Annual Research Progress Report 2023-2024

Edited and designed
by
Dr. Md. Latiful Islam

Bangladesh Fisheries Research Institute
Brackishwater Station
Paikgacha, Khulna

Annual Research Progress Report (2023-2024)

Edited and designed
by
Dr. Md. Latiful Islam
Chief Scientific Officer (cc)

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July 2024

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Population Dynamics of Important Fish and Shell Fishes in the Sundarbans Mangrove of Bangladesh

Annual Research Progress Report
(July 2023- June 2024)

Bangladesh Fisheries Research Institute
Brackishwater Station, Paikgacha, Khulna- 9280

Annual Research Progress report (2023-2024)

01. Preface: Not applicable

02. Executive Summary: Not applicable

03. Introduction: Not applicable

04. Bangladesh Fisheries Research Institute (An overview): Not applicable

05. Research Achievements of Brackishwater Station's Project: Not applicable

06. Project Title: Population Dynamics of important fish and shell fishes in the Sundarbans Mangrove of Bangladesh

07. Co-ordinator **:** Dr. Md. Latiful Islam, CSO

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09. Duration : July 2023-June 2024

10. Total Budget & Expenditure: (2023-2024)

Budget (Tk)**:** 9,00,000.00
Expenditure (Tk)**:** 8,94,876.00

11. 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.

12. Achievement in the past (2023-24):

12.1 Name of the 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.

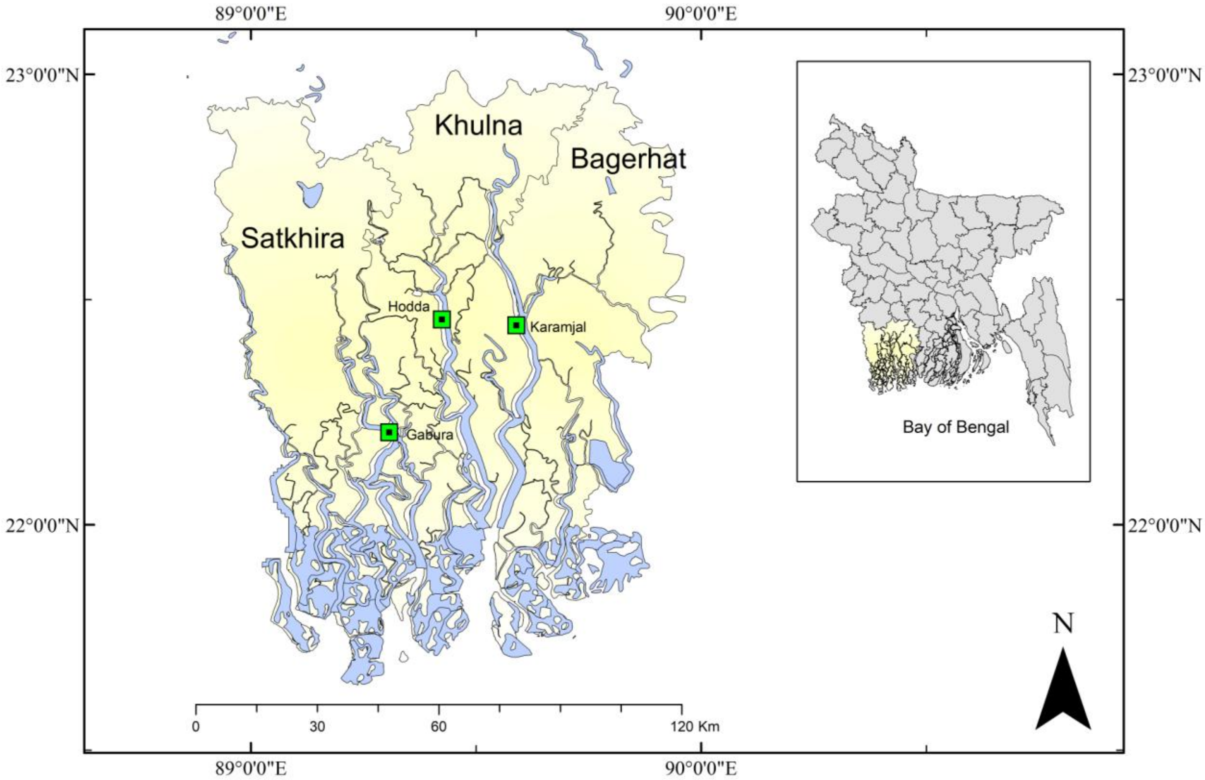

Figure 1: The sampling sites in the map

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:

