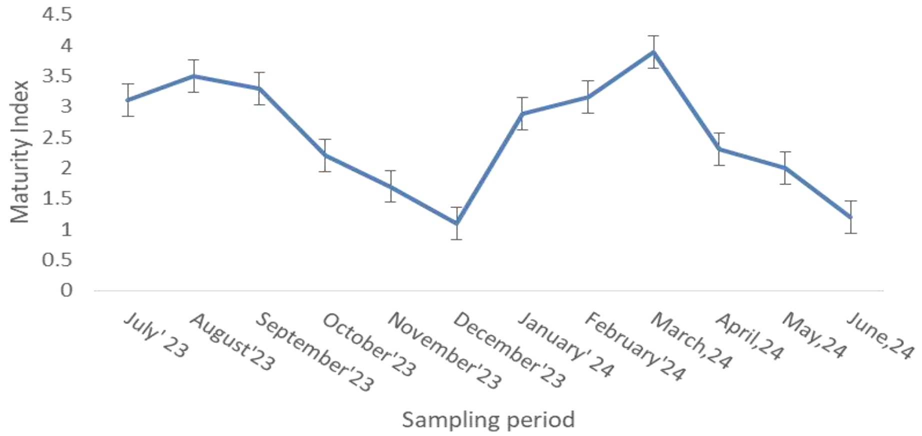

Figure 05. Maturity index of analyzed samples of *Perna viridis* recorded during study period.

Fig-06: Green mussel soft tissue

Fig-07 : Soft tissue weight

From the study maximum value was recorded in August, 2023and March,24 (3.5± 0.13and 3.89± 0.12) & lowest December, 2023 (1.1± 0.11) respectively.

Observation of gonadal histology of Green mussel

Methodology

For the development of breeding technology, green mussel gonad histology was started for observing gonadal maturity. Live green mussels were collected from Sonadia, Chowfaldandi and Kutubdia of Cox'sbazar every month. Gonadal development is being monitored regularly for taking necessary step of breeding purpose. For this purpose we sacrificed green mussel every month and we took gonad apart and preserved them in Bouin's fixative for 24 hours. After collecting a bunch of green mussel gonad sample, then we start the histological procedure. Histological analysis consist of some steps like tissue processing (dehydration&clearing) , embedding, trimming, sectioning, staining, mounting, microscopic observation etc.

Results

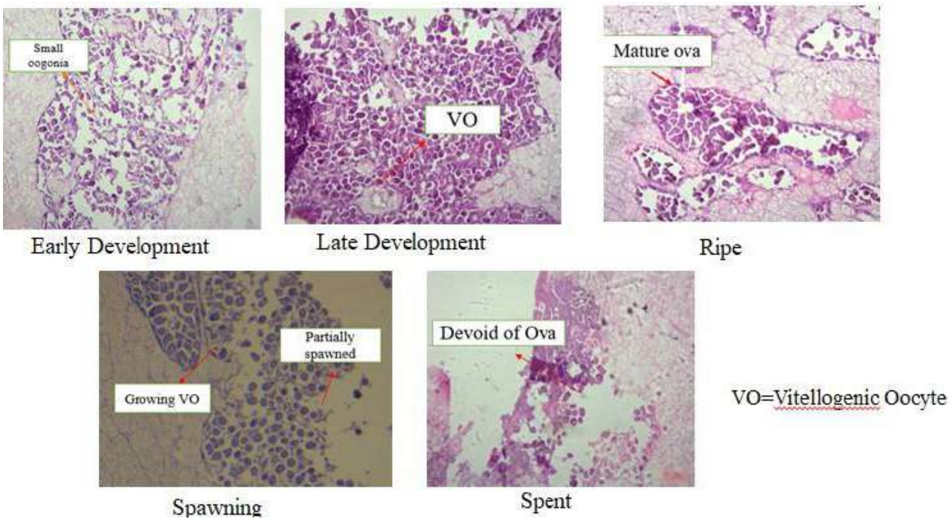

Fig-08: Photomicrographs of different ovarian stages of the female Green mussel

***p.viridis* at various stages in gamete development (H and E).**

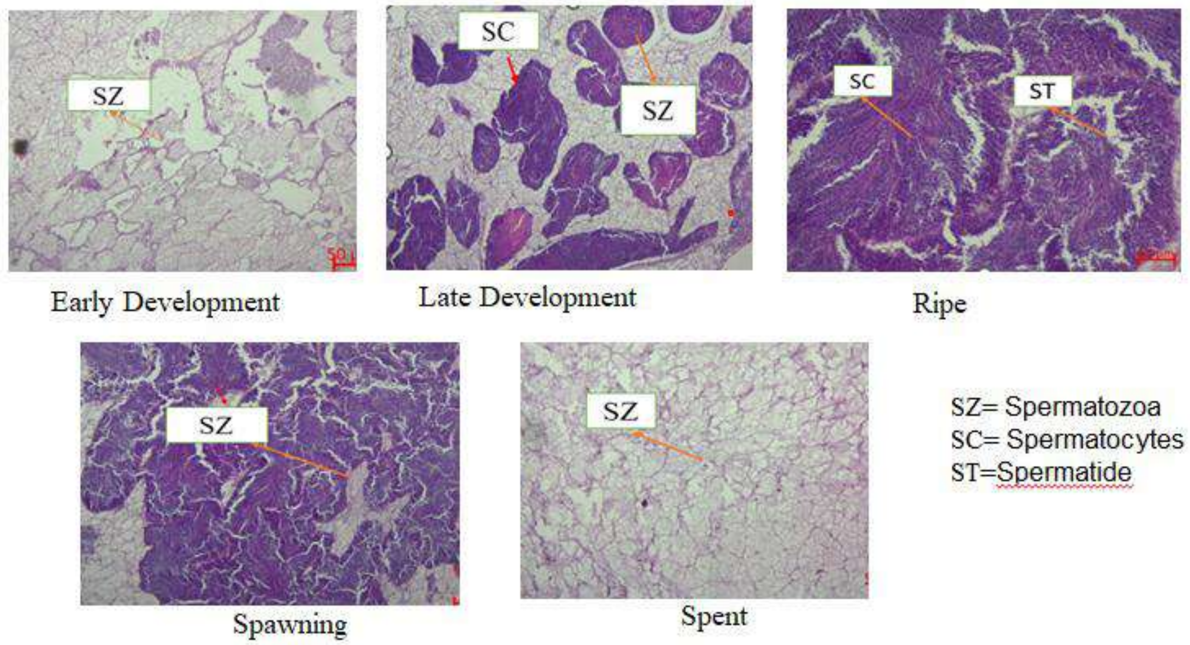

Fig-09 :Photomicrographs of different ovarian stages of the male Green mussel *p.viridis* at various stages in gamete development (H and E).

For developing the breeding program of green mussel we collected 10 broods from wild source, specifically from Inani beach, Kutubdia and Moheskhali channel. Then sacrificed the green mussel brood and collected the gonadal part for artificial breeding. After that, gonadal part stripped into transparent water jar and mixed all the strippd materials for fertilization but we didn't get any successful results. Besides that, we preserved some part of gonad for histological studied from July, 2023 to June, 2024. Early developing green mussel appeared in July,23 –September,23 and December,23 –March,24 and also appeared in June,24. Late developing green mussels occurred more or less over the year. Ripe green mussels occurred from July-November 2023 and January-May, 2024. Spawning commenced in August–November 2023 and March,24 –May,24 as evidenced by the presence of spawning green mussel in the histological preparations. There was a single prolonged spawning season with peak spawning was in October, 2023 and April, 2024. The absence of spawning green mussel from December, 2023 to February, 2024and July,23-August,23 as well as June,24 showed that no spawning activity took place during these months. Spent green mussels occurred from September,23 to November,23, and March,24 to May,24.

Table-3: Number of green mussel (female) in different stages of gonadal maturation from July, 2023-June, 2024 .

Month	No. of Female	ED	LD	Ripe	SW	Spent
July, 23	10	2	3	3	0	0
August, 23	10	1	3	4	0	0
September, 23	10	1	1	4	2	2
October, 23	10	0	1	1	5	3

November, 23	10	0	1	1	3	2
December, 23	10	1	2	0	0	0
January, 24	10	4	3	1	0	0
February, 24	10	2	4	2	0	0
March, 24	10	1	4	3	2	1
April, 24	10	0	1	2	4	1
May, 24	10	0	1	1	3	5
June, 24	10	2	2	0	0	0

ED= Early developing, LD= Late developing, SW= Spawning.

Experiment-3. To develop culture techniques of oyster and green mussel in Cox's Bazar and other suitable areas

Methodology

A.Outdoor culture system

The experiment were carried out in open water floating structures such as raft culture system at Maheshkhali channel for 06 months. There were two culture methods were followed for developing culture technique of oyster and green mussel *i.e.* (a) off -bottom culture and (b) bottom culture method

(I) Off -bottom culture method

Generally off-bottom culture methods were followed due to rough wave action in sea. According to our sea wave condition this experiment were implemented by off-bottom culture methods. Experimental structure were setup with three cultures subtract: Plastic fruit basket, stainless still basket and net bag. Three different stocking densities of oyster and green mussel were laid on trays and allowed to grow until marketable size. Sampling of oyster and green mussel were done once in a month.

(II) Bottom culture method

Bottom cultures also were implemented with three cultures subtract: Plastic tray, Bamboo tray and net bag tray. Experimental design same as off -bottom culture method. Sampling were done monthly.

B. Indoor culture system

Growth performance of green mussel and the oyster with seaweed in polyculture system were measured at indoor condition. In hatchery, three 1000L tanks were used for the rearing of green mussel and oyster. Three different stocking densities of oyster and green mussel were allowed growing until marketable size. Oyster and green mussel growth sampling were done monthly.

Results:

A. Outdoor culture system

I. Off-bottom raft culture:

Off-bottom cultures were implemented into three cultures subtract: plastic fruit basket, plastic tray and net bag. Oysters and green mussel were suspended from floating structures such as raft. Oysters and green mussel were held in tray. The rafts can be any shape and materials were oil drums used the float of the raft. Different stocking densities of oyster and green mussel were laid on trays and allowed to grow until marketable size. Oyster and green mussel growth were sampling monthly.

Green mussel (*Perna viridis*):

Initially 31.45±1.25 g green mussels were stocked in different culture substrate such as fruit basket, plastic tray and plastic net bag. After the last sampling of the experiment in fruit basket substrate, higher final weight were found in T_1 (76.49±4.18 g) followed by T_2 70.49±5.08 g) and T_3 (59.86±2.28 g) respectively (Table 4). Comparatively higher final weight were found in T_1 (85.14±3.45 g) than T_3 (81.12±4.73 g) and T_2 (80.23±3.15 g) groups where green muscle cultured in plastic tray substrate (Table 5). However, plastic net bag substrate higher final weight were found in T_1 (84.16±1.14g) followed by T_2 (78.19±6.05 g) and T_3 (76.19±3.04 g) respectively (Table 6).Among three culture substrate significantly higher yield was observed in net bag substrate.

Table 4. 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)	31.48± 1.51^a	30.48± 1.51^a	32.85± 1.09^a	NS	2.17	0.185
Final weight(g)	76.49±4.18^a	70.49±5.08^b	59.86±2.28^c	*	3.44	0.012
Survival rate (%)	94.59±2.18^a	90.49±2.08^b	85.86±2.28^c	*	3.58	0.026
Yield (kg/m^2)	2.56±1.04^c	2.89±1.22^a	2.78±4.12^b	*	2.38	0.013

Table 5. Different stocking densities of green mussel were considering as plastic tray substrate

Green mussel	Plastic tray substrate			Significance	CV (%)	p-value
	T_1	$T_{2,}$	T_3			
Initial weight(g)	39.12± 1.51^a	39.01± 1.15^a	40.12± 1.55^a	NS	1.37	0.261
Final weight(g)	85.14±3.45^a	80.23±3.15^c	81.12±4.73^c	*	3.34	0.013
Survival rate (%)	88.14±3.45^a	80.23±3.15^{ab}	78.12±4.73^c	*	2.18	0.026
Yield (kg/m^2)	02.51±1.34^c	2.97±4.23^b	3.24±1.42^a	*	3.18	0.011

Table 6. Different stocking densities of green mussel were considering as plastic net bag substrate

Green mussel	Plastic net bag substrate			Significance	CV (%)	p-value
	T_1	$T_{2,}$	T_3			
Initial weight(g)	38.15± 1.05^a	39.15± 1.05^a	35.52± 1.53^a	NS	2.57	0.381
Final weight(g)	84.16±1.14^a	78.19±6.05^b	76.19±3.04^c	*	3.24	0.013
Survival rate (%)	84.16±1.14^a	78.19±6.05^b	75.19±3.04^c	NS	3.18	0.143
Yield (kg/m^2)	02.32±0.24^c	02.44±0.82^b	03.66±9.91^a	*	1.13	0.016

Fig 10. Green mussel in net bag

Fig 11. Green mussel population

Fig 12. Green mussel sampling & data collection

Fig 13. Green mussel harvest from plastic fruit basket substrate

Oyster (*Crassostrea belcheri*)

In the beginning of the experiment, 50.50±1.45 g weights of the oysters were stocked in fruit basket, plastic tray and plastic net bag. The end of the experiment, higher final weight of oyster were found in T_3 (66.86±2.28 g) followed by T_1 (65.26±2.53g) and T_2 (62.49±3.08 g) in fruit basket substrate systems (Table 7).When considering plastic tray substrate, higher growth were found in T_1 (72.14±3.45g) followed by T_2 (70.23±3.15g) and T_3 (65.12±4.73 g) respectively (Table 8). However, comparatively bigger oyster were found in T_2 (73.66±3.05g) than T_1 (72.34±2.52g) and T_3 (71.22±3.64g) in net bag substrate culture methods (Table 9).

Table 7. Different stocking densities of oyster in fruit basket substrate

Oyster	Fruit basket substrate			Significance	CV (%)	p-value
	T_1	$T_{2,}$	T_3			
Initial weight(g)	$50.10±1.65^a$	$51.18±1.12^a$	$49.88±1.09^a$	NS	4.17	0.175
Final weight(g)	$65.26±2.53^b$	$62.49±3.08^c$	$66.86±2.28^a$	*	2.44	0.024
Survival rate (%)	$95.59±2.18^a$	$91.49±2.08^b$	$85.86±2.28^c$	*	6.47	0.031
Yield (kg/m^2)	$0.86±0.21^b$	$0.821±1.37^c$	$1.81±1.81^a$	*	3.28	0.021

Table 8. Different stocking densities of oyster in plastic tray substrate

Oyster	Plastic tray substrate			Significance	CV (%)	p-value
	T_1	$T_{2,}$	T_3			
Initial weight(g)	$49.12±2.51^a$	$46.22±1.15^a$	$45.12±1.55^a$	NS	1.46	0.062
Final weight(g)	$72.14±3.45^a$	$70.23±3.15^b$	$65.12±4.73^c$	*	1.32	0.025
Survival rate (%)	$88.14±3.45^a$	$80.23±3.15^b$	$78.12±4.73^c$	NS	3.58	0.046
Yield (kg/m^2)	$1.25±0.14^c$	$1.5±0.31^a$	$1.4±1.15^{ab}$	*	1.48	0.029

Table 9. Different stocking densities of oyster in net bag substrate

Oyster	Net bag substrate			Significance	CV (%)	p-value
	T_1	$T_{2,}$	T_3			
Initial weight(g)	48.15±1.05 [a]	49.25±1.05[a]	47.52±1.53[a]	NS	2.15	0.051
Final weight(g)	72.34±2.52[b]	73.66±3.05[a]	71.22±3.64[c]	NS	5.29	0.029
Survival rate (%)	84.16±1.14[a]	78.19±6.05[b]	71.19±3.04[c]	*	3.59	0.027
Yield (kg/m^2)	1.23 ±1.02[c]	1.5 ±0.12[b]	2.14±2.14[a]	*	2.32	0.034


Fig 14. Oyster sampling & data collection


Fig 15. Oyster stoking in fruit basket


Fig 16. Oyster population in net bag


Fig 17. Oyster stoking in net bag

II. Bottom culture

Bottom cultures were implement into three cultures subtract: plastic tray, bamboo tray and net bag tray. Oysters and green mussel were suspended from floating structures such as raft. Oysters and green mussel were held in tray. The rafts can be any shape and materials were oil drums used the float of the raft. Different stocking densities of oyster and green mussel were laid on trays and allowed to grow until marketable size. Oyster and green mussel growth were sampling monthly.

Green mussel (*Perna viridis*)

Initially 50.12±1.15 g, green mussels were stocked in different culture substrate such as plastic tray, bamboo tray and net bag tray. After the last sampling of the experiment in plastic tray substrate, higher final weight were found in T_3 (66.86±2.28g) followed by T_1 (65.26±2.53g) and T_2 (62.49±3.08g) respectively (Table 10). Comparatively higher final weight were found in T_1 (72.14±3.45g) than T_2 (70.23±3.15g) and T_3 (65.12±4.73g) groups where green muscle cultured in bamboo tray substrate (Table 11). However, bamboo net bag substrate higher final weight were found in T_2 (73.66±3.05g) followed by T_1 (72.34±2.52g) and T_3 (71.22±3.64g) respectively (Table 12).Among three culture substrate significantly higher yield was observed in bamboo tray substrate.

