

# **Research Progress 2014-15**



## Freshwater Station & Sub-station

### **Stock improvement and dissemination of commercially important tilapia and climbing perch koi through genetic selection**

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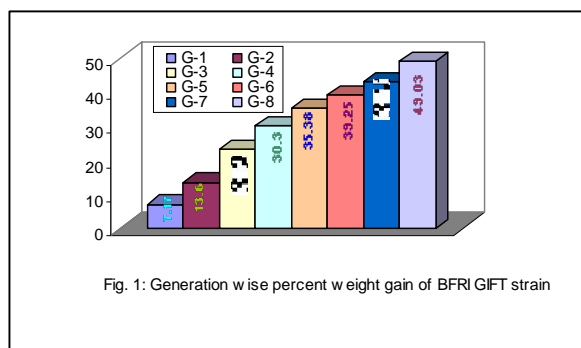
#### **Objectives**

- Continuation of stock improvement of BFRI-GIFT strain using family selection protocol
- Continuation of stock improvement of Thai koi using brood stock replacement technique
- Evaluation of growth performance of improved BFRI GIFT and Thai koi
- Distribution of improved BFRI-GIFT strain and Thai Koi among selected hatchery operators

#### **Achievement**

##### ***Expt. 1. Evaluation of growth performance of BFRI GIFT (F-8) at on station management***

The aim of this study was to evaluate of growth performance of the BFRI GIFT strain after seven generation of selection. This trial was carried out for a period of five months in a pond having an area of 1000 m<sup>2</sup>. There were two treatment groups, where offspring of founder population was treated as treatment-1 and offspring of F-8 Generation, which produced from 50 families was considered as treatment-2. In each treatment group 500 fingerling were stocked in a pond for communal rearing. Fingerlings of treatment-1 were marked through cauterization of pelvic fin. During rearing, fish were fed with supplementary feed contained 25% crude protein at the rate of 5-8% of estimated body weight. All the fishes under two treatments were harvested after five months rearing. After harvesting, individual weight of all fishes in both treatments were recorded. Data analysis was done through statistical programme (SAAS). Preliminary analysis showed that the upgraded fish had 49.03% greater harvest weight than that of the founder population (non selected population) in F<sub>8</sub> generations (Fig.1).

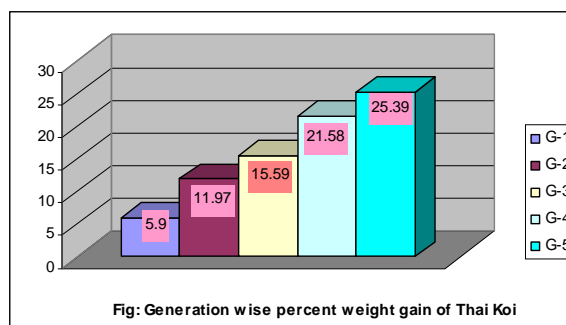


##### ***Expt. 2. Stock improvement of A. testudineus (F<sub>5</sub>) through brood stock replacement techniques***

In another study, stock improvement program of *A. testudineus* was also undertaken during 2014-15 through brood stock replacement technique. The largest 400 individuals (200 male and 200 female) of F<sub>4</sub> generation were selected and stocked in a breeding pond for the production of F<sub>5</sub> generation. The fishes were mated in 5 pair cross in a single hapa to ensure equal numbers of male and female fish. After induced breeding, about 20g of hatchlings from each hapa were mixed together and reared in a single nursery pond for 4 weeks. As such four nursery ponds were maintained where each nursery pond contained 200g larvae (from 10 hapas out of a total of 40 hapas). After nursing, 500-600 fry randomly selected from each batch (each nursery pond) and put into the brood stock replacement pond in which 200 pairs of founder brood fish contribute fingerlings in this desired stock.

**Expt. 3. Growth performances evaluation of improved koi (F<sub>5</sub>) at on-farm management**

For evaluating the growth performance of non selected parental group of Thai koi and improved F-5 generation of Thai Koi, an experiment was conducted for a period of three months with three replications during April to June. The fry of koi were stocked in March 2015 at the stocking density of 75,000/ha at Dhokhola village, Gouripur, Mymensingh. There were two treatments with two replicates. Treatment-I was designed with F-5 generation of Thai koi, while treatment-II with non selected parental group of Thai Koi. After stocking, the fry were fed 30% crude protein enriched feed at the rate of 5-15% of estimated body weight. After three months of rearing, the fish were harvested. The harvesting means weight of T<sub>1</sub> and T<sub>2</sub> were 102.87±4.56 and 82.70±5.60g, respectively and results were statistically significant (p>0.05). The F-5 generation of Thai koi showed 25.39% higher growth than non selected group.