$BW = a\ x TL^{b}$ …..………………………..……………………….…………………………….. (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-

$$C_t = P_t / T_t \quad \text{……} \quad (2)$$

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;

$$L_t = L_\propto \left(1 - e^{-K(t-t_0)}\right) \quad \text{……} \quad (3)$$

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);

$$\phi' = \text{Log } K + 2 \text{ Log } L_\propto \quad \text{……} \quad (4)$$

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.

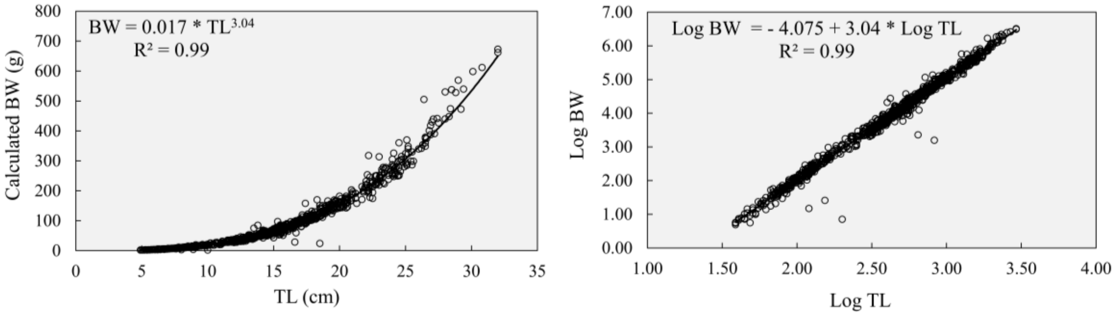

Figure 2. The relationship between Total lenth (TL) and body weight (BW) of *P. hasta* in the Sundarbans mangrove forest of Bangladesh

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.

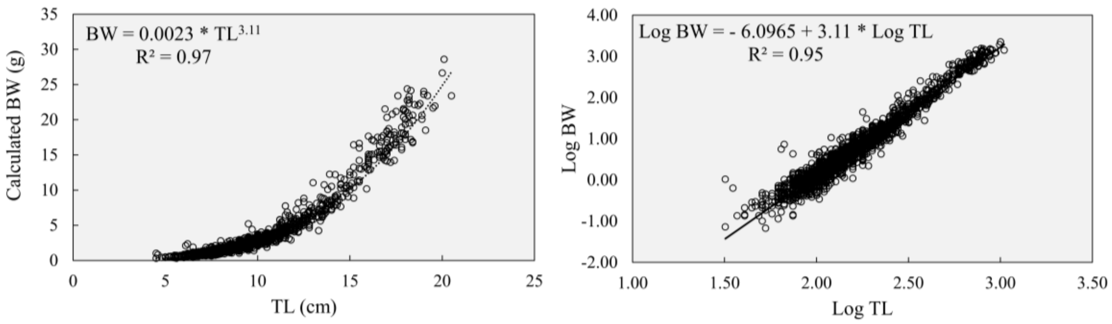

Figure 3. The relationship between Total lenth (TL) and body weight (BW) of *C. ramcarati* in the Sundarbans mangrove forest of Bangladesh

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.

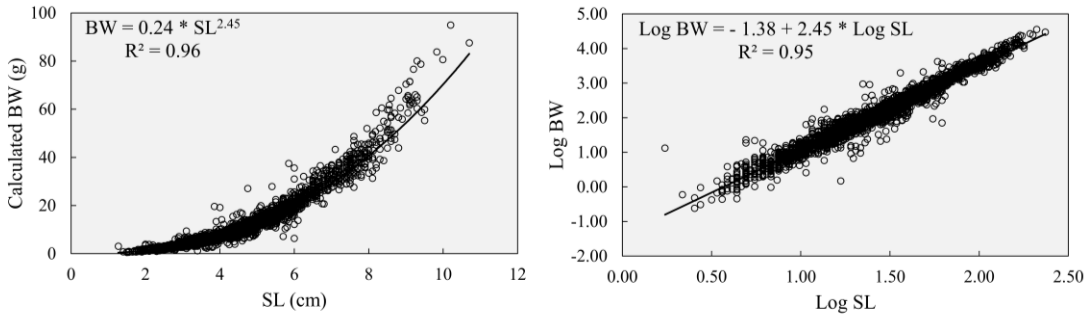

Figure 4. The relationship between shell length (SL) and body weight (BW) of *T. Telescopium* in the Sundarbans mangrove forest of Bangladesh