Table 10. Different stocking densities of green mussels in plastic tray substrate

Green mussel	Bamboo tray substrate			Significance	CV (%)	p-value
	T_1	$T_{2,}$	T_3			
Initial weight (g)	50.10± 1.65^a	51.18± 1.12^a	49.88±1.09^a	NS	1.57	0.481
Final weight (g)	65.26±2.53^b	62.49±3.08^c	66.86±2.28^a	*	2.24	0.012
Survival rate (%)	95.59±2.18^a	91.49±2.08^b	85.86±2.28^c	NS	3.15	0.152
Yield (kg/m^2)	0.86 ±0.38^c	0.82 ±0.22^b	1.7 ±2.01^a	*	1.18	0.021

Table 11. Different stocking densities of green mussels in bamboo tray substrate

Green mussel	Plastic tray substrate			Significance	CV (%)	p-value
	T_1	$T_{2,}$	T_3			
Initial weight (g)	49.12± 2.32^a	46.22±1.15^a	45.12±1.55^a	NS	1.37	0.145
Final weight (g)	72.14±3.45^a	70.23±3.15^b	65.12±4.73^c	*	3.41	0.034
Survival rate (%)	88.14±3.45^a	80.23±3.15^b	78.12±4.73^c	*	3.54	0.041
Yield (kg/m^2)	1.25±0.41^c	1.5±04.32^a	1.4±2.01^b	*	4.38	0.023

Table 12. Different stocking densities of green mussels in plastic net bag substrate

Green mussel	Bamboo net bag substrate			Significance	CV (%)	p-value
	T_1	$T_{2,}$	T_3			
Initial weight (g)	48.15±1.05^a	49.25±1.05^a	47.52±1.53^a	NS	1.33	0.251
Final weight (g)	72.34±2.52^b	73.66±3.05^a	71.22±3.64^c	NS	2.24	0.123
Survival rate (%)	84.16±1.14^a	78.19±6.05^b	71.19±3.04^c	*	3.28	0.023
Yield (kg/m^2)	1.32±7.^{c}	1.52±2.72^b	2.23±1.05^a	*	1.23	0.018

Fig 18. Green mussel population bamboo net bag substrate

Fig 19. Green mussel stoking in bamboo tray substrate

Oyster (*Crassostrea belcheri*)

In the beginning of the experiment, 51.13±1.25 g weights of the oysters were stocked in bamboo cage, bamboo tray and plastic net bag. The end of the experiment, higher final weight of oyster were found in T_1 (65.16±3.94g) followed by T_2 (62.89±3.08g) and T_3 (62.86±2.28g) in bamboo cage substrate systems (Table 13).When considering plastic tray substrate, higher growth were found in T_1 (80.16±0.33 g) followed by T_2 (78.45±3.17g) and T_3 (72.32±4.03 g) respectively (Table 14). However, comparatively bigger oyster were found in T_1 (81.49±2.52g) than T_2 (75.19±3.05g) and T_3 (71.19±3.04g) in bamboo net bag substrate culture methods (Table 15).

Table 13. Different stocking densities of oysters in bamboo cage substrate

Oyster	Bamboo tray substrate			Significance	CV (%)	p-value
	T_1	$T_{2,}$	T_3			
Initial weight(g)	51.48±1.51^a	49.48±2.09^a	46.85±1.09^a	NS	1.36	0.042
Final weight(g)	65.16±3.94^b	62.89±3.08^c	62.86±2.28^c	*	1.15	0.018
Survival rate (%)	95.59±2.18^a	91.49±2.08^b	85.86±2.28^c	NS	5.58	0.046
Yield (kg/m^2)	0.78±0.18^c	0.95±0.32^b	1.65±0.25^a	*	1.53	0.023

Table 14. Different stocking densities of oysters in bamboo tray substrate

Oyster	Plastic tray substrate			Significance	CV (%)	p-value
	T_1	$T_{2,}$	T_3			
Initial weight(g)	53.12±2.46^a	50.82±1.53^a	48.52±1.55^a	NS	3.17	0.155
Final weight(g)	80.16±0.33 ^{a}	78.45±3.17^b	72.32±4.03 ^{c}	*	3.44	0.026
Survival rate (%)	88.14±3.45^a	80.23±3.15^b	78.12±4.73^c	NS	6.17	0.231
Yield (kg/m^2)	0.85±0.41^c	1.36±02.32^b	1.32±3.42^a	*	2.18	0.024

Table 15. Different stocking densities of oysters in net bag substrate

Oyster	Bamboo net bag substrate			Significance	CV (%)	p-value
	T_1	$T_{2,}$	T_3			
Initial weight(g)	50.15±1.05^a	53.15±1.05^a	51.52±1.53^a	NS	2.15	0.091
Final weight(g)	81.49±2.52^a	75.19±3.05^a	71.19±3.04^a	*	5.19	0.023
Survival rate (%)	84.16±1.14^a	79.19±6.05^b	73.19±3.04^c	*	2.59	0.042
Yield (kg/m^2)	1.64±1.02^c	1.35±0.32^b	1.37±3.14^a	*	2.22	0.014

Fig 20. Bamboo tray substrate for oyster culture

Fig 21. Bamboo net bag substrate for oyster culture

B. Indoor culture system

Growth performance of green mussel and the oyster, seabass (*Latis calcarifer)* with seaweed

(*Ulva.sp*) in integrated (RAS) culture system were implemented at indoor condition at MFTS campus. Different stocking densities of oyster and green mussel were laid on trays and allowed to grow until marketable size. Oyster, green mussel, fish and seaweed growth were sampling monthly. Culture period were 180 days .

Green mussel (*Perna viridis*)

Initially 29.11±1.35 g green mussels were stocked in net bag culture substrate tray. After the last sampling of the experiment in plastic tray substrate, higher final weight were found in T_1 (78.51±1.34g) followed by T_2 (70.39±3.19g) (Table 16).

Table 16. Different stocking densities of green mussel in net bag substrate in fiberglass tank

Green mussel	Net bag substrate in fiberglass tank		Significance	CV (%)	p-value
	T_1(40 Indi.)	**T_2**, (50 Indi.)			
Initial weight (g)	31.38± 2.13^a	30.32± 1.23^a	NS	1.47	0.341
Final weight (g)	78.51±1.34^a	70.39±3.19^b	*	1.23	0.019
Survival rate (%)	85.71±.32^a	75.55±1.27^a	*	3.15	0.046
Yield (kg/m^2)	02.84±0.38^c	03.26±0.42^b	*	2.18	0.021

Oyster (*Crassostrea belcheri*)

Initially 50.654±1.45 g, oysters were stocked in net bag culture substrate tray. After the last sampling of the experiment in net bag substrate, After the last sampling of the experiment in net bag substrate, final weight were varied T_1 (78.31±1.24g) to T_2 (73.29±2.19g) (Table 17).

Table 17. Different stocking densities of oysters in net bag substrate in fiberglass tank

Oyster	Net bag substrate in fiberglass tank		Significance	CV (%)	p-value
	T_1(25 Indi.)	**T_2**, (.30 Indi.)			
Initial weight (g)	50.78± 1.23^a	50.42± 2.23^a	NS	1.27	0.321
Final weight (g)	78.31±1.24^a	73.29±2.19^b	*	1.22	0.023
Survival rate (%)	86.71±.34^a	83.19±1.13^a	*	3.15	0.024
Yield (kg/m^2)	01.95±0.32^c	02.36±0.42^b	*	1.12	0.016

Seabass:

Initially 250.11±1.35 g, seabass (*Lates calcarifer*) were stocked in fiberglass tank. After the last sampling of the experiment in fiberglass tank, After the last sampling of the experiment in plastic tray substrate, final weight were varied T_2 (395.31±3.64g) to T_1 (380.29±2.19g) (Table 18).

Table 18. Different stocking densities of seabass were considering in fiberglass tank

Seabass	Fiberglass tank		Significance	CV (%)	p-value
	T_1(4 Indi.)	**T_2**, (6 Indi.)			
Initial weight(g)	250.78± 1.23^a	250.82± 2.63^a	NS	1.27	0.321
Final weight(g)	395.31±3.64^a	380.29±2.19^b	*	1.22	0.023

Survival rate (%)	100±0.00[a]	100±0.00[a]	NS	3.15	0.321
Yield (kg/m^2)	01.58±0.34[b]	02.28±0.32[a]	*	3.48	0.023

Seaweed:

Initially 500.11±5.25 g, seaweed (*Ulva lactuca*) were stocked in cemented tank. After the last sampling of the experiment in cemented tank, final weight were varied T_1 (3940±2.19g) to T_2 (3560±1.24g) (Table 19).

Table 19. Different stocking densities of green mussel were considering as cemented tank

Seaweed	Cemented tank		Significance	CV (%)	p-value
	T_1	T_2			
Initial weight(g)	500.78± 2.23[a]	500.42± 4.23[a]	NS	3.27	0.121
Final weight(g)	3560±1.24[b]	3940±2.19[a]	*	1.22	0.013
Survival ret (%)	95.71±.62[a]	96.18±1.34[a]	NS	2.15	0.124
Yield kg/800L	03.56±0.32[c]	03.94±0.32[b]	*	1.18	0.021

Fig 22. Indoor green mussel sampling

Fig 23. Indoor oyster sampling

Fig 24. Indoor seaweed harvest

Fig 25. Indoor seabass sampling

Experiment 4. Developments of breeding techniques of oyster (*Crassostrea belcheri*) in captivity.

Methodology:

A. Indoor captive breeding:

Oysters of length ranging from 60-90 mm are ideal and 30% of them should be 60-75 mm of length in order to have assured availability of males. Firstly, the brood will be taken into count for sex determination. The ripe condition has a white creamy colour and has visible veining within the gonad tissue. While the Poor condition one is transparent in gonad area. However, the oyster with a little or no reproductive development will be avoiding because it more time period and high food demand. The breeding system of oyster will be proceeding in two ways.

1. Induced Spawning (Natural or chemical method)
2. Strip spawning

B. Outdoor natural close control breeding:

Off-bottom natural close control breeding was implemented four breeding hapa with 250µ mesh net cover. Oysters were suspended from floating structures such as raft. Oysters held in a net bag. The rafts can be any shape and materials were oil drums used the float of the raft. Oyster was stoked 10 individual per net bag. Oyster breeding hapa was sampling monthly.

Results:

A. Indoor captive breeding:

During histological tissue processing period striping breeding procedure protocol were followed. Mature ovum were collected but no sperm were collected. Due to improper striping process no fertilization was performed.

Fig 26. Mature oyster oyster sacrificed for artificial breeding

Fig 27. Microscopic observation of ovum and sperm

B. Outdoor natural close control breeding:

Off-bottom natural close control breeding was started October, 2023 and fortnightly monitoring oyster spat. During spat monitoring period oyster spat were found in January, 2024.

Fig 28. Oyster brood stock breeding hapa

Fig 29. Oyster breeding hapa

Fig 30. Oyster brood observation in breeding hapa

Fig 31. Oyster spat observation in breeding hapa

Experiment 4: Development of larval rearing techniques of oyster (*Crassostrea belcheri*)

Methodology:

A. Up-welling system in indoor environment

A closed recirculation system were designed for breeding and determined the effect of substrate conditioning on larval settlement and spat growth of oyster, *Crassostera sp*. The system were consisted of a submersible pump, a 1,200 L fiberglass tank (dimensions 1.06 × 2.13 × 0.76 m) will be used to hold setting units, a 105 L fiberglass tank (dimensions 50 × 70 × 30 cm) were used for food storage, and 10 sets of fiberglass tanks (diameter 25 cm × high 36 cm) were used as setting units. A screen with a mesh size of 180 µm was fixed by a fiberglass clamp to the bottom of each fiberglass tank.

B. Spat monitoring from wild condition

For the collection of spat, 4 types of rope materials such as tiles, kortal, bolder and oyster shell were used. Spat collector hang from the raft (each with 10-12 cm gap) having 1.5 m length. After the settlement of rope materials, a regular monitoring were done to observe spat frequency rate for every 15 days interval and water quality data were recorded. Different spat collector of oyster and green mussel were hung under raft.

Results:

A. Up-welling system in indoor environment

No successful breeding trail were occurred so no up welling operate

B. Spat monitoring from wild condition

For the collection of spat, 4 types of substrate (spat collector) such as tiles, kortal, bolder and oyster shell were used. Spat collector substrate hanged from the raft (each with 10-12 cm gap) having 1.5 m length following experimental design. After the setting the spat collector fortnightly regular monitoring was done to observe spat settlement. Water quality data were also recorded fortnightly. Different spat substrate of oyster and green mussels were hung from raft. Among the four treatments, higher number of total spat were found in T_1 (53.00±2.00) followed by T_3 (42.00±2.00), T_4 (41.00±3.00) and T_2 (27.00±3.00) respectively.

Table 20. 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)	17±1.00	36±2.00	53±2.00
T_2 (Bolder)	15±2.00	12±2.00	27±3.00
T_3 (Kortal)	18±3.00	24±3.00	42±2.00
T_4 (Oyster shell)	30±2.00	11±1.00	41±3.00

Fig 32. Spat collector (Kortal)

Fig 33. Spat collector (Tiles)

Fig 34. Spat collector (Bolder)

Fig 35. Spat collector (oyster shell)

Development of mariculture practice of some important fin fishes (Seabass, Mullet) in the south-east coast of Bangladesh

Researchers:

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Objectives of the project (2023-24)

i. To evaluate the production and survivability of different initial size groups of mullets (*Mugil cephalus*) in an open seawater floating cage culture system
ii. To optimize the nutritional requirements of mullet (*Mugil cephalus*) fingerlings in an indoor rearing system

Achievement:

Study-1: Evaluation of the production and survivability of different initial size groups of mullets (*Mugil cephalus*) in an open seawater floating cage culture system

Methodology:

A total of 06 circular cages were prepared with double-layered, knotless net, 12 plastic drums per cage for better bouncy. Mooring (9ft long, 3.5-inch diameter) was done to resist the stronger tidal and wave action.

The details of the fixed net cages were as follows-

- **Shape:** Round
- **Inner Diameter:** 4.8 meters
- **Outer Diameter**: 5.25 meters
- **Depth:** 1.3 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

✓ **Experimental Species:** Mullet (*Mugil cephalus*)
✓ **Experimental Design:** Completely Randomized Design (CRD)
✓ **Experimental Duration:** 4 Months
✓ **Stocking Density:** 50 ind./cage

Fig. 1: Releasing of mullet (*Mugil cephalus*)

Results and conclusion:

The data on growth and feeding gathered during the trial were summarized in Table-2. The highest weight gain percentage (%WG) was obtained at small size group, and differences were significant (p<0.05) than medium and larger size group. There was clear declining trend in growth rate values with increasing body weight. Specific growth rate (SGR) significantly (p <0.05) varies with different initial size group of mullet fishes and higher value found in smaller size group than other two groups. Feed conversion ratio (FCR) values were lowest in small group. The estimated FCR varied between 3.25 and 5.89, and normally plummet down with herbivore fish, differences among groups were found significant (p <0.05) in favour of smaller size group. Growth performance of mullet culture in cage appears to final biomass can be maximized at optimal stocking densities for smaller size of fish than for medium and larger fish.
In conclusion, based on the findings of the study, initial stocking size of fish is negatively correlated to final biomass and to increase biomass per unit volume, small size fish should be stocked into floating cages at the beginning of rearing season.

Table 1. Initial whole body and final flesh proximate composition (on % wet weight basis) of mullet reared in open seawater cage for 120 days.

Experimental groups[1]	Moisture	Crude protein	Lipid	Total ash
IBC[2]	70.11 ± 0.25	18.56± 0.14	1.42 ± 0.17	3.17 ± 0.48
FFC[3]				
C_1	72.45 ± 0.25	16.62 ± 0.32	2.55 ± 0.47	2.67 ± 0.26
C_2	72.34 ± 0.53	15.58 ± 0.40	2.01 ± 0.31	2.00 ± 0.17
C_3	72.65 ± 0.41	15.72 ± 0.25	1.83 ± 0.29	2.10 ± 0.31
***p*-value**	0.721	0.626	0.572	0.716

Data are expressed as mean (n=3)
Mean values in the same column with different superscripts differ significantly ($p<0.05$)
[1]C_1- initial smaller size group; C_2- initial medium size group; C_3 - initial larger size group.
[2]IBC, initial body composition; [3]FFC, final flesh composition

Table-2: Final Growth increment of different initial size groups of mullet reared in open seawater cages for 120 days

Parameters	Cage (C_1)	Cage (C_2)	Cage (C_3)
Initial weight (g)	$30.13^a \pm 0.15$	$50.30^b \pm 1.02$	$70.35^c \pm 0.24$
Final weight (g)	$135.37^a \pm 8.82$	$121.34^b \pm 8.62$	$112.28^b \pm 6.21$
Weight gain (g)	105.24 ± 8.69	71.04 ± 9.58	41.93 ± 9.08
% Weight gain	$349.22^a \pm 13.53$	$141.24^b \pm 15.53$	$59.6^c \pm 9.28$
SGR	**$1.25^c \pm 0.01$**	$0.73^b \pm 0.02$	$0.39^a \pm 0.01$
FCR	**$3.25^c \pm 0.6$**	$5.02^b \pm 0.4$	$5.89^a \pm 0.35$

Study-2: Optimization of nutrient requirements of mullet fingerlings in the indoor rearing system

I. Optimization of protein requirements of mullet fingerlings

Experimental diet formulation and preparation

Semi-purified ingredients were used for formulation (Table 3) and preparation of four iso-lipidic (6% CL), iso-caloric (380 kcal digestible energy/100 g), and hetero-nitrogenous (25–40% CP) semi-purified experimental diets, *viz.*, CP_{25} (25% protein), CP_{30} (30% protein), CP_{35} (35% protein), and CP_{40} (40% protein). All the ground ingredients except oils and the vitamin-mineral mixture were mixed uniformly, followed by the addition of the required quantity of water to form dough, which was then kept in a heat-resistant plastic bag and pressure-cooked under steam for 25 minutes. After cooling, the rest of the raw materials were added, and the dough prepared after thorough mixing was then hand-pressed through a pelletizer to prepare 1 mm-diameter pellets. After that, the pellets were dried at room temperature for one day, followed by oven drying at 40^0C until they reached around 10% of the moisture level. The dried pellets were then broken to adjust to the mouth size of the experimental fish, followed by packaging in polythene bags, labeling, and storage at 4^0C until used for feeding.