**Expt. 4. Evaluation of production performances of koi with shing & GIFT in semi-intensive culture management**

Production potential of koi with shing and GIFT, an experiment was carried out for a period of four months from March to June 2015 at Dohakhola village in Gouripur upazilla under Mymensingh in six farmers ponds having an area of 10 decimal each. The stocking density of koi was same in all treatments. But shing and GIFT density were varied with treatment. Design of experiment is shown in Table 1.

**Table 1.** Stocking density and species combination of fish under different treatments

Treatments	Fish species/ha			Total
	Koi	Shing	GIFT	
T-1	1,25,000	37,500	5000	1,67,500
T-2	1,25,000	32,500	10,000	1,67,500
T-3	1,25,000	27,500	15,000	1,67,500

In all the treatments, the fish were fed with pelleted feed (30% crude protein) at the rate of 5-20% of estimated body weight. All ponds were completely harvested after four months of rearing, first by seine netting followed by draining out of the ponds. Koi reached at an average final weight of 140.10±5.60, 132.66±5.11 and 129.73±4.07g in treatments-1, 2 and 3, respectively. The final weights of Shing were 32.47±7.11g in treatment-1, 35.40±6.59 and 37.51±6.95g in treatment-3. Relatively, identical growth of monosex GIFT in terms of weight was attained in all the treatments. It grew to an average weight of 210±9.75g, 208±7.51 and 206± 6.21g in treatments-1, 2, and 3, respectively. The survival rates of various species in three treatments were fairly high. The survival rate of koi, shing and monosex GIFT were ranged from 82-88, 72-79 and 87-94%, respectively. The gross production of fish in three treatments was calculated from the growth and survival of each fish species. The highest gross production of 17995 kg/ha were obtained in treatment-1 and the lowest of 16805 kg/ha in treatment-2.

## Stock improvement and dissemination of Thai pangas (*Pangasianodon hypophthalmus*)

**Researchers:** Dr. Md. Shaha Ali, SSO  
Md. Golam Sajed Riar, SO

### Objectives

- Stock improvement of pangas through rotational group breeding techniques
- Comparative growth study of improved & existing stocks of pangas
- Quality seed production and distribution of improved breeds

### Achievements

#### *Expt. 1. Stock improvement of Thai Pangas (Pangasianodon hypophthalmus) using rotational group breeding techniques*

**Production of base population:** Breeding of pangas (Batch-1) was initiated in 17 July 2013. At the end of the March, 200 pairs of immature brood (above 2.0 kg Male and above 3.0 kg Female) were stocked in BFRI, FS pond complex to mature them for breeding. For production of base population at least 60 pairs out of 200 pairs of brood were selected in breeding program and separated them randomly into 4 groups (Group-A, B, C and D). Within the randomly selected group, 15 pairs (sex ratio of female and male 1:1) of brood were mated separately to make 15 families in each group. All mating of the same group were performed in the same day. From each family, a sub sample of fertilized eggs (100 g fertilized eggs/pair) were taken and incubated in circular units and spawn from each group were stocked in separate 20 decimal earthen nursery ponds (replicated for each group). From each group, 5000 fingerlings were selected and reared under maintaining separate groups. 40% mortality was occurred in rearing ponds so that 3000 fingerlings were available for stocking in grow-out ponds. For brood stock development, fingerlings were stocked at the rate of 50 individuals per decimal. Status of base population in rearing conditions is shown in Table 1. During all phases of the growing period, the fish were fed 30% protein rich feeds and at the age of 1.5 years at least 1500 to 2000 breeders were ready for individual (mass) selection. At the age of 1.5 years or more, randomly selected 20% fish (sex ratio female o male 1:1) were kept in brood ponds until they will be used for the production of F<sub>1</sub> generation in the year 2016. The present status of base population of pangas in brood ponds is shown in Table 2.