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

12.2 Name of the 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).

12.3 Name of the 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).

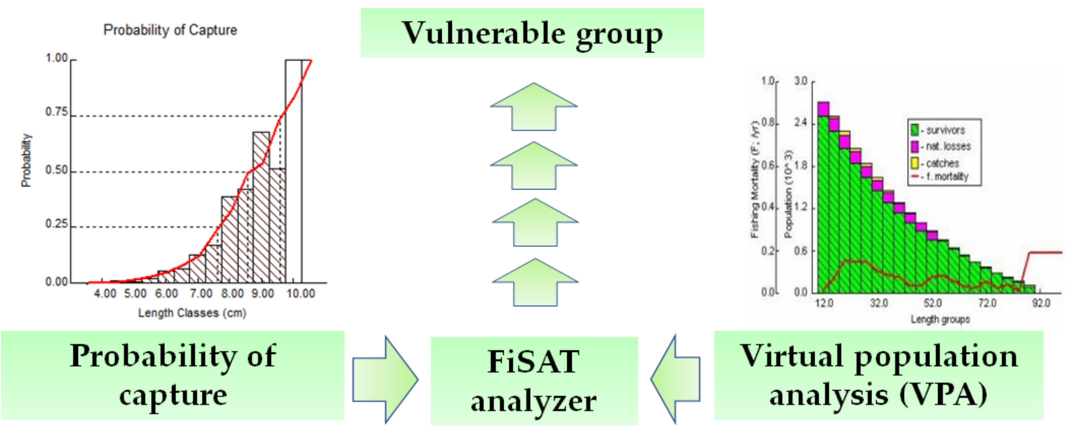

Figure 5. Strategies to calculate vulnerable size group of a species

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.

12.4 Name of the 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

12.4 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.

12.5 Photo gallery:

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

12.6 References:

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13. List of scientific Publication:

- Two conference papers have been presented and abstracts have been published.
- A full-length scientific paper is accepted for publication in Heliyon (IF: 3.4; 2024).

14. Problems and Constraints encountered (if any): No specific constraint faced during reporting period.

15. Signature of Principal Investigator(s) and date: 15/07/2024

16. Signature of the Chief Scientific Officer and date: 15/07/2024

Potentiality of Aquatic Weed as Alternative Feed Ingredients for the Development of Cost-Effective Fish Feed for Coastal Aquaculture

Annual Research Progress Report
(July 2023- June 2024)

Bangladesh Fisheries Research Institute
Brackishwater Station, Paikgacha, Khulna- 9280

<u>**Annual Research Progress report (2023-2024)**</u>

01. Preface: Not applicable

02. Executive Summary:

03. Introduction: Not applicable

04. Bangladesh Fisheries Research Institute (An overview): Not applicable

05. Research Achievements of Brackishwater Station's Project:

06. Project Title: Potentiality of aquatic weed as alternative feed ingredients for the development of cost-effective fish feed for coastal aquaculture

07. Co-ordinator : Dr. Md. Latiful Islam, CSO

08. Researcher(s) Md. Masudur Rahman, SO (PI)

Md. Golam Mostafa, SO (Co-PI)

Saima Sultana Sonia, SO (Co-PI)

09. Duration : July 2023-June 2024

10. Total Budget & Expenditure: (2023-2024)

Budget: Tk. 9,00,000.00

Expenditure: Tk. 8,96,708.00

11. 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.

12. **Achievement in the past: (2023-24)**

12.1 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**

12.2 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

12.3 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

$$\mathbf{HIS} = \frac{\textbf{Liver weight}}{\textbf{FBW}} \times \mathbf{100}$$

Viscerosomatic index (VSI): The net amount of filet produced was also monitored closely.

$$VSI = \frac{\text{weight of animal viscera}}{\text{FBWanimal weight}}$$

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) 1.49×10^{12} /l.