Experimental facilities and feeding trial

After acclimatization, 120 fish (avg. b. wt. 22.50 ± 2.02 g) were arbitrarily dispersed in four experimental groups such as CP_{25}, CP_{30}, CP_{35} and CP_{40} with three replicates under each according to completely randomized design (CRD). Ten fish were stocked in each circular tank (100 L water volume). Respective diets were used for feeding the fish two times per day (10.00 and 18.00 h). Fortnightly, the body weight was checked to adjust the feeding rate.

Results and conclusion

Initial whole-body moisture, protein, lipid and ash contents of fish were 70.11, 18.56, 1.42 and 3.17%, respectively (Table-4). Though no significant variation ($p>0.05$) was found for final whole-body moisture, crude protein and ash content, whole body lipid of fish was significantly ($p < 0.05$) affected in relation to dietary protein levels (Table-4). Significantly highest and lowest whole body lipid content ($p < 0.05$) found in CP_{35} and CP_{25} group respectively whereas CP_{25} was statistically similar ($p > 0.05$) to CP_{30} group.

Growth performances such as FBW, WG and SGR were affected with dietary protein and showed insignificant ($p > 0.05$) increasing trend with increasing protein up to 35% protein level then decreased with increased protein level in diet. FCR of mullet juveniles were affected significantly ($p < 0.05$) in relation to graded level of CP in the diet (Table-5). There was significantly ($p < 0.05$) decreasing of FCR in relation to enhancing dietary protein up to 35% level, then increased ($p < 0.05$) with the further increase in protein level.
Based on WG, second-order polynomial regression analysis revealed that the optimum dietary crude protein requirement of mullet juveniles under the rearing condition of 15 ppt salinity was 37.00% (Fig. 2)

Table 3. Formulation and proximate composition of the experimental diets fed to mullet fingerlings cultured in saline water of 15 ppt for 60 days

[1]CP_{25} (25% dietary crude protein), CP_{30} (30% dietary crude protein), CP_{35} (35% dietary crude protein), CP_{40} (40% dietary crude protein) [2]Ingredients procured from Himedia Pvt. Ltd., India; [3]Procured from Seacod Oil by Sanofi India Ltd., India; [4]Purchased from local retail shop [5]Vitamin-mineral mixture [6]Carboxymethyl cellulose, purchased Himedia Pvt. Ltd., India; [7]Nitrogen free extract; [8]Gross energy; [9]Digestible energy; [10]Protein to energy ratio

Table 4. Whole-body proximate composition (on % wet weight basis) of mullet fingerlings cultured in saline water of 15 ppt and fed with hetero-nitrogenous experimental diets for 60 days

Experimental groups[1]	Moisture	Crude protein	Lipid or ether extract	Total ash
IBC[2]	70.11 ± 0.25	18.56±0.14	1.42 ± 0.17	3.17 ± 0.48
FBC[3]				
CP_{25}	72.56 ± 0.15	18.94 ± 0.12	3.23^{ab} ± 0.41	4.97 ± 0.06
CP_{30}	72.82 ± 0.74	20.08 ± 0.45	3.42^{ab} ± 0.11	4.89 ± 0.09
CP_{35}	72.51 ± 0.27	21.37 ± 0.15	4.98^{b} ± 0.09	4.22 ± 0.09
CP_{40}	72.60 ± 0.12	22.15 ± 0.04	5.32^{a} ± 0.16	4.33 ± 0.10
***p*-value**	0.421	0.326	0.005	0.716

Data are expressed as mean (n=3)
Mean values in the same column with different superscripts differ significantly ($p<0.05$)
[1]CP_{25}-CP_{40}, 25-40% dietary crude protein
[2]IBC, initial body composition; [3]FBC, final body composition

Table 5. Growth and nutrient utilization of mullet fingerlings cultured in saline water of 15 ppt and fed with hetero-nitrogenous experimental diets for 60 days

Experimental groups[1]	IBW[2] (g)	FBW [3](g)	WG[4] (g)	SGR[5] (%/day)	FCR[6]
CP_{25}	22.41 ± 2.14	26.97 ± 1.04	4.56 ± 0.05	0.64 ± 0.03	3.20^{c} ± 0.12
CP_{30}	23.12 ± 2.02	31.35 ± 1.25	8.23 ± 0.03	0.75 ± 0.03	4.41^{b} ± 0.06
CP_{35}	22.78 ± 2.04	35.11 ± 1.22	12.33 ± 0.25	0.94 ± 0.11	4.04^{ab} ± 0.24
CP_{40}	24.10 ± 3.02	34.39 ± 2.08	10.29 ± 0.10	0.81 ± 0.04	4.55^{a} ± 0.09
***p*-value**	0.058	0.257	0.274	0.191	0.028

Data are expressed as mean (n=3)
Mean values in the same column with different superscripts differ significantly ($p<0.05$)
[1] CP_{25}-CP_{40}, 25-40% dietary crude protein
[2]IBW, Initial body weight; [3]FBW, Final body weight; [4]WG, weight gain; [5]SGR, specific growth rate; [6]FCR, feed conversion ratio; [7]PER, protein efficiency ratio

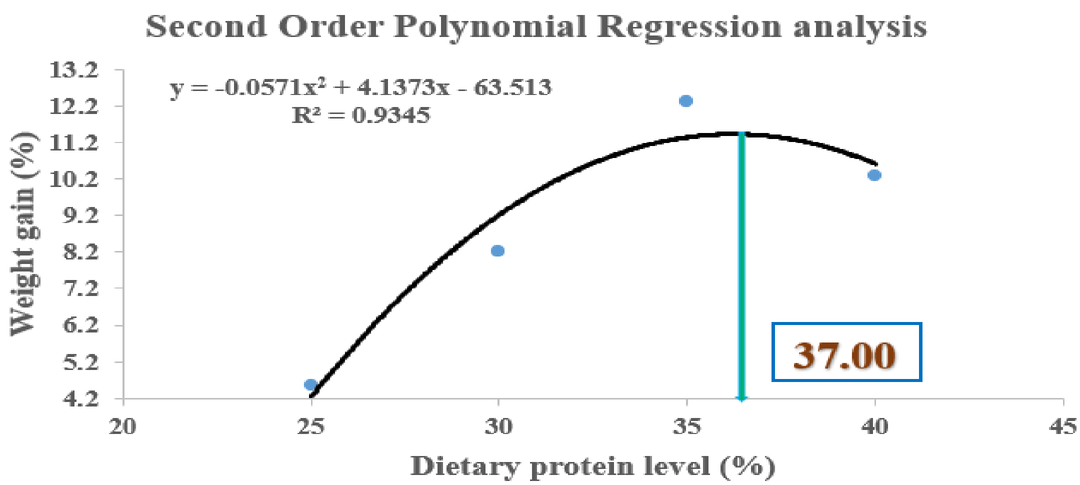

Fig. 2: Optimization of dietary protein level of mullet based on weight gain

II. Optimization of lipid requirements of mullet fingerlings

Experimental diet formulation and preparation

Semi-purified ingredients were used for formulation (Table-6) and preparation of four iso-nitrogeous (40% CP), and hetero-lipidic (3-12% CL) semi-purified experimental diets *viz.*, CL_3 (3% lipid), CL_6 (6% lipid), CL_9 (9% lipid) and CL_{12} (12% lipid). All the ground ingredients except oils and vitamin-mineral mixture were mixed uniformly followed by addition of required quantity of water to form dough, which was then kept into a heat-resistant plastic bag and pressure-cooked under steam for 25 min. After cooling, the rest of the raw materials were added, and dough prepared after thorough mixing was then hand pressed through a pelletizer to prepare 1 mm diameter pellets. After that, the pellets were dried at room temperature for one day followed by oven drying at 40^0C until achieving around 10% of the moisture level. The dried pellets were then broken to adjust with mouth size of experimental fish followed by packaging polythene bags, labelling and storage at 4^0C until used for feeding.

Experimental facilities and feeding trial

After acclimatization, 120 fish (avg. b. wt. 21.30 ± 0.03 g) were arbitrarily dispersed in four experimental groups such as CL_3, CL_6, CL_9 and CL_{12} with three replicates under each according to completely randomized design (CRD). Ten fish were stocked in each circular tank (100 L water volume). Respective diets were used for feeding the fish two times per day (10.00 and 18.00 h). Fortnightly, the body weight was checked to adjust the feeding rate.

Table 6. Formulation and proximate composition of the experimental diets fed to mullet fingerlings cultured in saline water of 15 ppt for 60 days

[1]CL_3(3% dietary crude lipid), CL_6 (6% dietary crude lipid), CL_9 (9% dietary crude lipid), CL_{12} (12% dietary crude lipid)
[2]Ingredients procured from Himedia Pvt. Ltd., India; [3]Procured from Seacod Oil by Sanofi India Ltd., India; [4]Purchased from local retail shop;[5]Vitamin-mineral mixture;[6]Carboxymethyl cellulose, purchased Himedia Pvt. Ltd., India; [7]Nitrogen free extract; [8]Gross energy; [9]Digestible energy; [10]Protein to energy ratio

Results and conclusion

Initial whole-body moisture, protein, lipid, and ash contents of fish were 70.11, 18.56, 1.42, and 3.17%, respectively (Table 7). Though no significant variation (p > 0.05) was found for final whole-body moisture, crude protein, and ash content, the whole-body lipid of fish was significantly ($p < 0.05$) affected in relation to dietary lipid levels and showed an increasing trend with increasing lipid content up to the maximum level.

Growth performances such as FBW, WG and SGR were affected with dietary lipid and showed insignificant ($p > 0.05$) increasing trend with increasing lipid up to 9% lipid level then decrease with increased lipid level in diet. FCR of mullet juveniles were affected significantly ($p < 0.05$) in relation to graded level of CL in the diet (Table-8). There was significantly ($p < 0.05$) decreasing of FCR in relation to enhancing dietary lipid up to 9% level and then decrease ($p < 0.05$) with the further increase lipid level in diet.

Based on WG, second order polynomial regression analysis revealed that the optimum dietary crude lipid requirement of mullet juveniles under the rearing condition of 15 ppt salinity was 8.80% (Fig 3).

Table 7. Whole-body proximate composition (on % wet weight basis) of mullet fingerlings cultured in inland saline water of 15 ppt and fed with hetero-energic experimental diets for 60 days

Experimental groups[1]	Moisture	Crude protein	Lipid or ether extract	Total ash
IBC[2]	70.11 ± 0.25	18.56±0.14	1.42 ± 0.17	3.17 ± 0.48
FBC[3]				
CL_3	73.73 ± 0.15	18.11 ± 0.12	4.59^{a} ± 0.07	4.26 ± 0.19
CL_6	73.52 ± 0.74	18.28 ± 0.36	5.13^{ab} ± 0.19	4.38 ± 0.10
CL_9	73.41 ± 0.27	18.68± 0.17	5.47^{ab} ± 0.18	4.91± 0.08
CL_{12}	72.98 ± 0.12	18.15 ± 0.04	6.21^{b} ± 0.52	4.93 ± 0.11

***p*-value**	0.512	0.446	0.003	0.643

Data are expressed as mean (n=3)
Mean values in the same column with different superscripts differ significantly ($p<0.05$)
[1]Cl_3-CL_{12}, 3-12% dietary crude lipid
[2]IBC, initial body composition; [3]FBC, final body composition

Table 8. Growth and nutrient utilization of mullet fingerlings cultured in saline water of 15 ppt and fed with hetero-energic experimental diets for 60 days

Experimental groups[1]	IBW[2] (g)	FBW [3](g)	WG[4] (g)	SGR[5] (%/day)	FCR[6]
CL_3	23.26 ± 1.02	29.27 ± 1.04	6.01 ± 0.05	0.58 ± 0.03	5.02^a ± 0.15
CL_6	21.30 ± 1.03	31.77 ± 1.25	10.47 ± 0.03	0.89 ± 0.03	3.05^{ab} ± 0.09
CL_9	22.20 ± 2.04	30.41 ± 1.22	8.21 ± 0.25	1.12 ± 0.05	4.56^b ± 0.34
CL_{12}	22.32 ± 2.02	30.21 ± 2.08	7.89 ± 0.10	0.97 ± 0.05	5.17^a ± 0.12
***p*-value**	0.057	0.257	0.274	0.201	0.036

Data are expressed as mean (n=3)
Mean values in the same column with different superscripts differ significantly ($p<0.05$)
[1]CP_{20}-CP_{50}, 20-50% dietary crude protein
[2]IBW, Initial body weight; [3]FBW, Final body weight; [4]WG, weight gain; [5]SGR, specific growth rate; [6]FCR, feed conversion ratio; [7]PER, protein efficiency ratio.

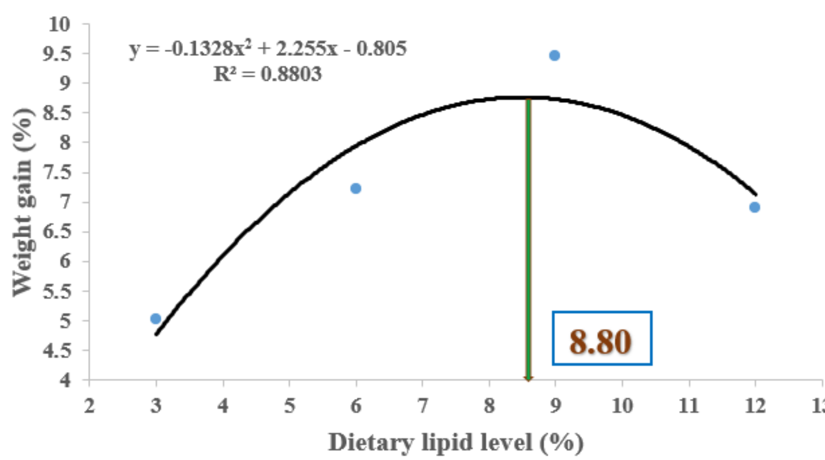

Fig. 3: Optimization of dietary lipid level of mullet based on weight gain

10. Problems/constraints encountered if any:

a) Unavailability of suitable size mullet fry and fingerlings
b) Sometimes net mooring is being torn by the other boat
c) Occurrence of different types of natural calamities
d) Causation of vandalism by local people and fishermen.

11. Proposed work during the next period

a) Evaluation of the production and survivability of different initial size groups of seabass, mullet in an open seawater floating cage culture system.
b) Optimization of nutrient requirements of seabass, mullet fingerlings in the indoor rearing system.

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

0Researchers: Dr. Shafiqur Rahman, PSO
Ahmad Fazley Rabby, SSO
Turabur Rahman, SO
Zahidul Islam, SO

Objectives (2023-2024)

- To domesticate the Blue Swimming Crab (*P. pelagicus*) and Horseshoe Crab (*Tachypleus* sp.) under captive/ hatchery conditions
- To develop breeding technology of Horseshoe Crab (*Tachypleus* sp.) in captive/ hatchery conditions
- To develop larval, nursery and grow out management technique of Blue Swimming Crab
- To develop incubation technique and larval management Horseshoe Crab (*Tachypleus* sp.)

Achievements:

Experiment 1: **To domesticate the Blue Swimming Crab (*P. pelagicus*) and Horseshoe Crab (*Tachypleus* sp.) under captive/ hatchery conditions**

Domestication of Horseshoe crab was done in a system where a canister filter was used for filtration of the water in the habitat of the tank. Cannister filter remove the ammonia, uneaten particles and fecal metal of the horseshoe crabs which betters the environment. Another thing is that, with the use of canister filter seawater can be reused, however 50% of the sea water in the tank of the horseshoe crabs were exchanged with new fresh sea water. Horseshoe crabs were fed with bi valve (calm) meat at the ratio of 2-3% of their body weight. 3 individual of the *T. gigas* and 16 individuals of the *C. rotundicauda* were domesticated in the system. In the new system of the horseshoe crab habitat, no mortality observed unlike previous systems.

Figure 1: Habitat of Horseshoe crabs facilitated with canister filter

Total 35 Blue swimming crabs were collected and habituated in MFTS Hatchery. Water temperature was maintained at 25-30^{o} C, water salinity was 30 ppt with continuous aeration and daily feeding ratio was @ 3% of body weight with fresh marine squid, bivalve (clam) or fish meat (flat fish) alternately. Total 8 berried female crabs were identified when water level was reduced among 16 domesticated crabs. Berried females were then transferred individually to 500-liter tank with aerated sea water at 30 ppt salinity. Size of berried crab was average 7.2 cm carapace length and 143gm total Weight.

Figure 2: Cemented tank habitat

Figure 3: Fiber tank habitat

Experiment 2: **To develop breeding technology of Horseshoe Crab (*Tachypleus* sp.) in captive/ hatchery conditions**

Domesticated horseshoe crabs were stimulated through electric simulation where 4V of electric shock were applied in both male and female. This electric simulation was applied during full moon day like their natural breeding time. Domesticated horseshoe crabs, *T. gigas* produced eggs and sperm which then kept in the petri dish for fertilization and then incubation. Domesticated *C. Rotundicauda* produced eggs however they did not release sperm. After on month of incubation none of the eggs hatched. So, electric shock was applied in the following month, which resulted in lesion in the shock area of the horseshoe crab which eventually died. Kept that in consideration, horseshoe crabs from natural sourced were collected on spot and then shocked. During November to March of the fiscal year of 2023-24, total 86 *C. Rotundicauda* were stimulated and got 152 eggs in those months. Four types of incubation systems were used sand system, wet tissue system, RAS incubator, clay system. Eggs were monitored for any changes in the eggs but there were no sign of egg developmental stage. After 10 days of incubation, fungal attack and deformities were observed.