**Table 1.** Status of base population of Thai pangas in rearing pond

Group	Pond size (Dec.)	SD (Nos)	Initial		Final	
			Length (cm)	Weight (g)	Length (cm)	Weight (g)
A	20	1500	14.23± 0.43	25.60± 3.49	32.33± 0.87	467.33± 5.09
B	20	1500	14.21± 0.35	25.00± 2.03	32.00± 0.44	432.45± 3.45
C	20	1500	15.28± 0.59	28.80± 2.73	33.56± 0.48	420.23± 4.34
D	20	1500	14.35± 0.60	27.30± 3.23	32.56± 0.44	450.45± 6.89

**Table 2.** Present status of base population of pangas in brood ponds

Group	Pond size (Dec.)	SD (Nos)	Initial		Final	
			Length (cm)	Weight (g)	Length (cm)	Weigh (g)
A	25	200	30.25± 3.43	525.60 ±17.49	51.45±13.44	1345.00±89.56
B	25	200	31.21± 2.35	535.00 ±16.03	49.89±9.56	1302.00±98.78

C	25	200	30.28±2.59	528.80 ±16.73	50.67±12.45	1290.70±101.89
D	25	200	32.35±3.60	557.30 ±14.23	52.89±11.78	1389.67±95.23

***Expt. 2. Comparative growth study of improved and existing stocks of Thai Pangas (Pangasianodon hypophthalmus) in farmers ponds***

For evaluation of growth performance of each generation, comparative growth trial were conducted using fingerlings from base population groups of pure Thai pangas with existing local stocks of pangas in the farmers field of Mymensingh and Kurigram region. The stocking density was maintained 120 fingerlings/decimal and the fish were feed commercially available peleted feed at the rate of 3% body weight daily. The fish was sampled at monthly intervals to assess growth performance and adjust the feed ration. After 6 months the fish were harvested and data were presented in Table 3.

**Table 3.** Comparative growth performance of improved (BP) and existing stocks of Thai pangas in farmers ponds at Kurigram district, Bangladesh

Stock	Stocking density/dec.	Initial weight (g)	Final weight (g)	Trial period (day)	Daily weight gain (g)/d	Comments
Pure Thai pangas (BP)	120	18.56±3.56	884.67±17.89	180	4.80	Pure Thai pangas (BP) showed 10-11% higher growth compared to local Thai stock
Local stock	120	20.34±6.45	803.56±18.33	180	4.35	

**Distribution of improved breeds (Base population of Thai pangas) to fish farmers:** Thirty thousands (improved breeds, size 3-4 cm) of base population of Thai pangas were distributed to the hatchery owners in Mymensingh, Barishal and Kurigram regions as per objectives of the IAPP.

### Upgradation of carp broods for quality seed production and dissemination

**Researchers:** Dr. Md. Shaha Ali, SSO  
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#### Objectives

- To collect wild germplasm and evaluate growth performance within the collected wild germplasms and existing stocks.
- To develop live gene bank with quality brood stocks through implementation of effective breeding plan.
- To produce quality seed of improved breeds and disseminate to the farmers/hatchery and nursery owners/entrepreneurs.

#### Achievements

***Collection of wild breeds and comparative growth study within the collected stocks and existing stock***

**Halda sources:** Five hundred gram (500 g) wild breeds of Rohu, Catla and Mrigal (not yet identified) were collected from Halda River, Hathazari; Chittagong on 11<sup>th</sup> May 2013. Collected spawn were stocked in 15 decimal nursery ponds for primary nursing for one month. Following one month primary nursing the spawn were transferred to secondary nursery ponds and then rearing pond followed the existing rearing protocol.

**Jamuna sources:** Five hundred gram (500 g) wild breeds of Rohu, Catla and Mrigal were collected from Jamuna River, Matin Shaheber Ghat, Shirajgonj sader, er Shirajgonj on 05<sup>th</sup> June 2013. Collected spawn were stocked in 15 decimal nursery ponds for primary nursing for one month. After one month primary nursing the spawn were transferred to secondary nursery ponds and then rearing ponds followed the existing rearing protocol.

**Comparative growth study among Halda and Jamuna stock:** After eight month of rearing period we compare the growth of Halda and Jamuna Stock.