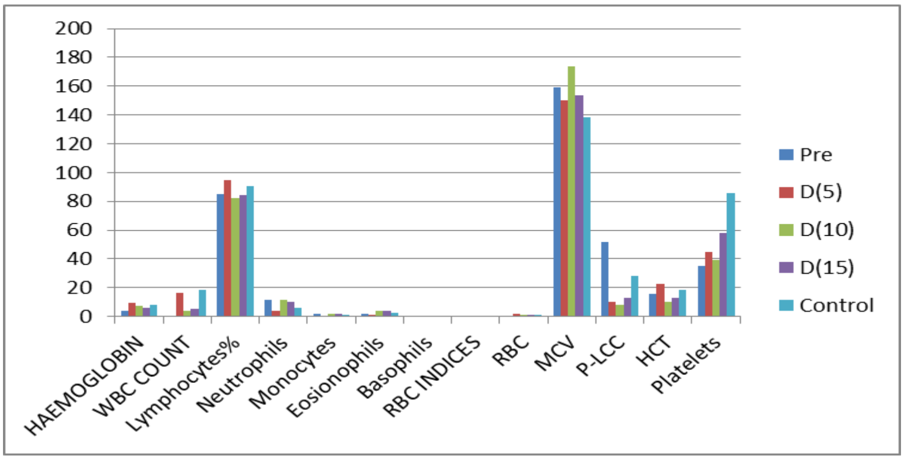

Fig 1: Haematological report of tilapia

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)

12.4 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.

12.5 Photo gallery:

Plate 1: *Chara baltica*

Plate 2: Mixing of feed ingredients

Plate 3: Formulated feed

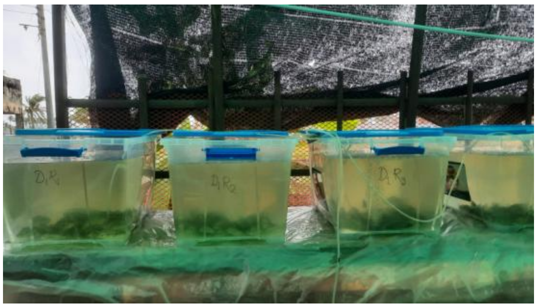

Plate 4: Culture trial

Plate 5: Feeding of fishes

Plate 6: Blood sample collection process

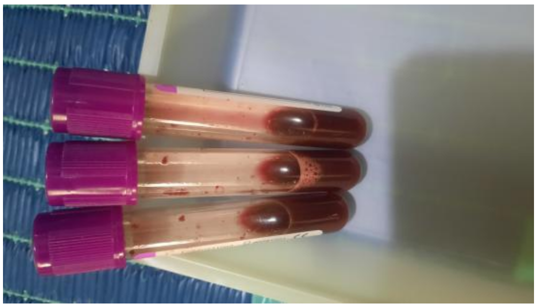

Plate 7: Collected blood

Plate 8: Hematological and biochemical test

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13. List of scientific Publication: One abstract has been published in the International Conference of FSB and two scientific papers are under preparation.

14. Problems and Constraints encountered (if any): No specific constraints encountered

15. Signature of Principal Investigator(s) and date: 15/07/2024

16. Signature of the Chief Scientific Officer and date: 15/07/2024

Development of Integrated Multi-Trophic Aquaculture Systems in South-West Coast of Bangladesh

Annual Progress Report
(July 2023- June 2024)

Bangladesh Fisheries Research Institute
Brackishwater Station, Paikgacha, Khulna- 9280

Annual Progress report (2023-2024)

01. Preface: Not applicable

02. Executive Summary: Not applicable

03. Introduction: Not applicable

04. Bangladesh Fisheries Research Institute (An overview): Not applicable

05. Research Achievements of Brackishwater Station's Project: Not applicable

06. Project Title: Development of Integrated multi-trophic aquaculture systems for south-west coast of Bangladesh

07. Co-ordinator **:** Dr. Md. Latiful Islam, CSO

08. Researcher(s) **:** A.K.M. Shafiqul Alam Rubel, SSO & PI

: Md. Motiur Rahman

: Md. Masudur Rahman, SO & Co-PI

09. Duration **:** July 2023-June 2024

10. Total Budget & Expenditure: (2023-2024)

Budget: Tk. 9,00,000.00

Expenditure: Tk. 8,95,253.00

11. 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. .