Table 2: Monthly artificial breeding and hatching status of Horseshoe Crabs

Species sampled	Sampling month	Domesticated/ Nature	No of male and female stimulated	No of collected eggs	Hatching status
C. rotundicauda	November	Nature	6	14	None
T. gigas	December	Domesticated	3	6	None
C. rotundicauda	January	Nature	15	37	None
C. rotundicauda	February	Nature	28	45	None
C. rotundicauda	March	Nature	37	56	None

Figure 4*: Freshly collected eggs are to be fertilized*

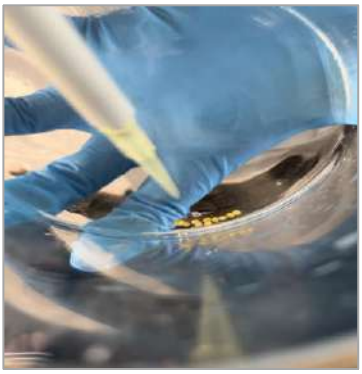

Figure 5: *Mixing of Sperm with eggs*

Figure 6: Un-hatched eggs after 29 days

Experiment 3: **To develop larval, nursery and grow out management technique of Blue Swimming Crab**

Larval rearing:

Blue swimming crabs normally spawn in full moon or new moon day and close to these days. They spawn early in the morning. So, when larvae were hatched brood crab was separated from the plastic jar where it spawned for better collection of the Zoea 1 from the plastic bucket. A torch was used to light the surface of the plastic bucket which helps to collect the healthy larvae to come on the surface of the water of the plastic bucket as ther are photophilic, and larvae which were in the bottom of the jar were siphoned out of the bucket. Then larvae were counted for calculating the viable spawning number. After that larvae were disinfected with 1ppm of formalin before stocking in the larvae rearing tank. Zoeae were stocked at a density of 30 individuals per liter in 3 circular fiber tanks respectively contain 300L seawater and fed with the rotifer *Brachionus rotundiformis* at a density of 10-15 rotifers/ml. The microalga *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 late zoea 1stage and upwards. The zoeae were reared at a salinity of 28-30 ppt and water temperature of 26-30.5^{o}C 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.

Feeding was done five times a day where rotifer was being used initially and from late zoea1 stage larve were fed with *Artemia* umbrella with a rotation of mix feed. At 9pm enzyme was being used for better digestion. Larval rearing was done three times in the year of 2023-24 according to last year's better result. That experiment was mimicked industrial production of crablets with less uses of inputs in the rearing period. Average survival rate of the crablets were 5.3%, 6.8% and 5.9% respectively. Total 4770 crablets were produced in those three trails.

Nursery rearing:

One nursery experiment was conducted where three treatments was used. In T_1- minced Bi-valve, shrimp and squid mix: T_2- only minced bi-valve and T_3- only minced artemia biomass was used. 21

days old Crablets were reared for one month in that experiment. Shelter was provided in all three treatments for hiding. 30-40% water was exchanged weekly. Left over feed was siphoned out from the treatment tanks daily. 50 crablets was the stocking density for those treatments and two replications was used for each treatment. Weight gain was calculated in 15 days interval. T_1 showed the better growth rate among all three treatments.

Table 2: Nursery experiment of Swimming crab larvae

Treatment	Avg. Initial Weight	Avg. Weight at day 15	Avg. Final Weight	Avg. Weight gain
T1R1	0.34g	2.1g	3.93	3.59
T1R2	0.37g	2.19g	3.87	3.5
T2R1	0.35g	1.63g	3.44	3.09
T2R2	0.34g	1.45g	3.27	2.93
T3R1	0.37g	1.96g	2.85	2.48
T3R2	0.35g	1.88g	3.12	2.77

Figure 7: Experimental nursey set-up of crablets

Figure 8: 21 days old crablets

Figure 9: Initial weight measurement of crablet

Figure 10: Crablets after one month of rearing

Grow-out of crablet:

Grow-out experiment will be done in salt field where shrimp are also being cultured. Two net holding structures were built however net was not tied to that structure because of no saline water in that area at that time. Grow-out experiment will be commenced as soon as water fills and crabs will be reared in the salt fields along with shrimps.

Figure 11: Preparing of net holding frame

Figure 12: Prepared structure in salt field

Experiment 4: **To develop incubation technique and larval management Horseshoe Crab (*T. gigas*)**

Fertilized eggs were incubated in four different systems were used sand system, wet tissue system, RAS incubator, clay system. Eggs were kept in the incubator for whole month and regularly checked any change in the eggs. However, none of the eggs were hatched. Incubation system will be changed in the upcoming fiscal year and larval rearing methodology will be developed onwards.

Figure 12: RAS incubator

Figure 14: Sand system

Figure 13: Clay system

Figure15: Wet tissue system

10. problems/constraints encountered if any:

i. Sperm of horseshoe crabs was quite low in amount to fertilize the eggs of horseshoe crabs
ii. Grow out set up depends on the season and work delayed due to long salt season

11. Proposed work during the next period

i. Artificial breeding of horseshoe crab will be conducted.
ii. Grow-out trails of blue swimming crab will be done extensivel

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

Researchers : Ahmad Fazley Rabby, SSO
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Objectives (2023-24)

- To estimate the life-history characteristics and stocks of commercially important marine fish species of Bangladesh
- To estimate the Biological Reference Points (BRPs) and Spawning Potential Ratio (SPR) of marine fish species
- To estimate stock of marine fish species using catch based MSY model (CMSY)

Achievements

Study-1: Estimating the life-history characteristics and stocks of commercially important marine fish species of Bangladesh

Total length and total weight of commercially important marine fishes with size at first sexual maturity of 12 species were determined from the land-mark base data i.e. *Auxis thazard* (Frigate tuna), *Euthynnus affinis* (Little tuna), *Katsuwonu spelamis* (Skipjack tuna), *Auxis rochei* (Bullet Tuna) *Thunnus tonggol* (Long tail 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) which results are followed in table 1:

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

Species name	Lm
Auxis rochei (Bullet tuna)	35.52 (31.08 - 38.88)
Auxis thazard (Frigate tuna)	24.58 (18.99 - 32.21)
Euthynnus affinis (Little tuna)	40.47 (28.37 - 45.73)
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)	58.86 (44.82 - 81.52)
Scomberomorus guttatus (Indo-pacific king mackerel)	55.29 (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 (Island Mackerel)	12.08 (12.53-20.56)
Rastrelliger kanagurta (Indian Mackerel)	14.89 (10.15-16.36)
Scomberoides tol (Needlescaled queenfish)	23.31 (17.87-30.18)

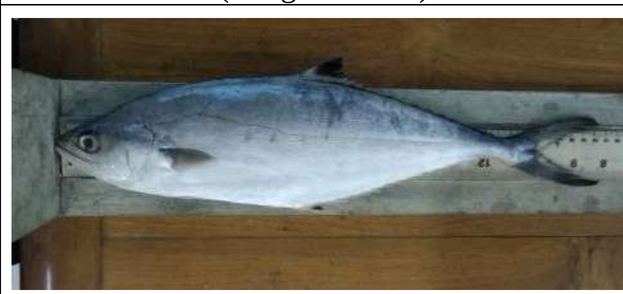
About 1480 (male=821 and female=659) individuals of *Auxis thazard* were examined. Estimated size at sexual maturity was at the length of 24.58 cm. Total of 1342 (male= 800 and female=542) individuals of *Auxis rochei* were examined. Estimated size at sexual maturity was at the length of 35.52 cm. Total of 2610 (male=1840 and female=770) individuals of *Euthynnus affinis* were examined. Estimated size at sexual maturity was at the length of 40.47 cm. Total of 1280 (male=846 and female=434) individuals of *Katsuwonus pelamis* were examined. Estimated size at sexual maturity was at the length of 33.30 cm. Sum of 1417 (male=950 and female=467) individuals of *Thunnus tonggol* were examined. Estimated size at sexual maturity was at the length of 34.92cm. Sum of 1346 (male=875 and female=471) individuals of *Scomberomorus guttatus* were examined. Estimated size at sexual maturity was at the length of 55.29 cm. Total of 1210 (male=810 and female=400) individuals of *S. commerson* examined. Estimated size at sexual maturity was at the length of 60.41 cm. About 2273 (male=1822 and female=451) individuals of *Scomberoides commersonnianus* were examined. Estimated size at sexual maturity was at the length of 58.86 cm. About 2348 (male=1482 and female=866) individuals of *Rastrelliger kanagurta* were examined. Estimated size at sexualmaturity was at the length of 14.89 cm. Total 1047 (male = 848 and female = 199) individuals of *Rastrelliger faughni* were examined. Estimated size at sexual maturity was at the length of 12.08 cm. Total 1284 (male 832 and female 452) individuals of *Scomberoides tol* were examined. Estimated size at sexual maturity was at the length of 23.31 cm. Total of 1334 (male =742 and female = 592) individuals of *Scomberoides lysan* were examined. Estimated size at sexual maturity was at the length of 27.84 cm.

Table 2: Descriptive statistics and assessed parameters of length–weight relationships (BW = a × TL^b) for 12 commercially important marine fishes in Bay of Bengal, Bangladesh during July 2023 to June 2024.

Species	N	Total length (cm)			Body weight (gm)			Regression parameter		95%CL of a	95%CL of b	r^2
		Min	Max	Mean	Min	Max	Mean	a	b			
Auxis thazard (Frigate tuna)	1480	12.5	42	32.54±5.6	276	681	342.6±155.7	0.0358	2.704	0.0163-0.782	2.48-2.926	0.892
Auxis rochei (Bullet tuna)	1342	20.5	79	40.25±12.3	78	2052	671.75±213	0.121	2.648	0.08- 0.19	2.43-2.75	0.99
Euthynnus affinis (Little tuna)	2610	28.5	71.2	46.09±11.1	320	4214	1369.7±150.3	0.0221	2.8364	0.016-0.031	2.74-2.93	0.965
Katsuwonus pelamis (Skipjack tuna)	1280	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)	1417	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	0.957
Scomberomorus guttatus (Indo-pacific king mackerel)	1346	36	110	53.57±13.5	334	6700	1068.45±465.2	0.0089	2.896	0.0062-0.013	2.80-2.98	0.98
S. commerson (Narrow-barred Spanish mackerel)	1210	42.4	124	61.43±27.74	450	10850	2234.74±876.8	0.0072	2.93	0.0061-0.0084	2.76-3.04	0.99
Rastrelliger kanagurta (Indian Mackerel)	2348	19	22	20.71±1.2	64	137	99.6±27	0.0042	3.18	0.0031-0.0047	2.89-3.21	0.98
Rastrelliger faughni (Island Mackerel)	1047	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)	1284	36	42	36.5±4.3	128	460	291.3±56.21	0.0056	2.98	0.0089-0.006	2.72-3.08	0.99
Scomberoides commersonnianus (Talang queenfish)	2273	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 (Double spotted queenfish)	1334	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; r^2, coefficient of determination; parentheses indicate the range of a mean value.

Figure 1: Picture of 12 commercially important identified marine fishes of Bay of Bengal, Bangladesh during July 2023 to June 2024.

 *Scomberomorus guttatus* Indo-Pacific king mackerel	 *Rastrelliger kanagurta* Indian mackerel
 *Scomberoides commeronnianus* Talang queenfish	 *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
 *Rastrelliger faughni* Island mackerel	 *Katsuwonus pelamis* Skipjack tuna

The research findings of commercially important marine fish species by the LBB method in the Bay of Bengal, Bangladesh are presented below:

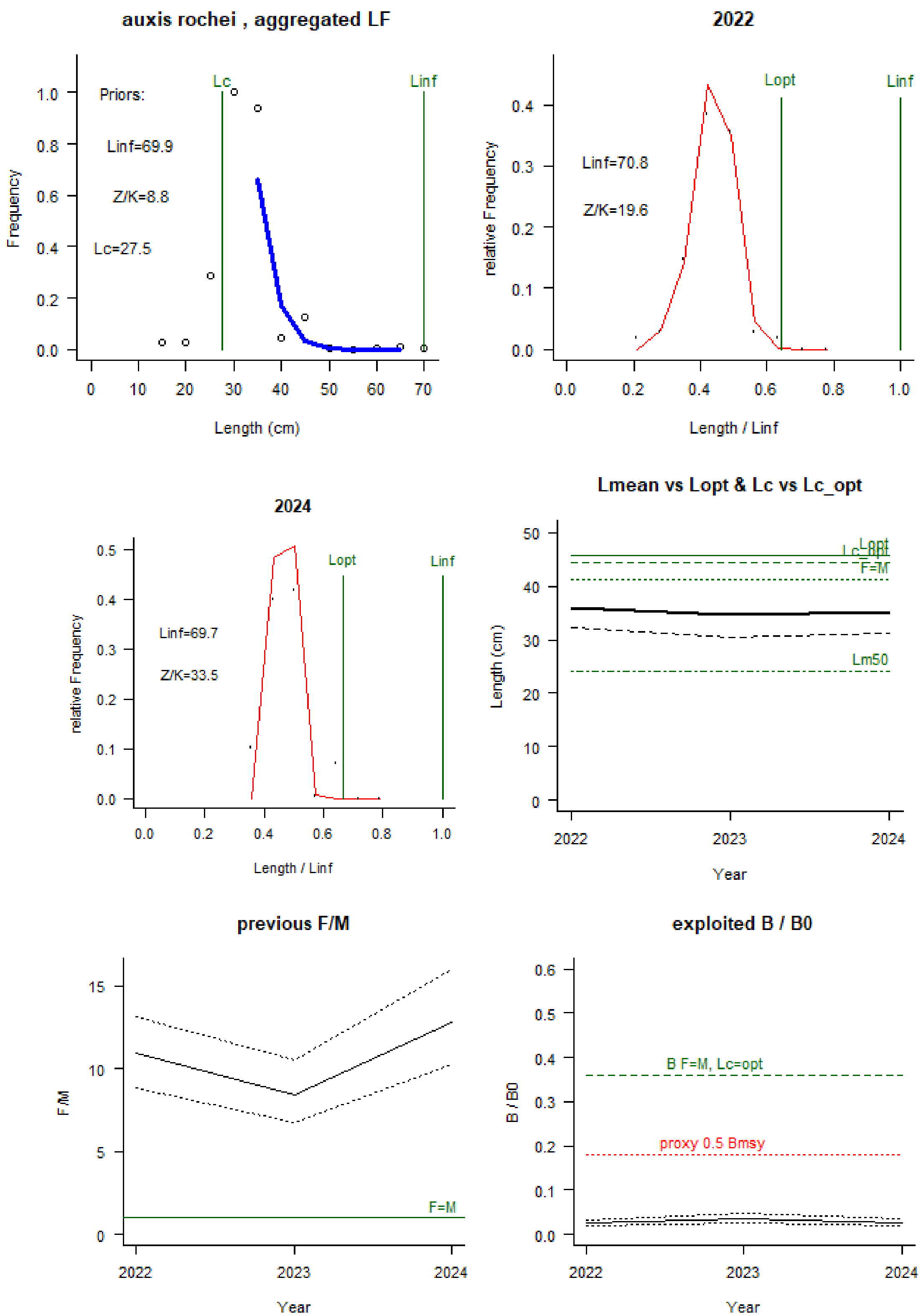


Fig 2: Graphical representation of LBB method for *Auxis rochei*

Here, Lc (length at first capture) was 27.5cm. L_{inf} is the limit body length of this species was 69.9 cm, and L_{opt} denotes the length at which the maximum sustainable catch is obtained.

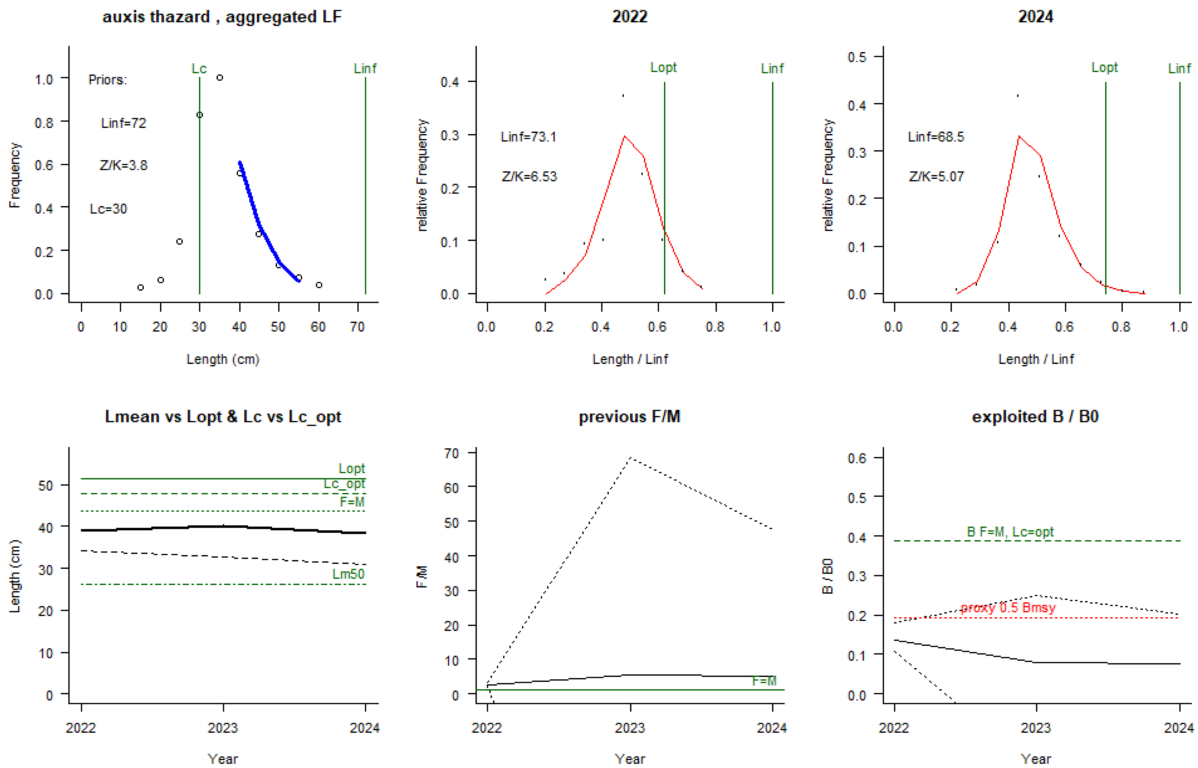


Fig 3: Graphical representation of LBB method for *Auxis thazard*

Here, Lc (length at first capture) was 30 cm. L_{inf} is the limit body length of this species was 60 cm, and L_{opt} denotes the length at which the maximum sustainable catch is obtained.