**Table 1.** Comparative growth study of Halda and Jamuna stock

Stocks	Rohu ( <i>L. rohita</i> )	Catla ( <i>C. catla</i> )	Mrigal ( <i>C. chirroshus</i> )	Comments
Daily weight (Halda) g/d	3.24	3.25	2.64	Higher growth were found in Halda compared with Jamuna
Daily weight (Jamuna) g/d	2.38	2.80	1.73	

**Quality seed production and distribution:** BFRI has the improved broodstocks which are used every year for the production of quality breeds and distributed to the fish farmers and hatchery owners. On the otherhand, the proposed developed wild and improved broods will also be used for mass seed production and distribution to the fish farmers/ and nursery operators and also to the hatchery owners for further mass seed production in their own hatcheries.

### Component B: Development of breeding and rearing technique of Crucian carp

#### *Developed induced breeding technique for Crucian carp*

This experiment consisted of four treatments (T1, T2, T3 and T4) with three replications of each. A total of 32 female and 32 male were selected from the brood rearing ponds. To observe the effective dose for artificial propagation, the females and males were injected with different doses of PG extract. Single dose had been used in treatment-T1 and Double dose had been used in treatments-T2, T3 and T4. After ovulation, eight females and eight males from each treatment were selected for stripping.

**Table 1.** Induced breeding of Crucian carp using PG

Treatment	Doses of first injection (mg/kg)		Interval (hr)	Doses of 2nd injection mg/kg		Ovulation time(hr)	Fertilization rate (%)	Hatching period (hr)	Hatchig rate (%)	Water temp (°C)	Comment
	M	F		M	F						
T1	--	--	--	2	6	-	--	--	20-22	T2 showed best result	
T2	--	1	6	2	5	90	44-48	78			
T3	--	2	6	2	6	56		45			
T4	--	3	6	2	7	35		30			

**Developed an effective nursing system for Crucian carp:** Primary nursing were conducted in the month of January to February in 5 decimal ponds and then transferred to 20 decimal secondary nursery ponds and the data of primary and secondary nursing given below:

**Table 2.** Result of Crucian carp primary nursing

Treatment	Stocking density/decimal	Nursing period (days)	Survival (%)	Initial weight (g)	Final average weight (g)
T1	7,000	30 days	72	0.00285	0.48
T2	10,500		69		0.40
T3	14,000		58		0.36
T4	17,500		48		0.33

**Table 3.** Result of Crucian carp Secondary nursing

Treatment	Stocking density/decimal	Nursing period (days)	Initial weight (g)	Final weight (g)	Survival (%)
T1	3,000	60 days	0.48	6.33±0.32	78
T2	4,000		0.40	5.01±0.89	75
T3	5,000		0.36	4.55±0.23	70

### **Development of induced breeding and culture techniques for Mekong giant catfish, *Pangasianodon gigas***

**Researchers:** Dr. Md. Khalilur Rahman, CSO  
Dr. Jubaida Nasreen Akhter, SSO  
Md. Rayhan Hossain, SO

#### **Objectives**

- To develop induced breeding technique for *Pangasianodon gigas*
- To develop rearing technique for *Pangasianodon gigas*

#### **Achievement**

**Introduction in BFRI, Mymensingh:** The BFRI has long experience on artificial breeding of *Pangasius* species and developed artificial breeding and culture technologies of *Pangasius sutchi* and *Pangasius pangasius* in 1993 and 2004, respectively. After receiving the information of introduction of *Pangasianodon gigas* in Bangladesh, BFRI showed their interest to work on this species. Accordingly, communication has been made to collect the species from the local Ramy Fish Farm Ltd, Boilor, Trishal, Mymensingh. A verbal gentleman agreement has been made between Ramy Fish Farm Ltd. and BFRI regarding the sharing of spawn of first production and giving training to the workers of Ramy Fish Farm Ltd. on artificial breeding techniques, spawn rearing and culture. After verbal agreement, authority of the Ramy Fish Farm Ltd. agreed to supply 50 individuals of *P. gigas* to the BFRI authority for conducting research on artificial breeding, spawn rearing and culture. Accordingly, 15 fishes of *P. gigas* were collected from the Ramy Fish farm. Ltd. to BFRI on 18 March 2015, then 12 fish on 28 March 2015 and again 12 fish on 17 April 2015 and finally 12 fish on 06 June 2015. However, one fish was died due to head injury during transportation by an open truck of 5 tons capacity.