12. 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% $KMnO_4$) 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).

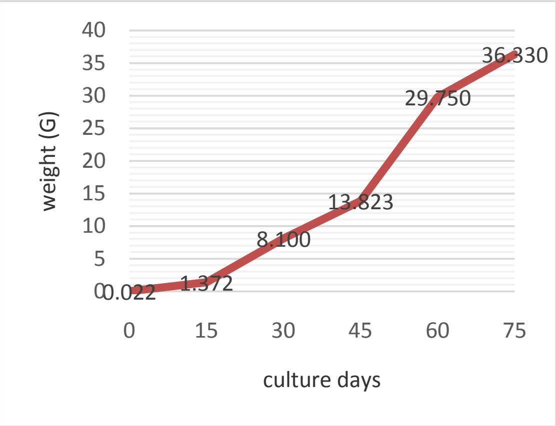


Fig 1. Growth performance of *P. monodon monodon*

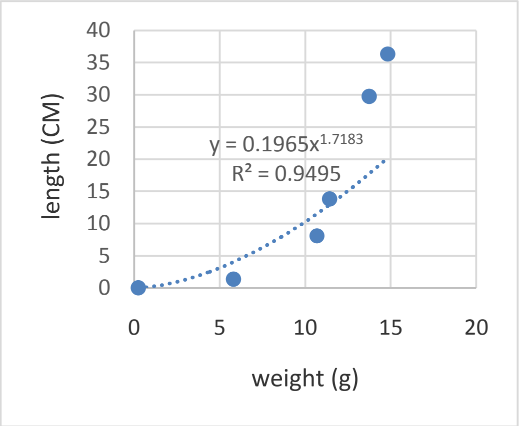


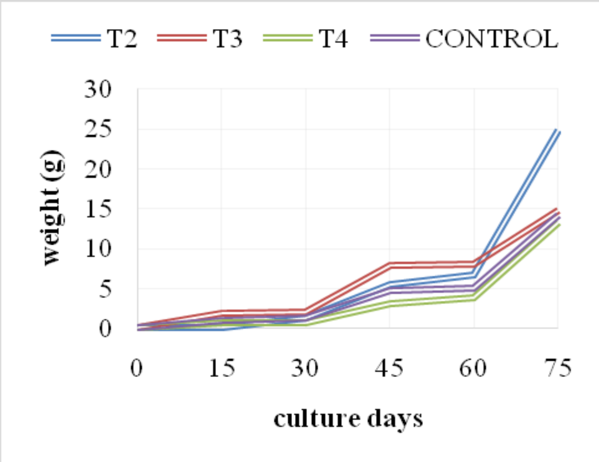
Fig 2. length-weight (g) relationship of *P.*

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).



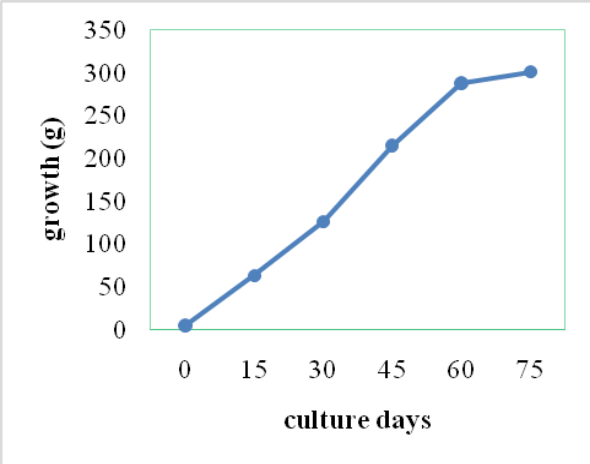


Fig 3. Growth performance of *M. rosenbergii*

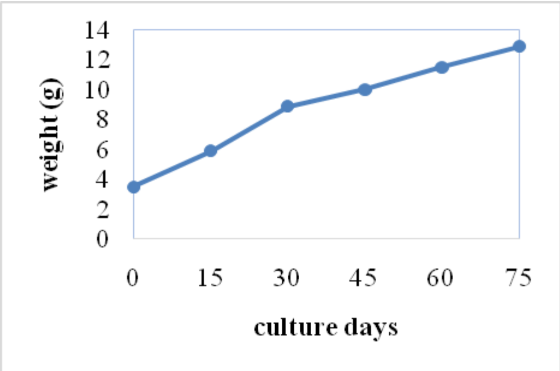
Fig 4. Growth performance of Nile Tilapia

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.