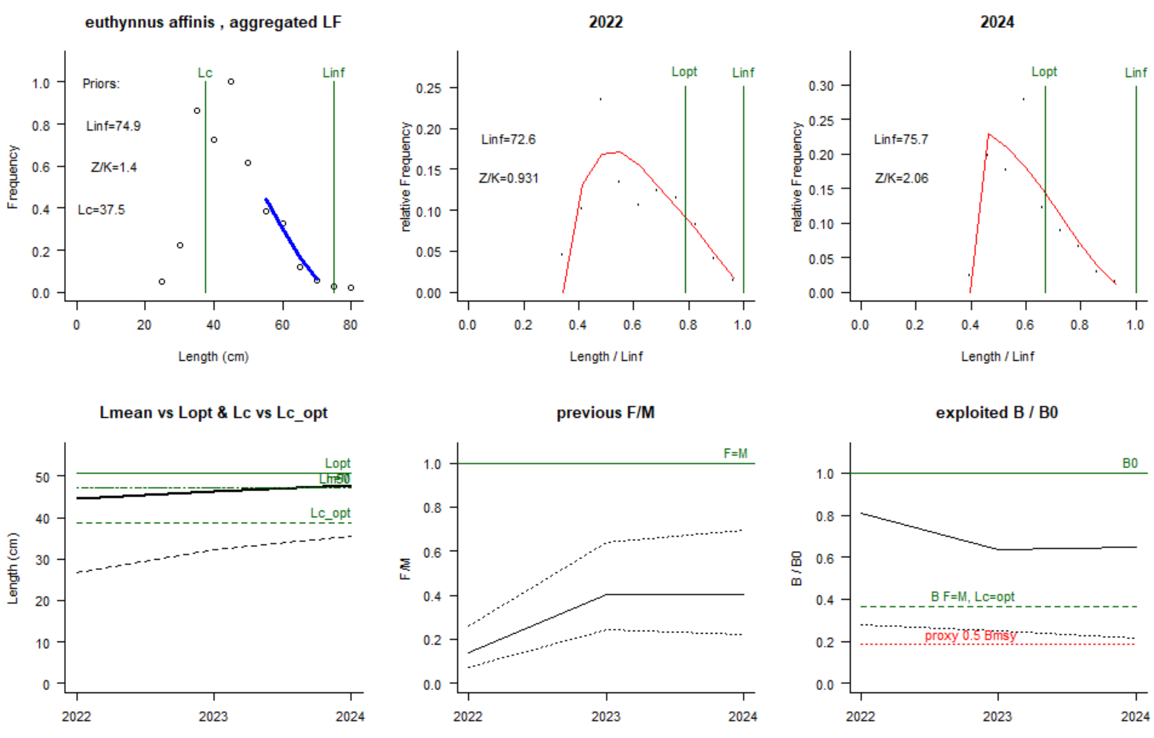


Fig 4: Graphical depiction of LBB method for *Ethynus affinis*

Here, Lc (length at first capture) was 37.5 cm. L_{inf} is the limit body length of this species was 74.9 cm, and L_{opt} denotes the length at which the maximum sustainable catch is obtained.

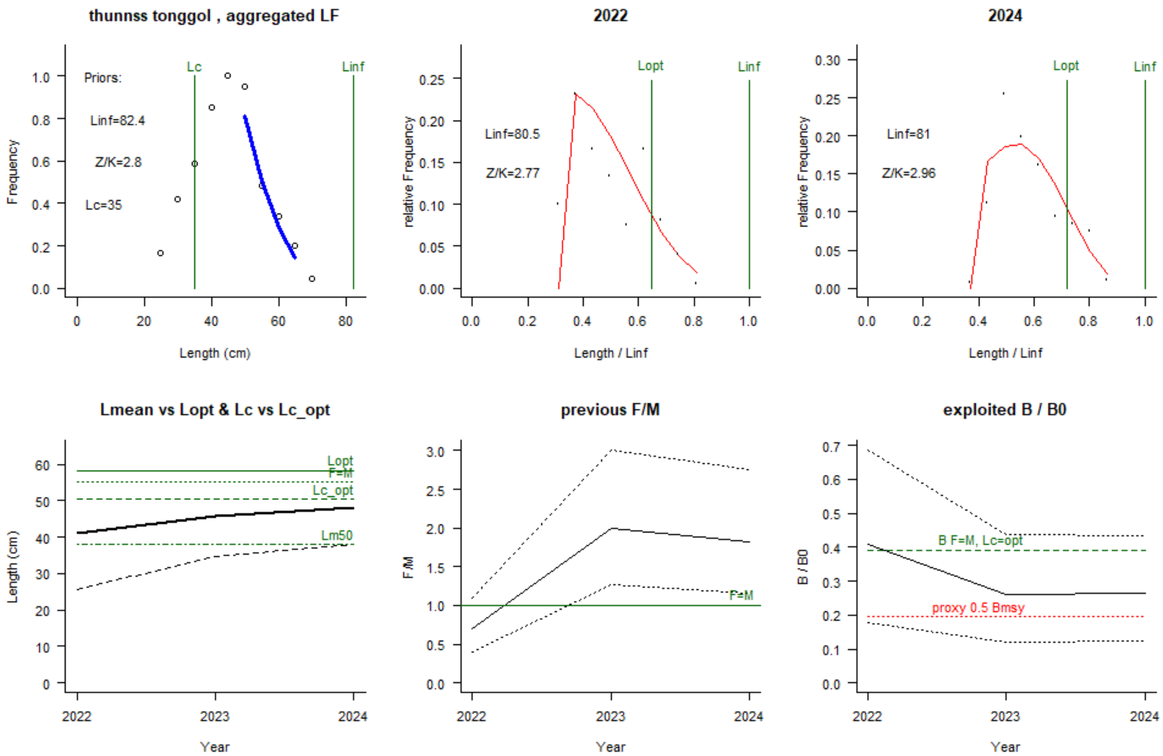


Fig 5: Graphical findings of LBB method for *Thunnus tonggol*

Here, Lc is the length at first capture was 35cm, L_{inf} is the limit body length of this species was 82.4 cm.

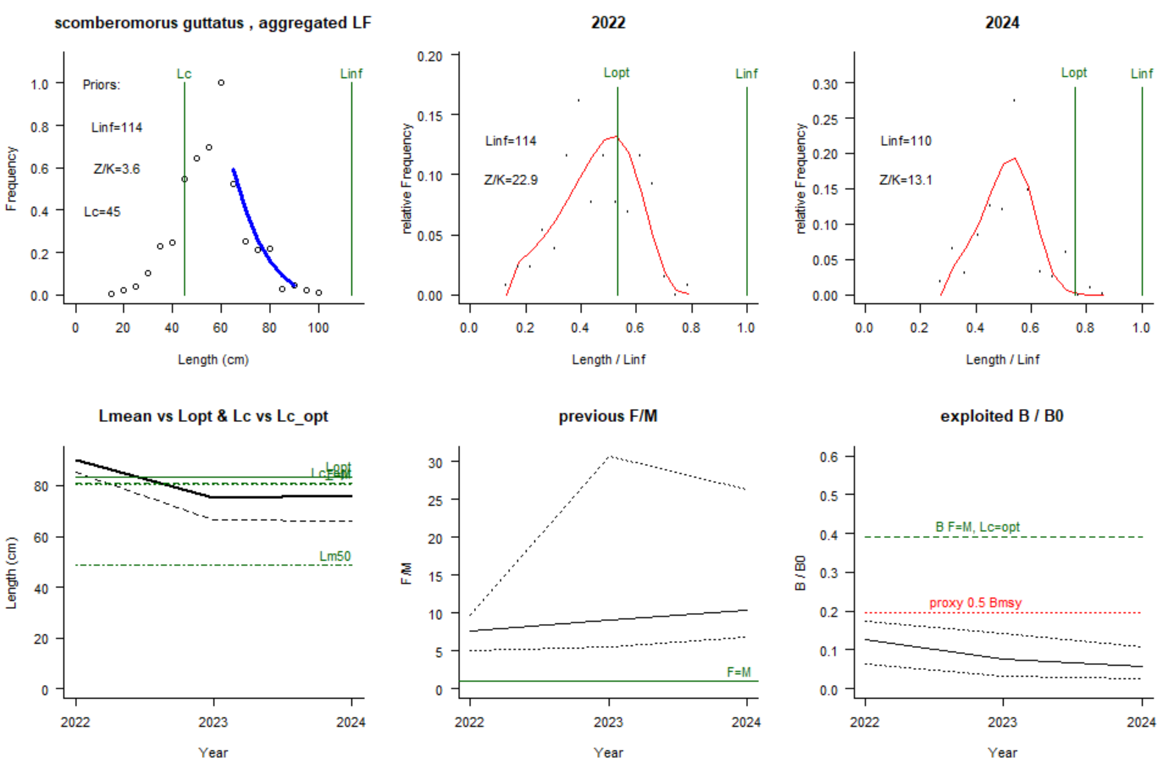


Fig 6: Graphical outcomes of LBB method for *Scombermorus guttatus*

Here, Lc is the length at first capture was 45cm, L_{inf} is the limit body length of this species was 114 cm.

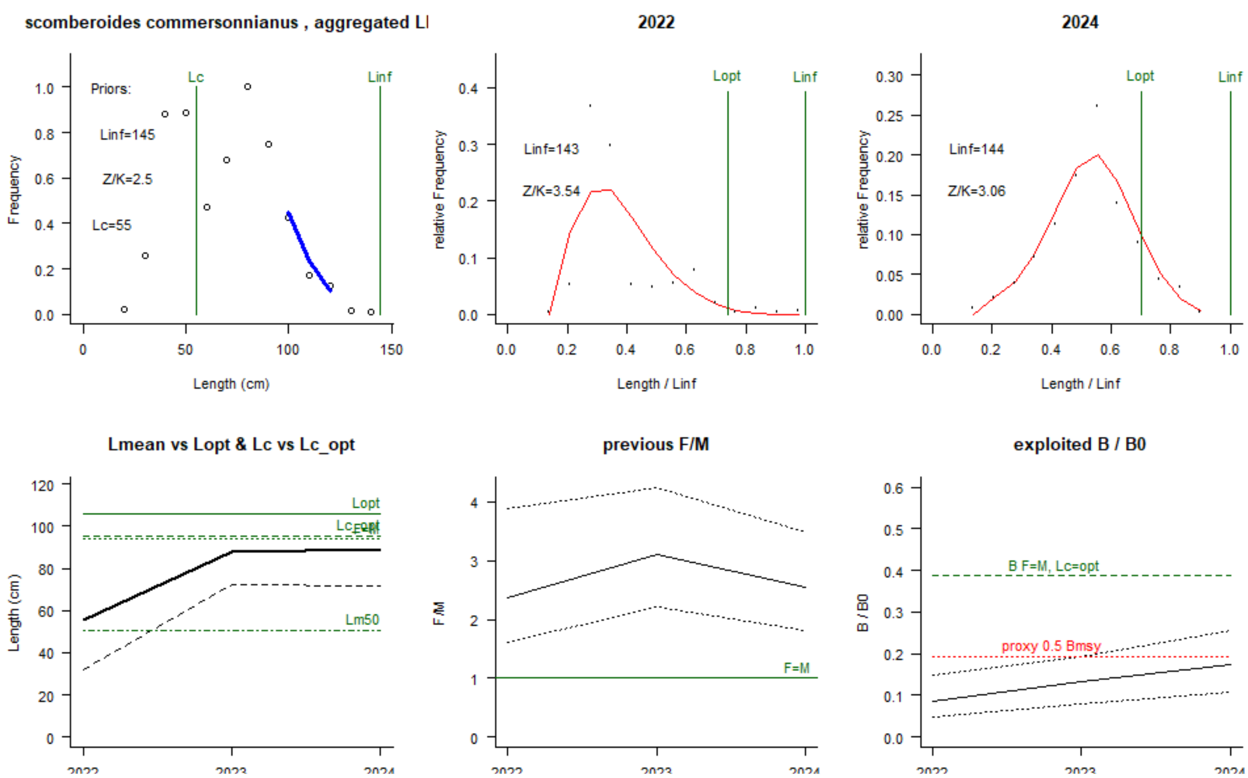


Fig 7: Graphical outcomes of LBB method for *Scomberoides commersonnianus*

Here, Lc is the length at first capture was 145 cm, L_{inf} is the limit body length of this species was 55 cm.

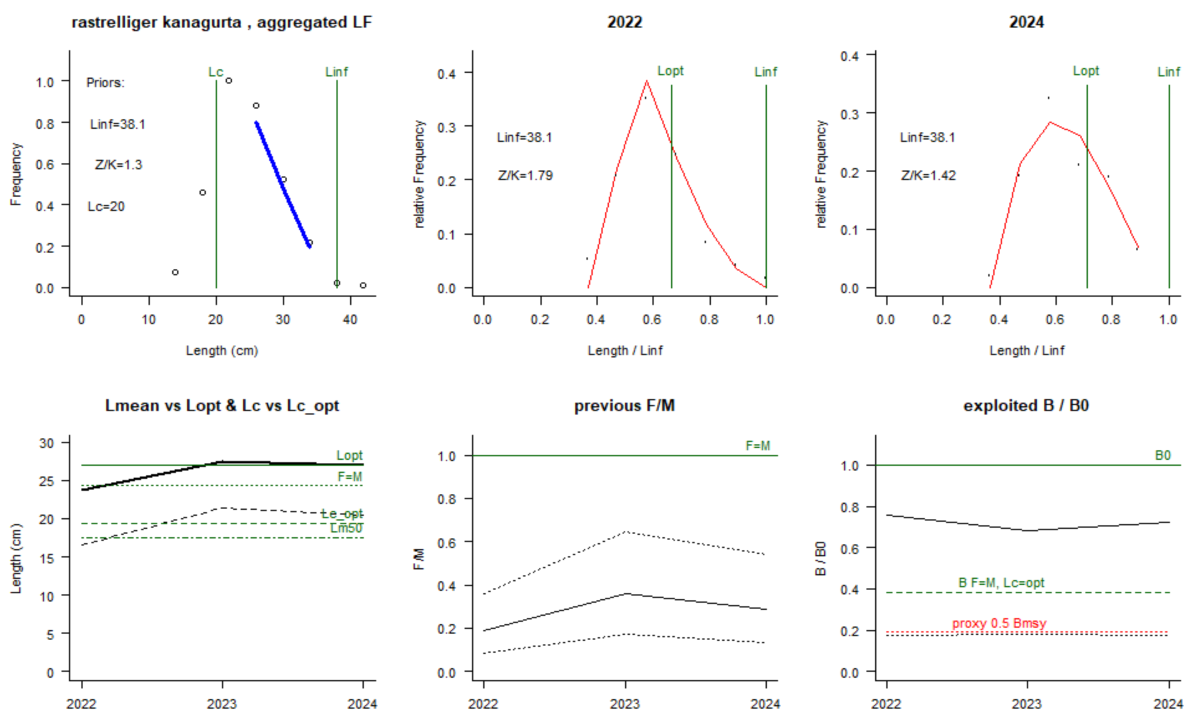


Fig 8: Graphical illustration of LBB method for *Rastrelliger kanagurta*

Here, Lc is the length at first capture was 20.0 cm, L_{inf} is the limit body length of this species was 38.10 cm.