**Stocking in pond:** A total of 15 fish collected on 18 March 2015, was stocked in a pond having an area of 40 decimal. One fish was died due to head injury during transportation. Rest fishes were stocked in another pond having an area of 150 decimal.

**Feeding and water management:** The ponds was filled up with fresh water from a deep tube well and treated with dry cow-dung at the rate of 5 kg/dec. After manuring, the pond was left for 7 days for growth of zooplankton. After growing plankton, *P. gigas* was stocked at a stocking rate of 10 kg/dec. Fish was fed with locally available commercial feed containing about 28% protein at the rate of 3% body weight daily. Water shower was provided daily in each pond for 2-3 hours. Moreover, freshwater from a deep tube well was provided once a week. Water quality parameters were recorded biweekly. Health and gonadal development were checked from April 2015. Female *P. gigas* was checked by observing external features of abdomen and, colour and shape of genital papillae while their male counterparts were checked by gentle pressing the abdomen to get milt.

**Inducing experiment by cPGE:** Trials on induced breeding of *P. gigas* were conducted on 12 June 2015. Both the ponds were netted at 09:00 hours and 05 gravid females and 02 males were selected primarily selected. Selected fishes were housed in a net hapa in the pond having a dimension of 8 m x 4 m. Water shower was provided by a submersible pump. At 20:00 hours in the evening, primarily selected fishes were checked again and finally 01 female and 01 male were selected for induced breeding trial. The female, having comparatively soft and bulging belly was selected as brood while the male counterpart was identified by observing elongated protrude genital papilla. Carp Pituitary Gland Extract (cPGE) was administered at the rate of 9 mg/kg BWt and 3 mg/kg BWt of female and male, respectively. Dose of the female was split into 2 portions. First dose was 3 mg/kg BWt and administered at 20:00 hours on 12/06/2015. After 12 hours interval 2<sup>nd</sup> dose was applied at 6 mg/kg BWt at 08:00 hours on the following day (13/06/2015). The male received a single dose at a rate of 3 mg/kg BWt at the time of 2<sup>nd</sup> dose of the female (Table 1).

**Table 1.** Details of induced breeding trials on *P. gigas* on 12 June 2015

Item	Criteria
Examination	Gonadal development Secondary sexual characters
Male ♂	01 Wt: 40 kg Single Dose
Female ♀	01 Wt: 45 kg, Double Dose
Dose 1 <sup>st</sup> ♀	3 mg/kg BWt
Interval	12 hours
Dose 2 <sup>nd</sup> ♀	6 mg/ kg BWt
Dose 1 <sup>st</sup> ♂	3 mg/kg BWt

At 16:00 hours on the following day, the injected fish was checked for stripping. However, no sign of ovulation was observed although the belly became more soft and bulging than the uninjected conditions. In case of male no milt was found when gentle pressure was applied on the abdomen. Both the brood was kept in the hapa and water shower was provided again. At 22:00 hours the injected fishes were checked again and no sign of ovulation was observed. Next day (14/06/2015), at 08:00 hours, the injected fishes were checked again and found no change. Then the fishes were treated with KMnO<sub>4</sub> to prevent secondary infection on body skin, lip of mouth and fins. Finally, the injected fishes were released in the pond.

## Establishment of Cryo-milt Bank for carps and catfishes

**Researchers:** Dr. Md. Khalilur Rahman, CSO  
Dr. Jubaida Nasreen Akhter, SSO  
Md. Rayhan Hossain, SO

### Objectives

- Developing appropriate collection techniques and transport systems of fish milt
- Cryo-Milt Bank for *Labeo rohita* and *Pangasius hypophthalmus (sutchi)*
- Estimation of breeding success of *L. rohita* and *P. sutchi* using cryo-milt

### Achievement

**Brood rearing:** A pond of 0.2 ha area of the Freshwater Station of BFRI has been prepared by repairing dykes in December 2014. Every year brood pond is damaged due to the activity of rate and fresh water eel. Therefore in dry season brood pond need repair. The pond was disinfected by applying lime at the rate of 1 kg/dec. The pond was filled up with water from a deep tube well. Water level was maintained at 1.8 to 2.0 m. A total of 50 broods of *Pangasius hypophthalmus (sutchi)* averaging 4.0 kg and 35 broods of *Labeo rohita* averaging 1.5 kg have been reared in the pond. Among the 50 *P. hypophthalmus* brood, 35 were female and 15 were male, while number of male and female of *L. rohita* were 15 and 20, respectively. Fishes were fed with commercially available pelleted feed containing about 28% protein at the rate of 2% body weight daily. Weekly water showering was provided in the pond for proper gonadal development of fishes. Moreover, water shower increases concentration of DO in pond water. Monthly sampling was done to check gonadal development of fishes. Female *P. hypophthalmus* and *L. rohita* were checked by observing external features of abdomen and, colour and shape of genital papillae while their male counterparts were checked by gentle pressing the abdomen to get milt.