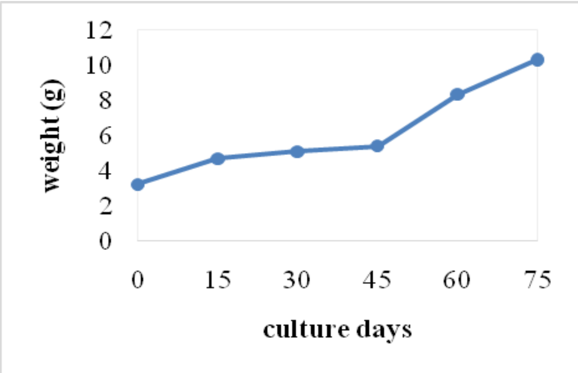


Fig 5. Growth performance of *Mystus gulio* **Fig 6.** Growth performance of *Planiliza parsia*

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

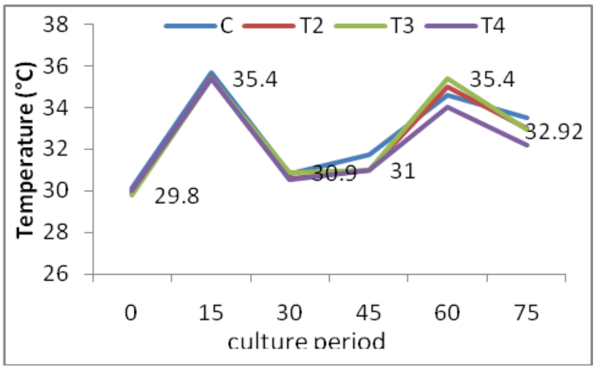
Parameters/ Treatments	Sampling frequencies	pH	Salinity (ds/m)	Organic Matter (%)	(%) N	P (µg/g)	K (meq/ 100g)	S (µg/g)	Zn (µg/g)	B (µg/g)	Ca (meq/ 100g)	Fe (µg/g)
C/T1	starting	7.9±0.01	14.6±1.27	2.05±0.39	0.10±0.05	5.1±0.30	0.68±0.06	219.9±0.1	0.6±0.39	2.91±0.41	20.5±3.99	39.14±7.40
T2	starting	7.05±0.05	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 (°C)	**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) **°C** 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).



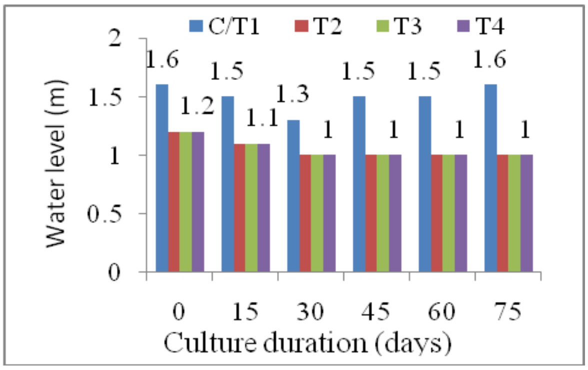


Fig. 7. Temperature variation during culture period   Fig. 8. Water level in ponds during culture period

12.2. The economic benefit analysis of IMTA system: As experiment is still ongoing thus economic analysis yet not done.

12.3. 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.

12.4 Photo gallery of activities: 2023-2024



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

12.5 References:

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13. List of scientific Publication: One scientific paper is under preparation.