Table 3: Estimated results of Six Marine fish species using length-frequency (LF) data assessed by length-based Bayesian biomass (LBB) method in between 2023-2024

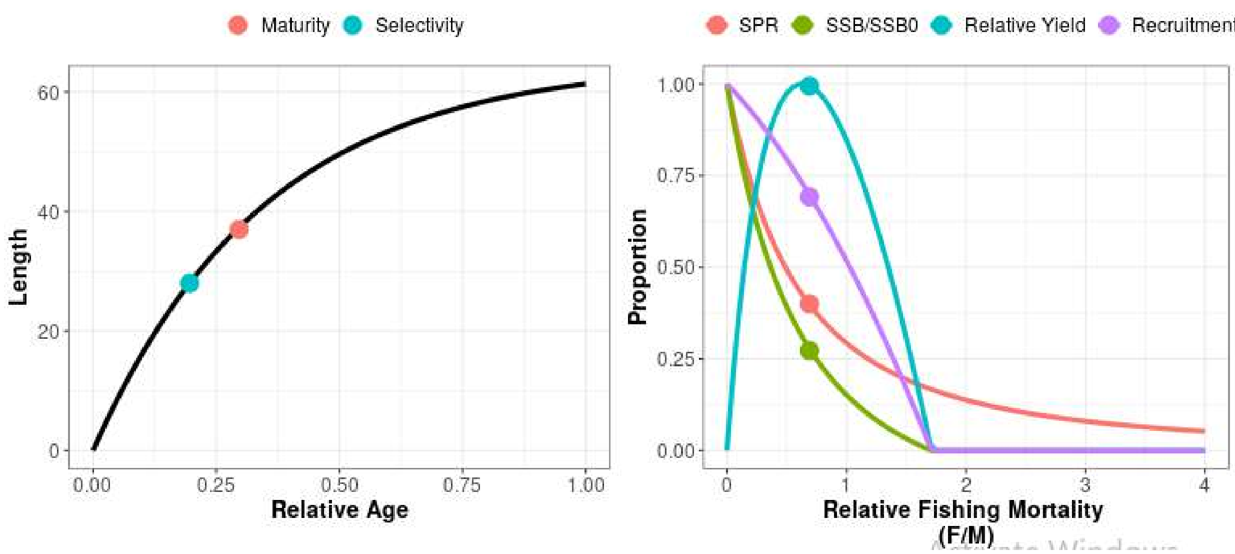
Species Name	**L_{mean}/L_{opt}**	**L_c/L_{c_opt}**	**M/K**	**L_{95th}/L_{inf}**	**B/B0**	**B/B_{MSY}**	**F/M**	**F/K**	**Z/K**	**Y/R'**	**Present Status**
E. affinis	0.92	0.92	1.48	0.94	0.64	1.8	0.4	0.61	1.9	0.031	Healthy stock
T. tonggol	0.84	0.75	0.85	0.84	0.26	0.67	1.8	2	3.2	0.047	Grossly overfished
A. rochei	0.76	0.7	1.64	0.75	0.027	0.074	4.12	7.31	8.8	0.0032	Grossly overfished
A. thazard	0.71	0.65	1.05	0.83	0.068	0.19	3.82	4.02	5.05	0.016	Grossly overfished
S. guttatus	0.94	0.82	0.97	0.84	0.059	0.15	1.8	1.2	2.8	0.0098	Grossly overfished
S. commersonnianus	1	1.1	1.23	0.96	0.73	1.9	0.29	0.37	1.7	0.026	Healthy stock
Rastrelliger kanagurta	0.86	0.75	1.05	0.92	0.17	0.45	2.36	2.49	3.55	0.044	Grossly overfished

Table 4: Life-history characteristics of 12 Marine Fishes using FiSAT II

Species	n	L_{inf}	K	M
Auxis rochei (Bullet tuna)	**1342**	46.3	0.40	0.79
Auxis thazard (Frigate tuna)	**1480**	47.32	0.98	1.35
Euthynnus affinis (Little tuna)	**2610**	75.45	0.56	0.86
Katsuwonus pelamis (Skipjack tuna)	**1280**	62.6	1.06	1.14
Thunnus tonggol (Long tail Tuna)	**1417**	70	0.49	0.67
Scomberomorus guttatus (Indo-pacific king mackerel)	**1346**	109	0.31	0.53
S. commerson (Narrow-barred Spanish mackerel)	**1210**	119.4	0.48	0.67

Rastrelliger kanagurta (Indian Mackerel)	**2348**	24.5	1.6	2.41
Rastrelliger faughni (Island Mackerel)	**1047**	23.5	1.9	2.56
Scomberoides tol Needlescaled queenfish	**1284**	42.5	0.84	1.57
S. commersonnianus (T. queenfish)	**2273**	118	0.16	0.39
Scomberoides lysan D. spotted queenfish	**1334**	56	0.67	0.84

Study-2: Determination of the Biological Reference Points (BRPs) of marine fishes

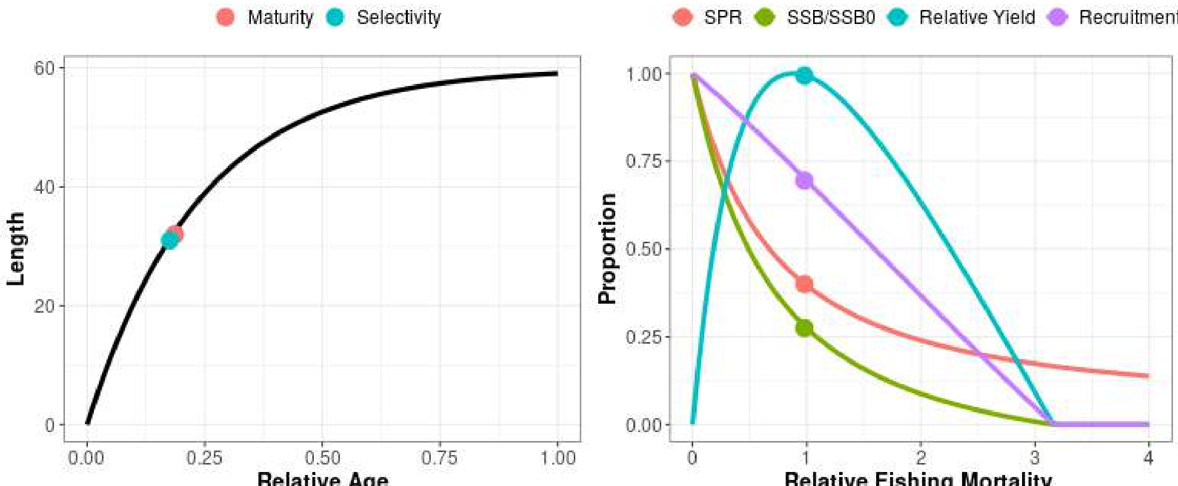


a b

Fig 9 : Estimation BRPs for *Auxis rochei*

a) Growth curve with relative age, and b) SPR and relative yield curves as a function of relative fishing mortality.

In figure a, selectivity is in the maturity point i.e fishes were captured before mature stage. In figure b, The M/k value was >1.50, which means all specimens unlike some are mature others remain immature with the length composition consisting of widely varying age and near asymptotic size.

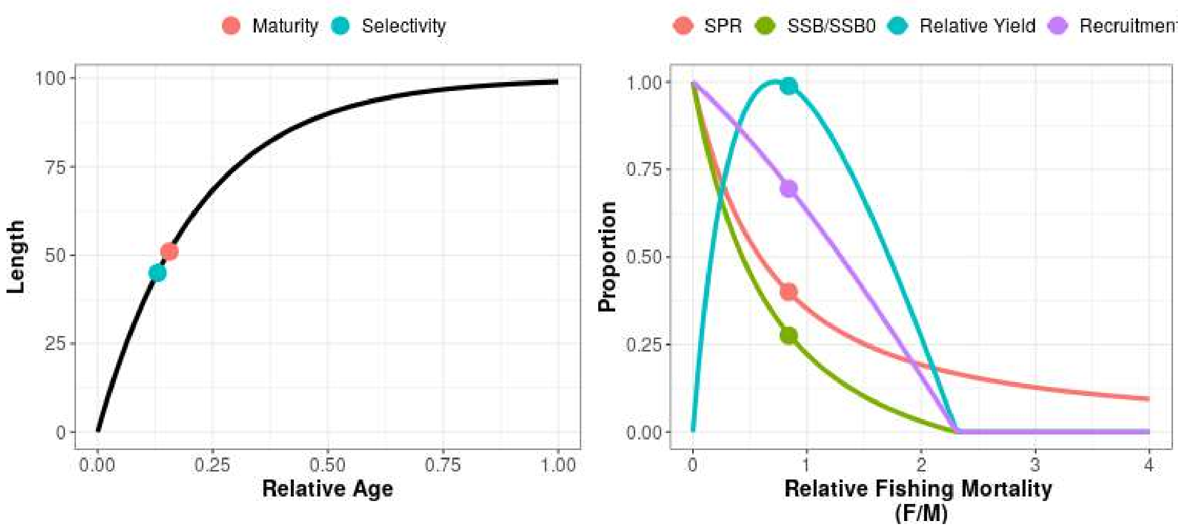


a b

Fig 10 : Calculation of BRPs for *A. thazard*

a) Growth curve with relative age b) SPR and relative yield curves as a function of relative fishing mortality

In figure a, selectivity point cross the maturity point i.e fishes were caught before they reach their spawning time. In figure b, The M/k value was higher than 0.50, which means some fishes are mature with the length composition consisting of widely different age and near asymptotic size.



a b

Fig 11 : Estimation BRPs of *Scombermorus guttatus* using the length-based SPR software

a) Growth curve with relative age b) SPR and relative yield curves as a function of relative fishing mortality

In figure a, selectivity point cross the maturity point i.e fishes were captured before mature stage. In figure b, The M/k value was greater than 0.50, which means little fishes are mature with the length composition consisting of widely different age and near asymptotic size.

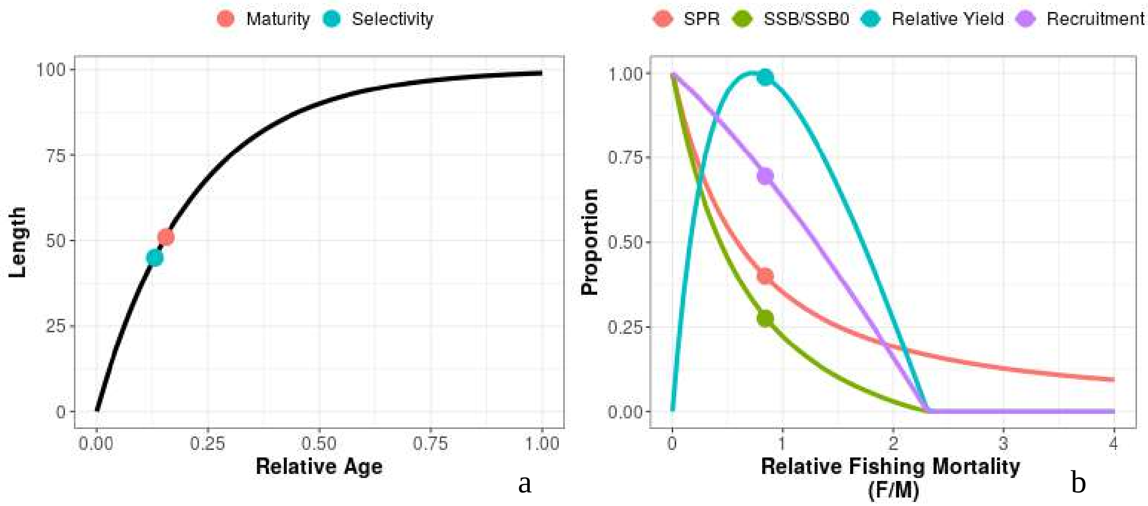


Fig 12 : Estimation BRPs of *Euthynnus affinis* using the length-based SPR software

a) Growth curve with relative age b) SPR and relative yield curves as a function of relative fishing mortality

In figure a, selectivity point is in front of the maturity point i.e fishes were captured before mature stage. In figure b, The M/k value was larger than 1.48, which means fishes are partly mature with the length composition consisting of different age and near asymptotic size.

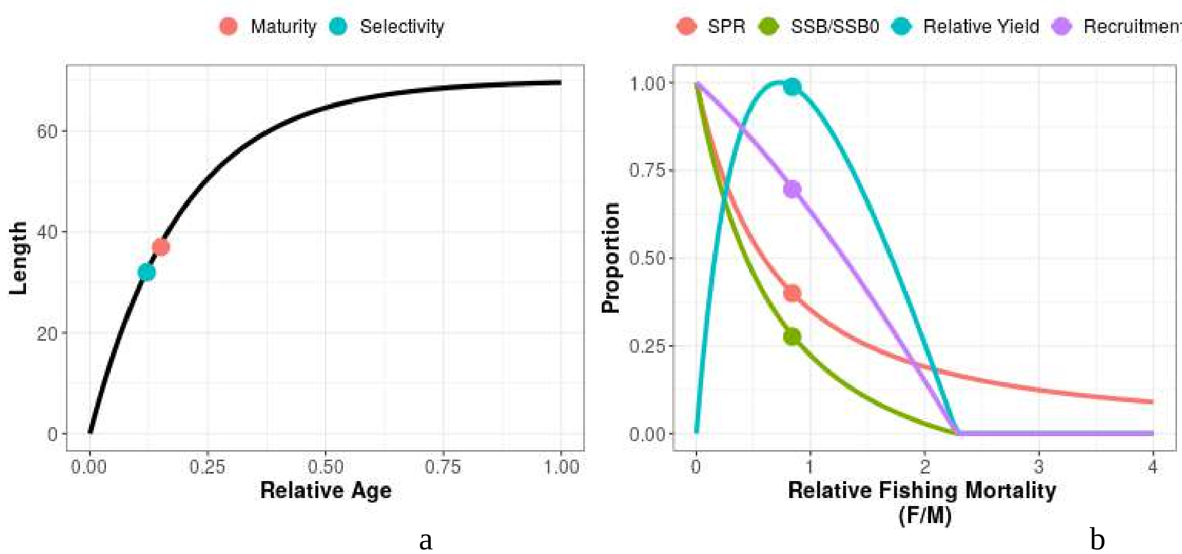
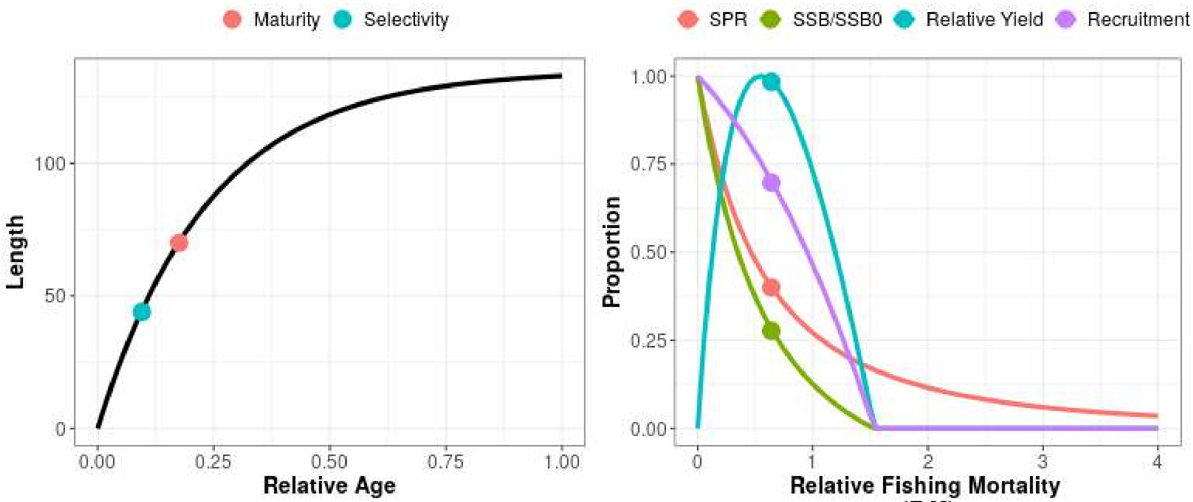


Fig 13: Determination of BRPs for *Thunnus tonggol* by the length-based SPR software

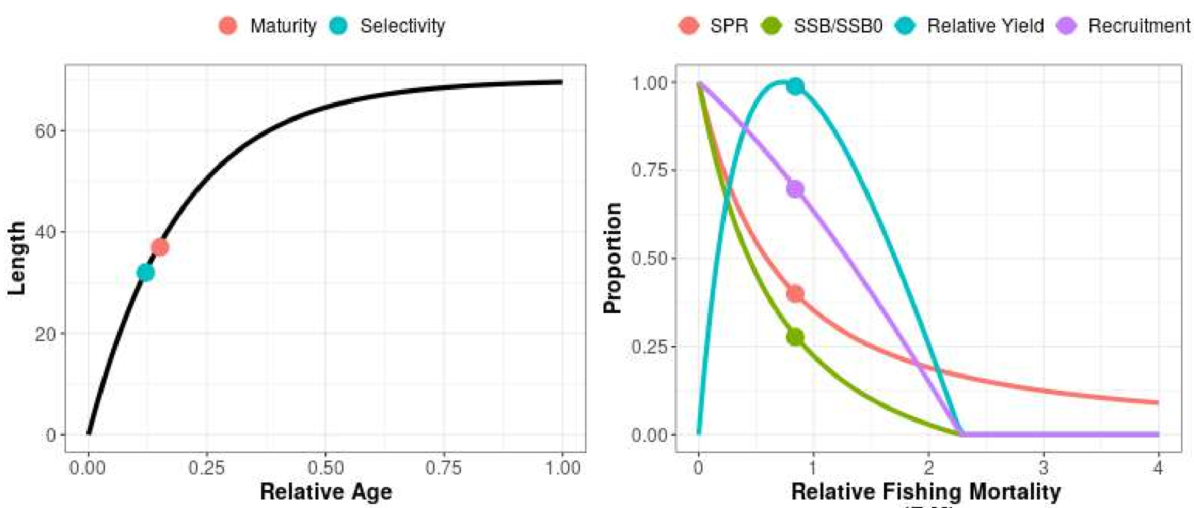
a) Growth curve with relative age b) SPR and relative yield curves as a function of relative fishing mortality

In figure a, selectivity point cross the maturity point i.e fishes were captured before maturation. In figure b, The M/k value was more than 0.85, which means fishes are slightly mature with the length composition consisting of widely different age and near asymptotic size.



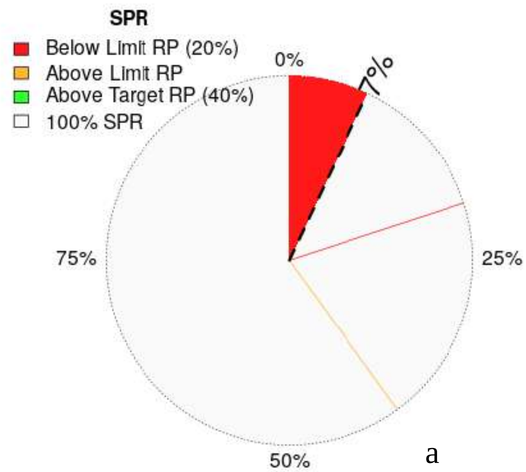
a b

Fig 14: Estimation BRPs of *S. commersonnianus* using the length-based SPR software
a) Growth curve with relative age b) SPR and relative yield curves as a function of relative fishing mortality
In figure, maturity point far behind the selectivity point i.e fishes were captured before attain its maturity. In figure b, M/k> 1.00, which means all fishes, are immature with the length composition consisting of different age and near asymptotic size.