**Collection of milt and motility examination and preservation:** Mature and healthy male brood fishes were selected to collect milt. Milt sample were expelled from the male fish by gentle abdominal pressure and collected into a clean and dry tube of 5 ml capacity. Contamination of sample with blood, water, urine or the feces were avoided as these contaminants significantly reduce the milt quality and cause poor post-thaw sperm motility. The milt samples were primarily placed in sealed ice box for 2-3 minutes then it was stored in a normal freezer for 20 minutes at 0 to 4°C and then milt was stored in a deep freezer at -15°C. Before storage, cryo-diluent was prepared. Dimethyl-sulphoxide (DMSO) was used as cryo-protectant and phosphate buffered saline was used as extender. Cryo-diluents solution was prepared by mixing 75% phosphate buffered saline, 10% DMSO and 15% skimmed milk. This composition was homogenously mixture by vortex mixture. Milt was stored in 5 ml cryo-tubes. Cryo-tubes were leveled as A, B and C for storing 4, 8 and 12 hours, respectively. The milt was mixed with the solution at the ratio 1:1(v/v); and stored in 5 ml leveled cry-tubes. This cry tubes ware gently shaken to allow milt to properly mix with solution, and then carry those cryo-tubes by ice box. Leveled cry-tubes ware placed in the refrigerator for 15 min to reduce their temperature to 4°C before fast freezing in the freezer. Then this tubes ware storage in the -15°C deep freezer.

In the thawing process, this cryo tubes ware first transferred into refrigerator for 20 min to allow the milt to thaw from -15°C to 4°C inside the refrigerator. There after, they ware transferred at room temperature and allowed to stand 5 min before mixing with female aggs. The sperm motility rates of the freshly collected semen sample were evaluated prior to cryopreservation. Sperm motility of fresh milt was recorded as 95%.

**Thawing and fertilization:** Samples of milt were removed from the deep freezer. The 5 ml tube was held in a water bath at a temperature of 37-40°C for around 7-10 seconds. As a general rule, samples are thawed when air bubbles within the straw can move freely within the liquid. Thawed milt was added to the eggs of *Pangasius hypophthalmus* and thoroughly mixed. After approximately 5 minutes, water was added to water-harden the eggs. Hatching rates were very poor that ranged between 5 and 20%.

**Short-term preservation and fertilisation:** Availability of milt usually depends on species, age, size, season and physiological condition of the fish. Generally, *P. hypophthalmus* breeds between May and August, while *L. rohita* breeds between May and July. Oozing female and running male are found during peak spawning season. Short-term milt preservation technique was applied with the milt of *P. hypophthalmus* on 06 August 2014. Milt was preserved in a deep freeze for 8 hours at -20°C and motility of spermatozoa was assessed by a microscope. Although August is the last month of spawning season but milt of *P. hypophthalmus* was found motile. Mortality rate of fresh milt ranged between 40%. After short-term preservation, mortality rate was only 10%. Fertilisation rate of *Pangasius hypophthalmus* was nil with the milt preserved at -20°C for 8 hours. Long-term preservation experiment was not conducted due to lack of instruments. No experiment was conducted with *Labeo rohita* during late spawning season.