14. Problems and Constraints encountered (if any): No specific constraint faced yet

15. Signature of Principal Investigator and date:

16. Signature of the Chief Scientific Officer and date: 15/07/2024

Domestication, Reproductive Biology, Breeding and Culture of Indigenous Brackishwater Prawns under Captive Conditions

Annual Research Progress Report

(July 2023- June 2024)

Bangladesh Fisheries Research Institute

Brackishwater Station, Paikgacha, Khulna- 9280

Annual Research Progress Report (2023-2024)

01. Preface: Not applicable

02. Executive Summary: N/A

03. Introduction: Not applicable

04. Bangladesh Fisheries Research Institute (An overview): Not applicable

05. Research Achievements of Brackishwater Station's Project:

06. Project Title: Domestication, reproductive biology, breeding and culture of indigenous brackishwater prawns under captive conditions

07. Co-Ordinator	**:** Dr. Md. Latiful Islam, CSO
08. Researcher(s)	**:** Dr. Md. Latiful Islam, CSO & Co-I
	: Md. Abu Naser, SO & PI
	: Shafiqul Alam Rubel, SSO & Co-I
	: Md. Hashmi Sakib, SSO & Co-I
09. Duration	**:** July 2023-June 2024

10. Total Budget & Expenditure: (2023-2024)

Budget (Tk)**:** 10,00,000.00

Expenditure (Tk)**:** 9,98,889.00

11. 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.

12. Achievement in the past (2023-24):

12.1 Name of the experiment/study:

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.

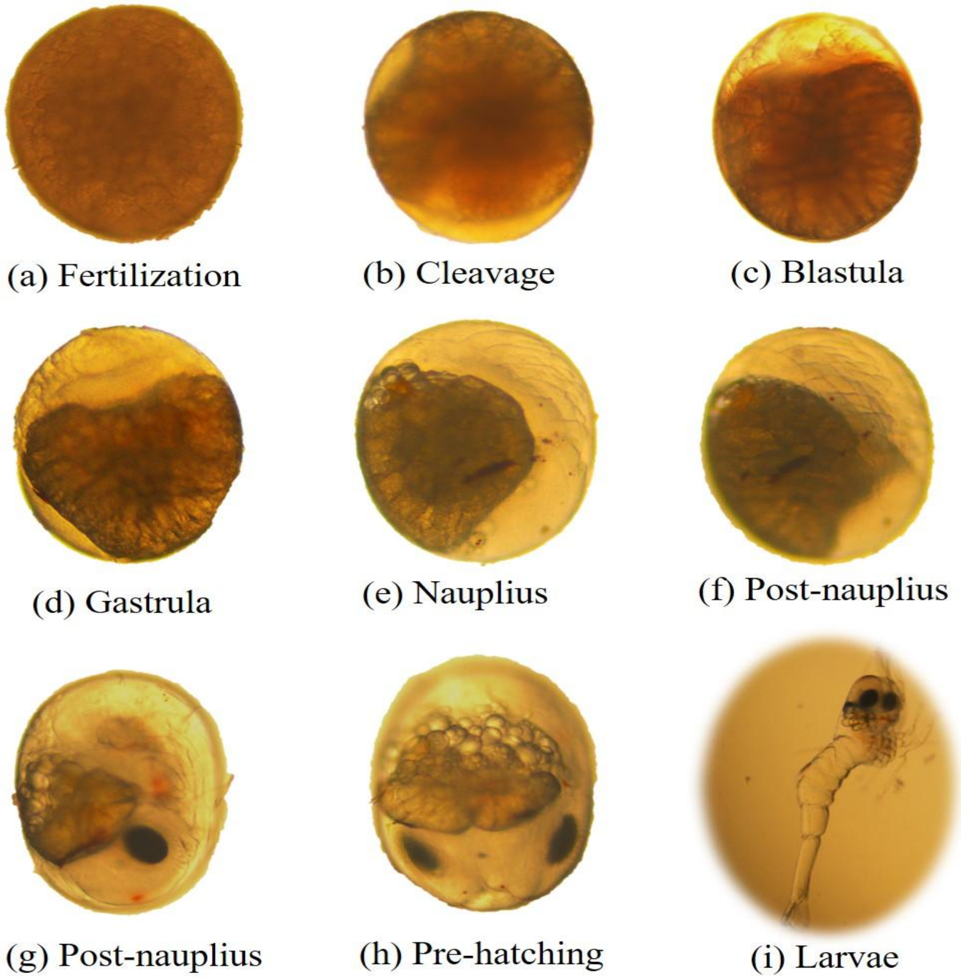


Figure 1: Embryonic developmental stages of *M. lamarrei*.