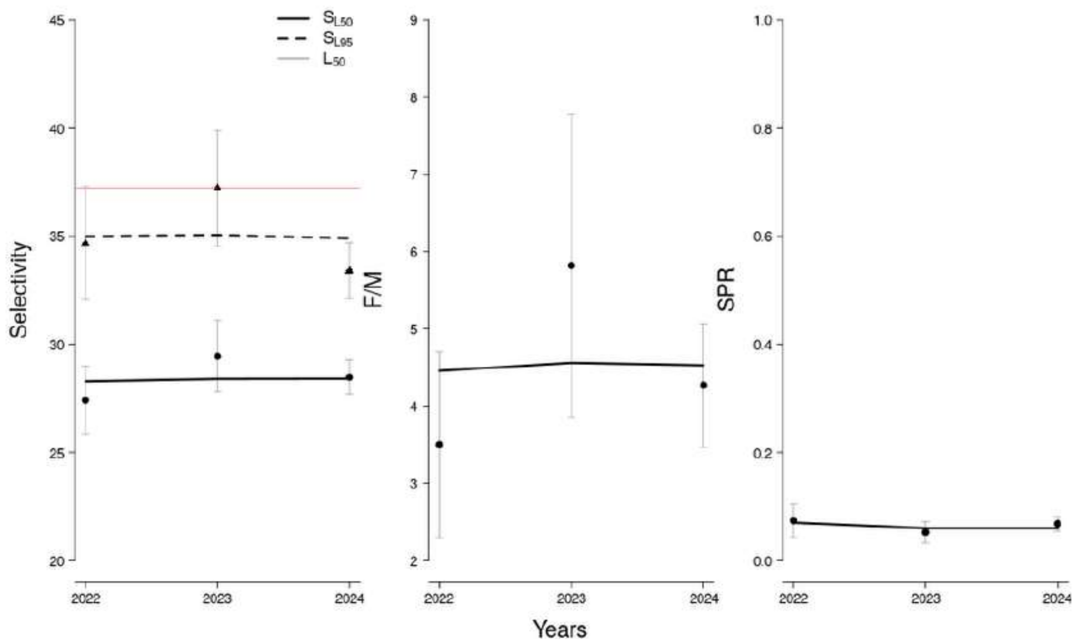


a b

Fig 15: Estimation BRPs of *Rastrelliger kanagurta* using the length-based SPR software
a) Growth curve with relative age b) SPR and relative yield curves as a function of relative fishing mortality
In figure, maturity point far behind the selectivity point i.e fishes were captured before attain its maturity. In figure b, M/k> 1.00, which means all fishes, are immature with the length composition consisting of different age and near asymptotic size.



a



b

Fig 16: a) Estimation of SPR and total stock of *A. rochei* b) Estimates of selectivity parameters SPR-based BRPs Plot showing estimates when size data are aggregated into a 3-year dataset (2022–2024). Red straight line indicates size at 50% maturity (L50%). Vertical lines indicate confidence intervals.

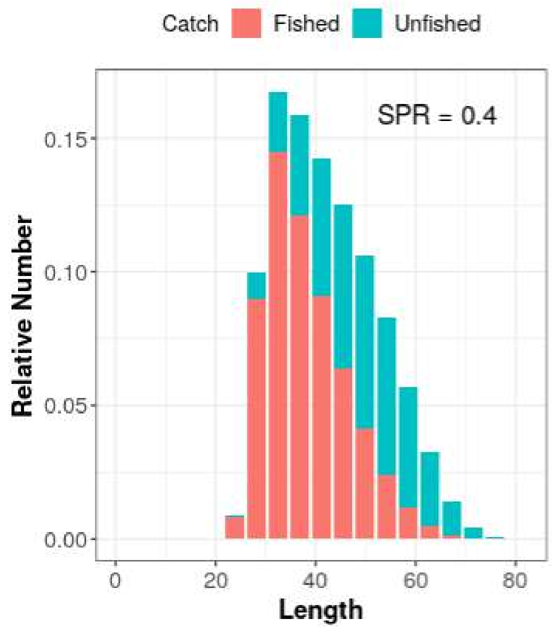
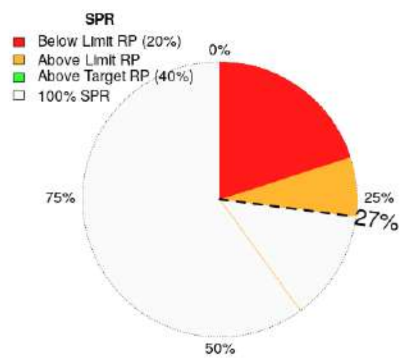


Fig 17: Expected (equilibrium) size structure of the catch and the expected unfished size structure of the vulnerable population (*Auxis rochei)*



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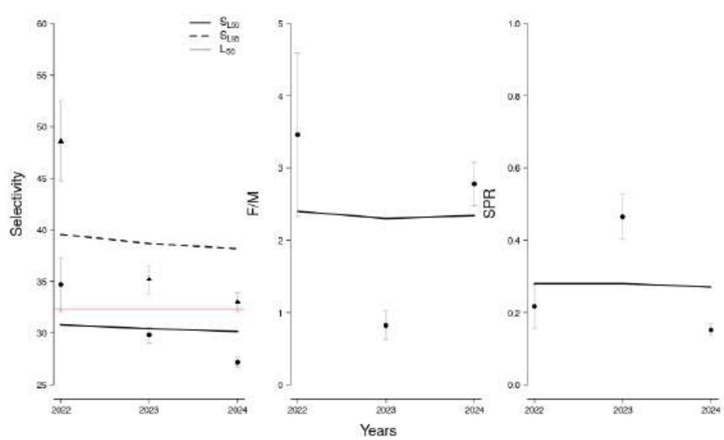


b

Fig 18: a) Estimation of SPR and total stock of *A. thazard* b) Estimates of selectivity parameters SPR-based BRPs Plot showing calculates when size data are aggregated into a 3 years dataset (2022–2024). Red straight line indicates size at 50% maturity (L50%). Vertical lines indicate confidence limits.

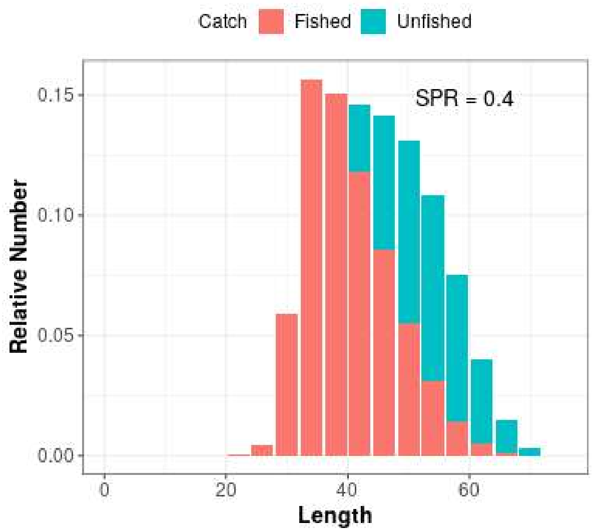
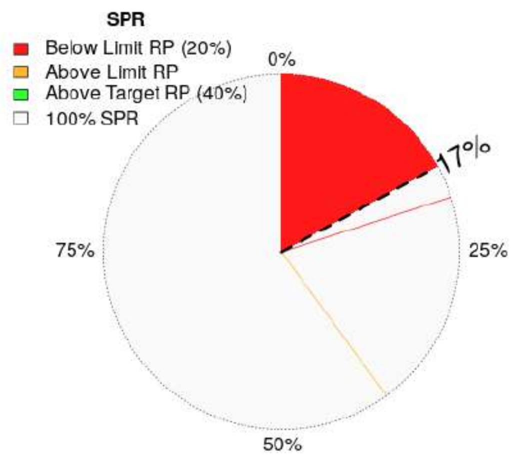
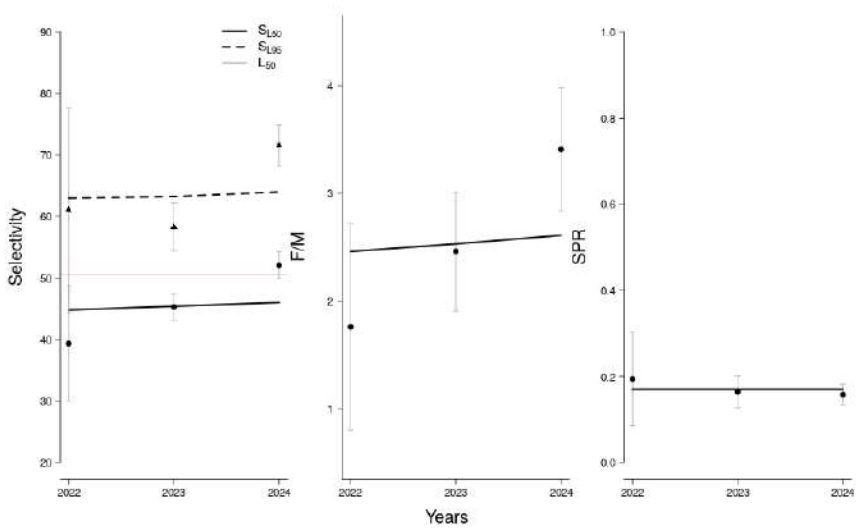


Fig 19: Expected (equilibrium) size structure of the catch and the expected unfished size structure of the vulnerable population for *A. thazard*



a



b

Fig 20: a) Estimation of SPR and total stock of *Scombermorus guttatus* b) Estimates of selectivity parameters

SPR-based BRPs Plot showing estimates when size data are aggregated into a 3-year dataset (2022–2024). Red straight line indicates size at 50% maturity (L50%). Vertical lines indicate confidence intervals.

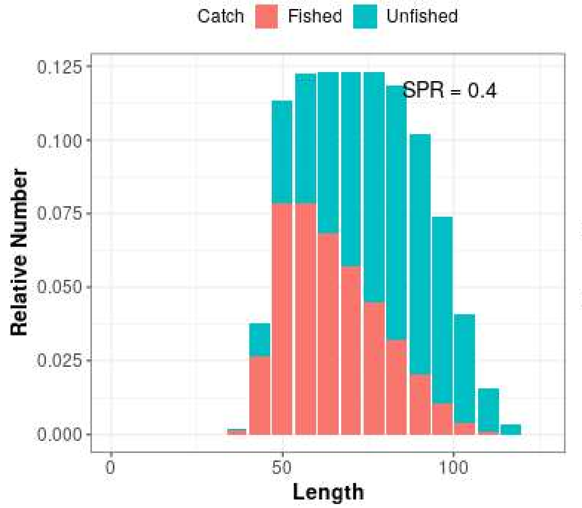


Fig 21: Expected (equilibrium) size structure of the catch and the expected unfished size structure of the vulnerable population for *Scombermorus guttatus*

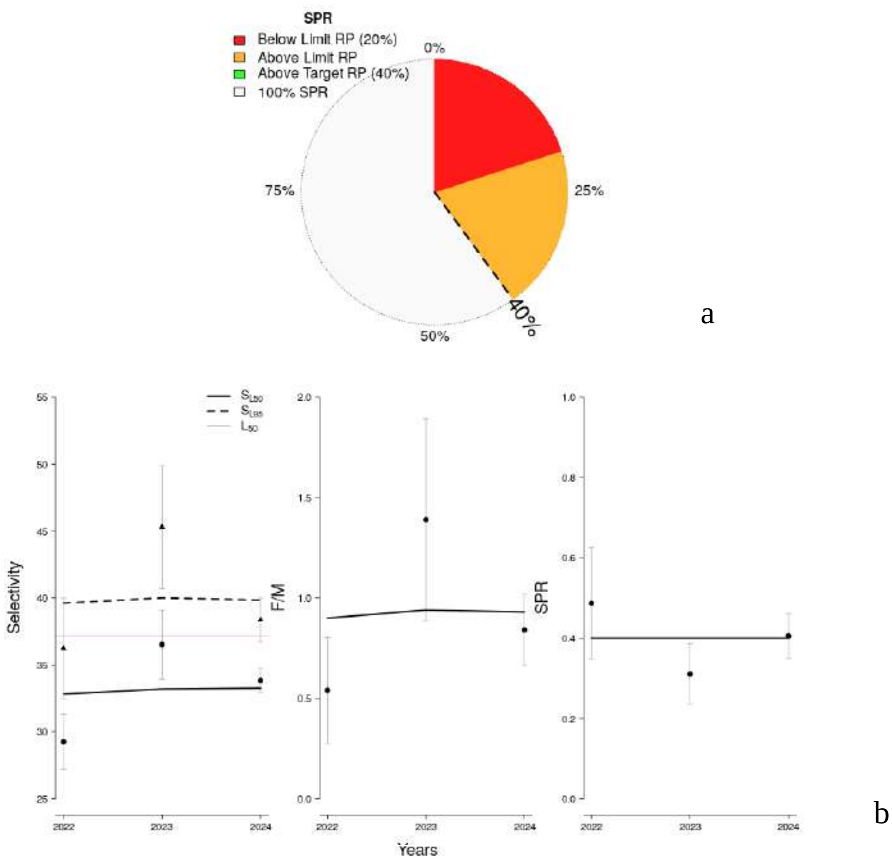


Fig 22: a) Estimation of SPR and total stock of *Euthynnus affinis* b) Estimates of selectivity parameters

SPR-based BRPs Plot showing estimates when size data are aggregated into a 3-year dataset (2022–2024). Red straight line indicates size at 50% maturity (L50%). Vertical lines indicate confidence intervals.

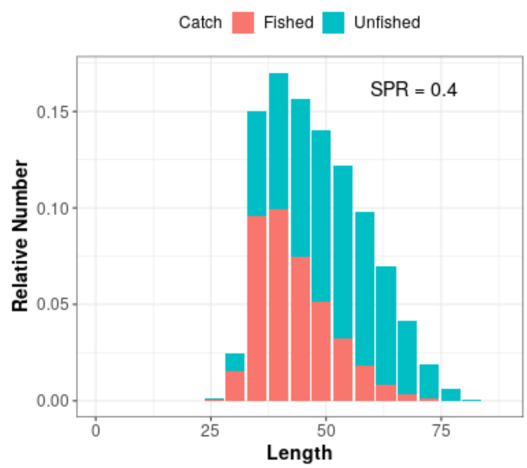
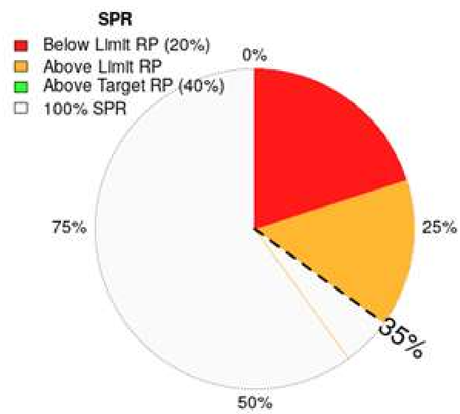


Fig 23: Expected (equilibrium) size structure of the catch and the expected unfished size structure of the vulnerable population for *Euthynnus affinis*



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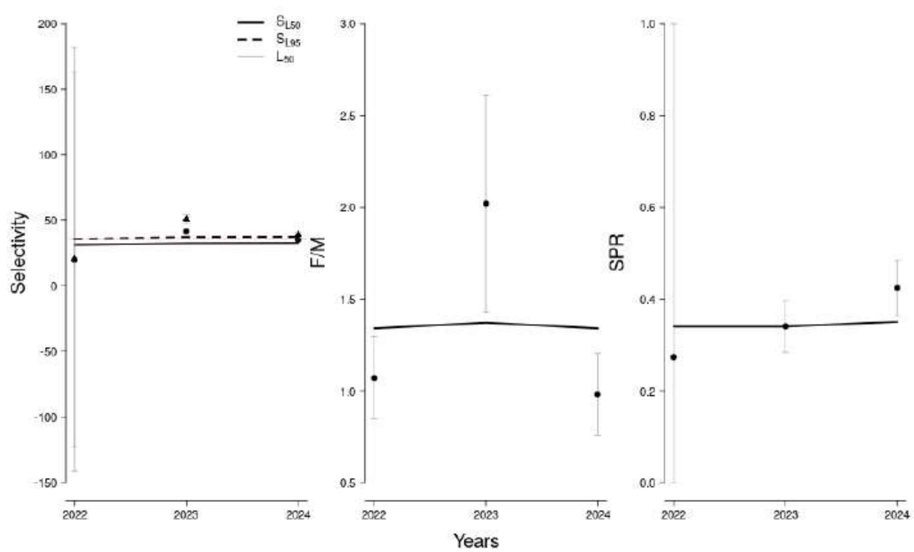

b

Fig 24: a) Estimation of SPR and total stock of *Thunnus tonggol* b) Estimates of selectivity parameters

SPR-based BRPs Plot showing estimates when size data are aggregated into a 3-year dataset (2022–2024). Red straight line indicates size at 50% maturity (L50%). Vertical lines indicate confidence intervals.