### Development of seed production and grow-out techniques for endangered fish species (*Chitala chitala*, *Monopterusuchia* and *Tor putitora*) in Bangladesh

**Researchers:** Dr. Durin Akhter Jahan, SSO  
Md. Mominuzzaman Khan, SO  
Joniara Rasid, SO

#### Objectives

- To evaluate the growth performance of *C. chitala* spawns under different stocking densities
- To evaluate growth performance of *C. chitala* fingerlings with low stocking densities
- To develop breeding technique for *M. cuchia*
- To observe the growth performance of *M. cuchia* using different feeds
- Broodstock management of Mohashol (*Tor putitora*)

#### Achievement

##### *Expt. 1. Evaluation the growth performance of C. chitala spawns under different stocking densities*

The experiment was conducted in hapa ecology for a period of 30 days. Three stocking density of *C. chitala* spawn, such as T<sub>1</sub>: 100; T<sub>2</sub>: 200 and T<sub>3</sub>: 300/m<sup>2</sup> were tested with 2 replications for each. Initial length and weight of *C. chitala* spawn was 2.15±0.01cm and 0.084±0.02. In all Treatment live fish spawn and zooplankton were applied. The growth performances of *C. chitala* spawn are presented in Table 1.

**Table 1.** Growth performance of chital spawn after 30 days rearing

Treatments	Initial length (cm)	Initial weight (g)	Final length (cm)	Final weight (g)	SGR (%)	Survival rate (%)
T <sub>1</sub> =100/m <sup>2</sup>	2.15 ±0.01	0.084 ±0.02	5.11±1.17	1.44 <sup>a</sup> ±0.62	7.47	91.33±5.13 <sup>a</sup>
T <sub>2</sub> =200/m <sup>2</sup>			4.97±1.14	1.28 <sup>b</sup> ±0.86	6.07	88.33±8.14 <sup>a</sup>
T <sub>3</sub> =300/m <sup>2</sup>			4.93±0.37	1.16 <sup>c</sup> ±0.70	5.75	86.67±8.74 <sup>a</sup>

**Expt. 2. Development of breeding technique for *M. cuchia***

For development of induced breeding technique, *M. cuchia* having weight range of 250-300g were collected from different area of Mymensingh during July-August 2013. After collection, the fish were stocked in cistern (1:1 ratio). Brood *M. cuchia* were fed with supplementary feed fish paste, earth worm, live fish and snail. Induced breeding trials were initiated during Mid April 2014 with administering PG and HCG as inducing agents. Two trials were conducted during breeding season. In first trial PG was administered for female 35, 40 & 45 mg and for male 10 mg per kg body weight. HCG was administered 1000 IU per kg body weight of female. In second trial PG was administered for female 10mg per kg body weight as first dose. After 6 hours interval PG was administered for female 20mg and male 10 mg per kg body weight as second dose. After administration of inducing agent, male and female fishes were kept in cistern according to dose application.

The experiment was conducted in pond and cistern ecology. In cistern ecology helencha were used as shade and shelter. In pond ecology polythine was used in the pond bottom. In both ecologies soil 1feet soil layer was used. Three replications were maintained for both ecologies. Mature male and female eels were stocked at 1:1 ratio. In cistern ecology supplementary feed, fish paste was supplied at the 3-5% of estimated body weight. In both ecologies live fish, snail earth worm used as supplementary feed. Breeding activity of *cuchia* in cistern was keenly monitored but not bred in cistern ecology but *M. cuchia* propagated in pond ecology

**Expt. 3. Growth performances of *M. cuchia* using different types of feed**

The experiment was conducted for a period of 120 days from March to June 2014 in cistern ecology with three treatments each having three replications. Mud eels  $30.50 \pm 2.98$  was stocked in each treatment at a density of 10/m<sup>2</sup> and fed with different feeds at the rate of 5% body weight was applied once in a day. In treatment 1, 2 and 3 fishes fed with 100 % fish paste, 60 % fish paste with 40% atta, and live fish and snail, respectively. Aquatic weed, helencha was used as shelter. Details results are shown in the following Table2.

**Table 2.** Growth performance of *M. cuchia* using different types of feed

Treatments	Initial wt. (g)	Final wt. (g)	SGR (%)	Survival rate (%)
T <sub>1</sub>	30.50 ±2.98	124.63 <sup>a</sup> ±20.27	1.17	95.33 <sup>a</sup> ±8.08
T <sub>2</sub>		111.38 <sup>a</sup> ±15.86	1.11	92.67 <sup>a</sup> ±6.43
T <sub>3</sub>		103.13 <sup>b</sup> ±10.08	1.02	90.33 <sup>a</sup> ±4.51

**Expt. 4. Broodstock management of mohashol (*T. putipora*)**

Brood mohashol was stocked in pond having an area of 20 decimal. The male and female mohashol was reared in separate pond. Stocking density was maintained 5/decimal. Brood fish fed with improved supplementary diet at the rate of 2-5% body weight. Deep tubewell water was added 1-2 hrs in every day to enrich dissolved oxygen in male and female brood rearing pond. After rearing, breeding trial was conducted during breeding season November to January. Due to temperature fluctuation fishes were not responded.