12.2 Name of the experiment/study:

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

12.3 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.

12.4 Photo gallery:

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

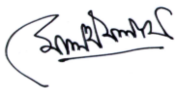
12.5 References: Not applicable

13. List of scientific Publication:

One abstract is published in the 2nd International Conference on Sustainable Fisheries (ICSF) 2022 "Fisheries for Achieving SDGs" at Faculty of Fisheries, Sylhet Agricultural University, Sylhet-3100, Bangladesh and One manuscript (Research Article) is under preparation.

14. Problems and constraints encountered (if any): N/A

15. Signature of Principal Investigator(s) and date: 15/07/2024



16. Signature of the Chief Scientific Officer and date: 15/07/2024

Domestication, breeding and seed production of some commercially important brackishwater fishes

Annual Research Progress Report

(July 2023- June 2024)

Bangladesh Fisheries Research Institute

Brackishwater Station, Paikgacha, Khulna- 9280

Annual Research Progress Report (2023-2024)

01. Preface: Not applicable

02. Executive Summary: N/A

03. Introduction: Not applicable

04. Bangladesh Fisheries Research Institute (An overview): Not applicable

05. Research Achievements of Brackishwater Station's Project:

06. Project Title: Domestication, breeding and seed production of some commercially important brackishwater fishes

07. Co-Ordinator : Dr. Md. Latiful Islam, CSO

08. Researcher(s) : Dr. Md. Latiful Islam (CSO & PI)

: Md. Golam Mostofa (SO & Co-I)

: Saima Sultana Sonia (SO & Co-I)

: Md. Abu Naser (SO & Co-I)

09. Duration : July 2023-June 2024

10. Total Budget & Expenditure: (2023-2024)

Budget (Tk)**:** 11,00,000.00

Expenditure (Tk)**:** 10,98,408.00

11. 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.

12. Achievement in the past: (2023-24)

12.1 Name of the experiment/study:

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.

12.2 Name of the experiment/study:

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 ($\mu g.kg^{-1}$ 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

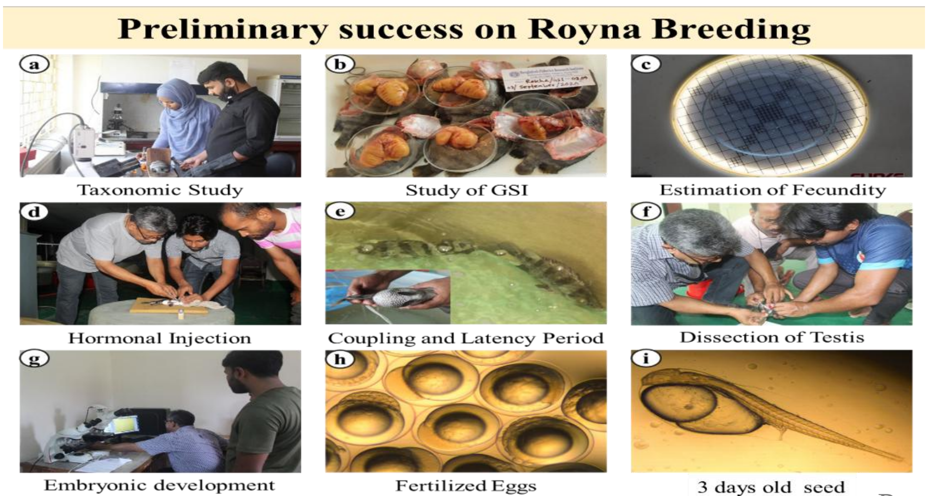
Among those three treatments, T2 has only been implemented with successful ovulation. Collected oocytes of Bhangon were mixed with the milt collected by dissecting of potential male for fertilization. Regretfully no fertilization occurred. The team investigated the possible causes behind infertility 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	**1st dose (µg/kg)**	**Interval (hours)**	**2nd dose (µg/kg)**
Female	LHRHa	30	18	70
Male	LHRHa	-	-	50 (single dose)

12.3 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.

12.4 References: Not applicable

13. List of scientific Publication:

Two articles have been published; two articles is under preparation.

14. Problems and constraints encountered (if any):

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15. Signature of Principal Investigator(s) and date: 15/07/2024

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16. Signature of the Chief Scientific Officer and date: 15/07/2024