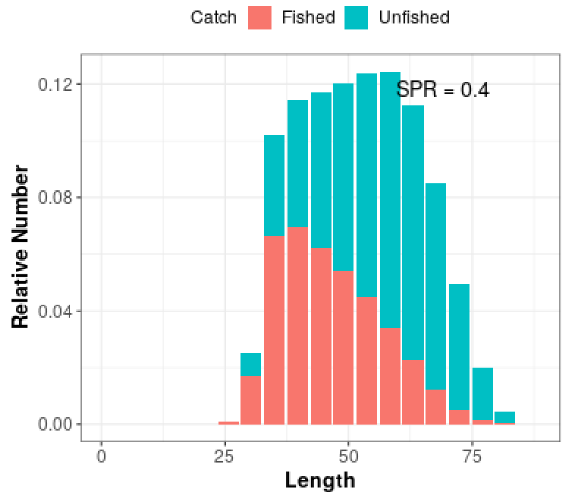

Fig 25: Expected (equilibrium) size structure of the catch and the expected unfished size structure of the vulnerable population for *Thunnus tonggol*

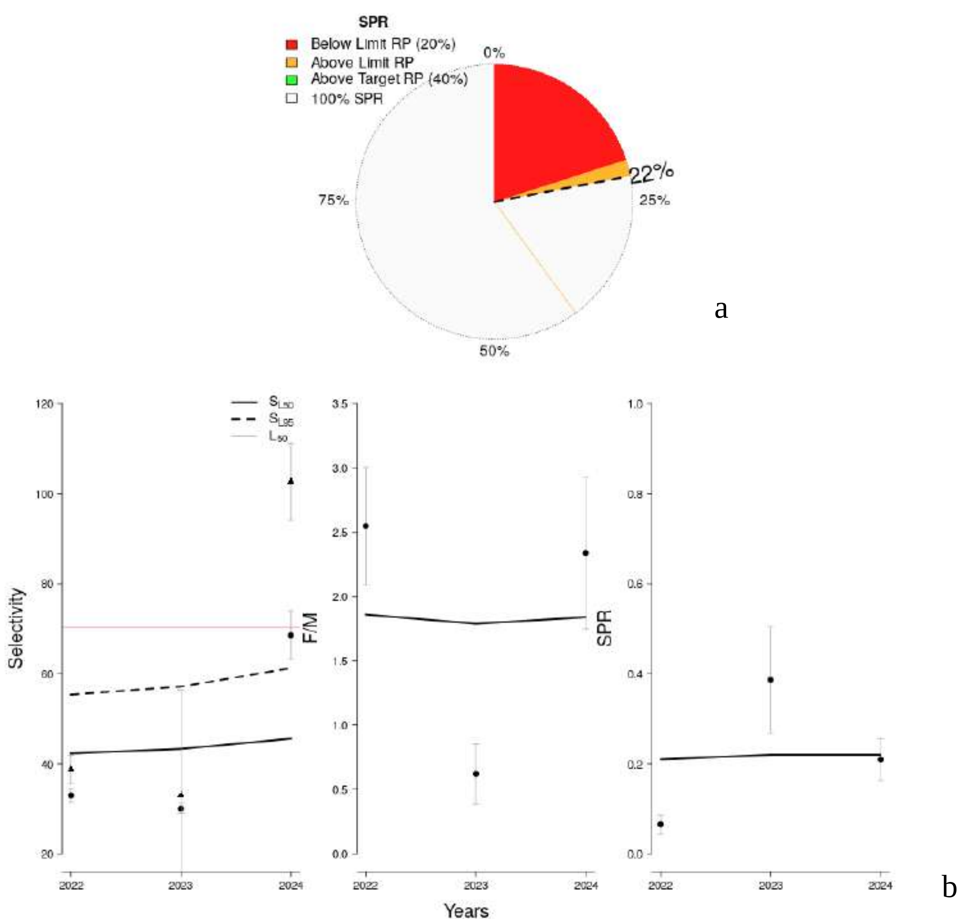

Fig 26: a) Estimation of SPR and total stock of *S. commersonnianus* b) Estimates of selectivity parameters

SPR-based BRPs Plot showing estimates when size data are aggregated into a 3-year dataset (2022–2024). Red straight line indicates size at 50% maturity (L50%). Vertical lines indicate confidence intervals.

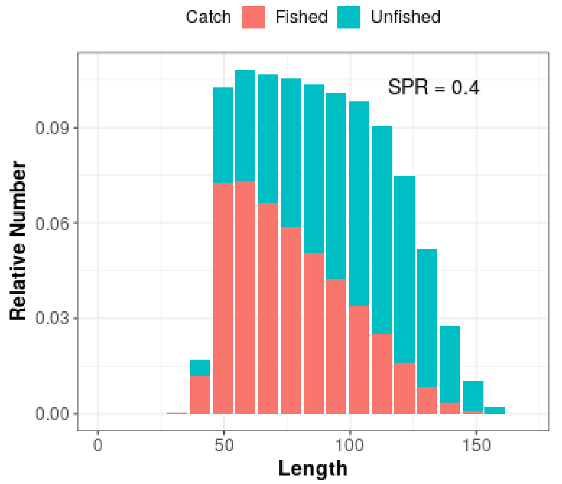

Fig 27: Expected (equilibrium) size structure of the catch and the expected unfished size structure of the vulnerable population for *S. commersonnianus*

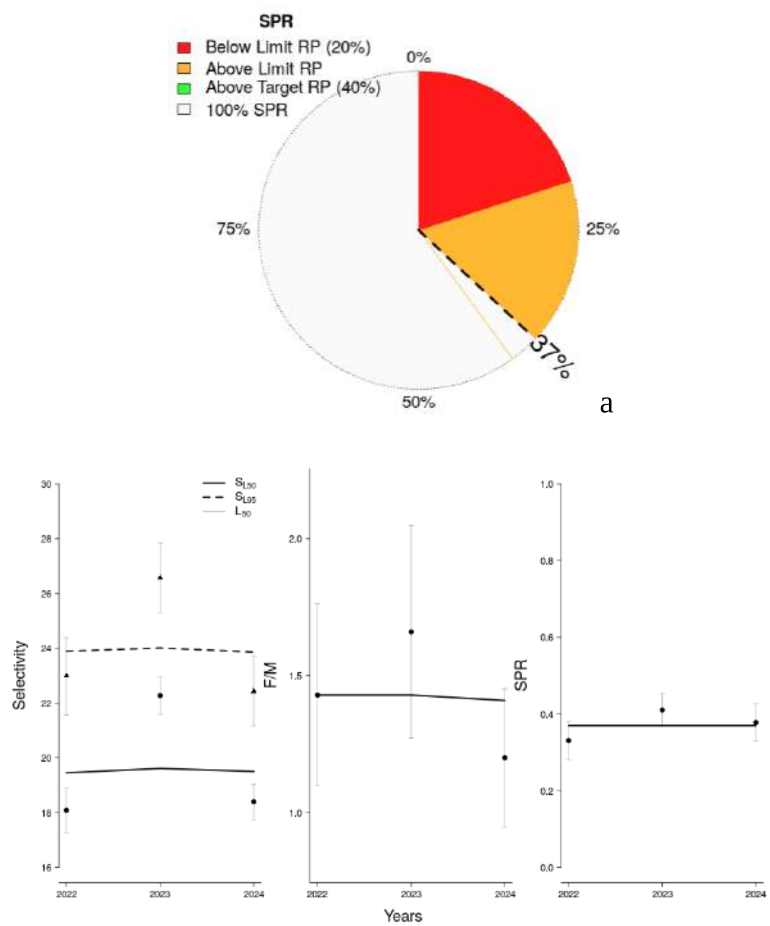

Fig 28: a) Estimation of SPR and total stock of *Rastrelliger kanagurta* b) Estimates of selectivity parameters

SPR-based BRPs Plot showing estimates when size data are aggregated into a 3-year dataset (2022–2024). Red straight line indicates size at 50% maturity (L50%). Vertical lines indicate confidence intervals.

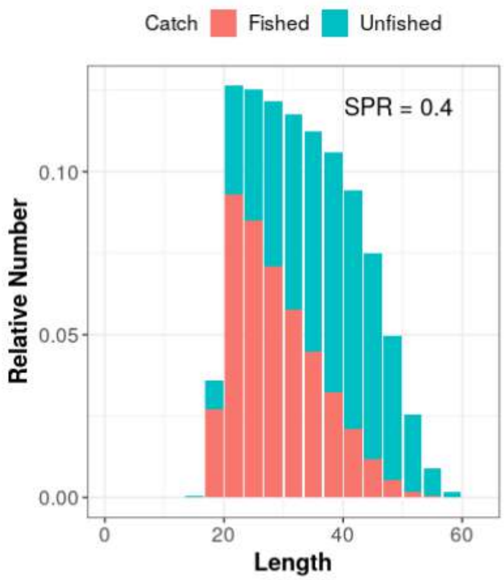

Fig 29: Expected (equilibrium) size structure of the catch and the expected unfished size structure of the vulnerable population for *Rastrelliger kanagurta*

Red bars represent the current scenario and indicate the frequency of observed length with a modeled spawning potential ratio below 20%. Green bars indicate the simulated target (SPR 40%) length-frequency distribution. The current harvest pattern results in a lower spawning potential ratio than the ecological target of 40%.

Table 5: The current status of analyzed Marine fishes

Species Name	SPR	SL50	SL95	F/M	M/K	Linf	L50	L95	Present Status
E.affinis	0.40	33.1	39.82	0.92	1.48	78	37.21	48.94	Maturity< Selectivity
T. tonggol	0.34	32.12	36.74	1.35	0.85	82.4	37.21	48.94	Maturity< Selectivity
S. commersonnianus	0.21	43.71	57.93	1.83	1.05	135	70.2	94.32	Maturity< Selectivity
A. rochei	0.06	28.35	34.99	4.51	1.5	68	37.21	48.9	Maturity< Selectivity
A. thazard	0.28	30.45	38.81	2.35	1.05	58	32.31	42.3	Maturity< Selectivity
S. guttatus	0.17	45.42	63.41	2.53	0.95	98	50.64	67.3	Maturity< Selectivity
R. kanagurta	0.37	19.52	23.92	1.42	1.23	40	14.12	30.18	Maturity< Selectivity

Problems/ Close up if any:

- Less co-operation of fisherman during sampling of fishes on landing site
- Lack of On-boat data for CPUE (RV Mean-Shandhani)

Proposed work during the next period: Regular sampling will be done at selected spot for collecting Land-based data of fish and after having the year round data; it will be analyzed to determine BRP and SPR of target species.

Development of breeding, seed production and nursery techniques of Seabass, *Lates calcarifer*

Researchers : Dr. Shafiqur Rahman, PSO
: Md. Aktaruzzaman, SO
Zahidul Islam, SO

Objectives of the project

i. To develop the brood of seabass in captive condition
ii. To assess the reproductive biology of cultured seabass
iii. To confirm the hormones and standardize the dosages in the breeding of Seabass
iv. To develop the seed production and larval rearing techniques of Seabass

Achievements

Experiment 1: Development of the brood of seabass in captive condition

The Asian seabass were collected from traditional coastal aquaculture farm/ gher near about chaufoldondi and Khuruskul area, Cox's Bazar. The fish were acclimatized in on station seabass hatchery pond environment at 8 ppt salinity for 72 hours before stocking. The average initial weight of the fish was 600g. Then 105 fish were distributed equally (35 fish) into three MFTS cistern ponds those being fed live tilapia as food. On the other hand, 52 fish were distributed into four brood stock rearing tank (25000 L water volume capacity) in a RAS system in MFTS seabass hatchery (Figure 1). These fish were categorized into four groups according to their weight. These groups are named as **Large Group (L), Medium Group (M), Small Group -01 (S-1)** and **Small Group -02 (S-2)**. Large Group contains 08 fish in tank with average weight 3.96 kg. Medium Group contains 11 fish in tank with average weight 2.48 kg while Small group (01) contains 14 fish with average weight 1.61 kg and Small Group (02) contains 19 fish with average weight 0.99 kg. The length and weight of these fish are given below respectively. Those being fed chopped squid/flat fish/ sardin/Bata fish daily as feed @ ad libitum.

Figure 1. Seabass rearing in RAS

Table-1.1 Length & weight of Asian seabass reared in brood stock tank-03 **(L)**		
Sl. no.	Length (cm)	Weight (g)
1	70	4400
2	71	4300
3	71	4873
4	67	4040
5	65	3736
6	62	3280
7	63	3500
8	60	3590
Mean	66.13 cm	3965

Table-1.2 Length & weight of Asian seabass reared in brood stock tank-02 **(M)**		
Sl. no.	Length (cm)	Weight (g)
1	60	2876
2	61	3200
3	58	2200
4	59	2100
5	63	2500

Table-1.4 Length & weight of Asian seabass reared in brood stock tank-01 **(S-2)**		
6	58	2000
7	59	2400
8	63	3000
9	59	2500
10	54	2000
11	59	2600
Mean	59.4 cm	2489

Table-1.3 Length & weight of Asian seabass reared in brood stock tank-04 **(S-1)**		
Sl. no.	Length (cm)	Weight (g)
1	57	1600
2	60	1800
3	53	1506
4	52	1738
5	50	1318
6	50	1338
7	52	1586
8	50	1398
9	51	1600
10	53	1718
11	52	1540
12	53	2046
13	52	1636
14	52	1836
Mean	52.6	1618.6

Sl. no.	Length (cm)	Weight (g)
1	46	988
2	50	1034
3	53	1300
4	48	1130
5	47	1200
6	47	1176
7	43	925
8	45	1070
9	50	1280
10	51	1100
11	49	1136
12	45	1220
13	47	1026
14	47	1000
15	39	600
16	39	816
17	37	660
18	41	750
19	33	460
Mean	45.11	993.21

Experiment 02: Assessment of the reproductive biology of cultured seabass

Morphological study & LOB (Live Ovarian Biopsy) were done to assess the reproductive biology of Asian Seabass. Sampling was done once in two months to determine their growth and gonadal development. Among the 52 fish 08 fish were adult, 11 fish were sub-adult and rest were immature. Among 08 adult fish 04 fish were female, 02 were male & rest were

Figure-02: Regular Sampling and Adult Asian Seabass

Figure-03: Genital Opening and developed gonad of Seabass (Reference: CIBA)

Dark Yellow color Genital Opening indicates mature female where Yellowish indicates developing gonad as female & Pinkish indicates immature gonad. Besides LOB was done for sex determination. A cannula was used for LOB.

Figure-04: Cannula & Cnnulation of Seabass

Experiment 03: Confirming the hormones and standardize the dosages in the breeding of Seabass.
We confirmed a synthetic hormone i.e LHRHa & standardized a dose. Then prepared a hormone solution & injected the hormone solution into the fish body.

Figure-05: Hormone injection of Asian Seabass for Induce Breeding.

Experiment 04: Development of seed production and larval rearing techniques of Seabass in Bangladesh.

We developed a seed production technique. We got 2.5 million eggs after 12 hours of inducing hormone. Among these eggs 1.5 million were fertilized. The egg fertilization rate was 60%. Then we transfer the fertilized eggs into the incubation tanks. After 36 hours of transformation the fertilized hatched out. We got 1 million larvae. The hatching rate was 67.1%.

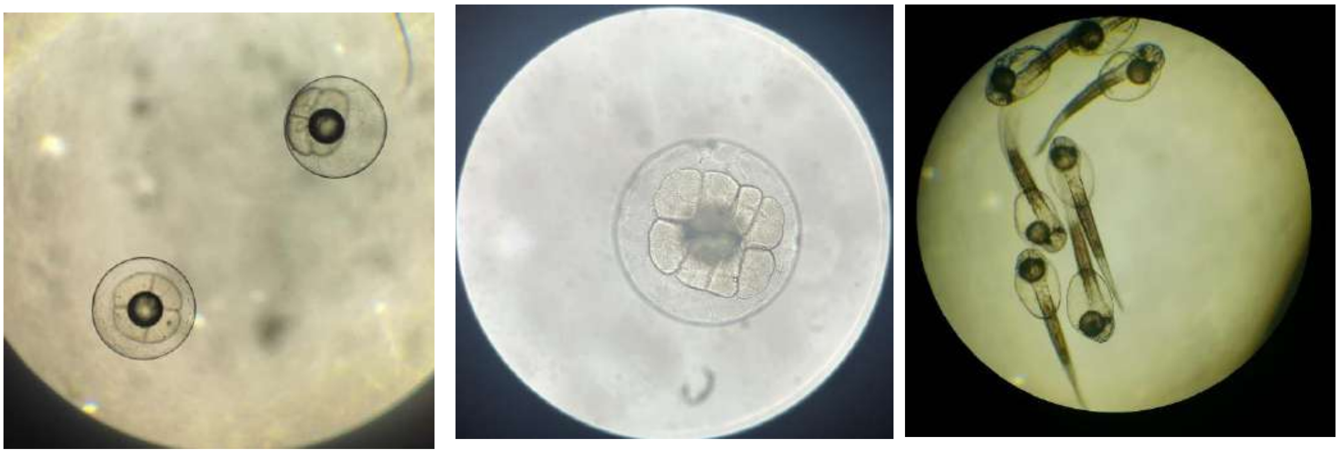

Figure-06: Fertilized egg & Larvae of Asian Seabass.

Installation of central temperature controlling system:

For controlling water temperature centrally an installation process i.e. central temperature controlling system is going on.

Chimney

Burner

Circulating Motor

Circulation Pipe

Circulation Pipe

Heating Coil

Figure-07: Central temperature controlling system (Central Boiler)

Problems/constraints encountered if any

- Lack of saline water facilities in the MFTS
- Unavailability of ready brood fish
- Lack of central temperature controlling system in the hatchery.

Proposed work during the next period

Collection of brood & development of brood is in progress. Fish are being fed daily. Collection of hormone for injection & installation of central temperature controlling system will be done. After completing this installation process fish will be injected with hormone for artificial propagation.

Scientific Publications (2021-23)

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Mahmuda Khandaker	Senior Librarian
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