## Development of feeds with probiotics and optimization of feeding strategies for important fish farming

**Researchers:** Dr. Md. Zulfikar Ali, SSO  
Mr. Mritunjay Paul, SO

### Objectives

- To optimize dietary protein to energy ratio (P/E ratio) for *Pangasianodon hypophthalmus*
- To evaluate the effect of selected probiotics on growth, feed and nutrient utilization and digestibility in *Pangasianodon hypophthalmus*
- To recommend the potential probiotics as feed additives in the formulated diets
- To develop and optimize feeds and feeding strategies in this fish farming

### Achievements

A series of feeding trials were conducted to develop and optimize of feeds with probiotics and feeding strategies for *Pangasianodon hypophthalmus*. Two feeding trials on: investigate the optimum dietary protein to energy ratio (P/E ratio (feeding trail-1) and evaluation of selected probiotics as feed additives in formulated feeds (feeding trail-2) in *Pangasianodon hypophthalmus* were conducted in a indoor rearing system of Freshwater Station, BFRI, consisting a series of cylindrical fiber glass tanks (70-L each) for 8 weeks. The follow up feeding trail in pond conditions on: development and optimization of feeds with probiotics in *Pangasianodon hypophthalmus* for 6 months (feeding trail-3) is also in progressing.

#### *Expt. 1. Optimizing dietary protein to energy ratio (P/E ratio) in Pangasianodon hypophthalmus*

Six experimental diets were formulated to contain two levels of protein (30 and 35%), each with three levels of lipid (5, 10 and 15%), in order to produce a range of protein to energy ratios. Protein to energy ratios ranged from 12.96 to 18.17 mg protein per kJ of GE. Fish meal and mustard oil cake were used as protein source. Lipid sources were a mix of equal amounts of cod liver oil and soybean oil. Starch and wheat flour were used as sources of carbohydrate. Alpha-cellulose was used as filler and carboxymethyl cellulose was used as a binder at a rate of 2%. A rate of 0.5% chromium (III) oxide was used as inert indicator for digestibility studies. Vitamin and mineral premix was added at a rate of 1%. The bite-sized (1.0 mm) pellet feeds was made with the help of hand pellet machine. The pelleted feeds were sun-dried or dried an oven at 40° C for two days. Each dietary treatment was conducted in triplicate tanks. The fish is being offered the test diets two times daily at the rate of 8-10% of their body weight and sub-divided into two equal feeds at 9.30 and 17.00 h. Feeding rate is being adjusted based on weekly sampling weights of fish.

Bio-chemical analyses to determine of crude protein, crude lipid, crude fiber and ash of diet ingredients, diets and whole body fish were analyzed following AOAC (1993) methods. Nitrogen-free extract (NFE) was calculated by difference. Gross energy was determined using an Automatic Oxygen Bomb Calorimeter (Gallenkamp & Co Ltd., England). Specific growth rate (SGR), %weights gain, food conversion efficiency (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU) will be calculated standard procedure.

**Table 1.** Mean growth performance and feed utilization of *Pangasianodon hypophthalmus* fed various P/E ratios for 8 weeks

Diet no. (Protein / Lipid), (%)	1 (25/5)	2 (25/10)	3 (25/15)	4 (30/5)	5 (30/10)	6 (30/15)
Initial body wt. (g)	2.58 <sup>a</sup> ± 0.04	2.55 <sup>a</sup> ± 0.07	2.58 <sup>a</sup> ± 0.04	2.55 <sup>a</sup> ± 0.07	2.53 <sup>a</sup> ± 0.04	2.55 <sup>a</sup> ± 0.07
Final body wt. (g)	14.70 <sup>d</sup> ± 0.71	16.15 <sup>c</sup> ± 0.35	17.10 <sup>bc</sup> ± 0.99	18.80 <sup>b</sup> ± 0.99	19.83 <sup>a</sup> ± 0.32	19.00 <sup>a</sup> ± 0.42
Weight gain (g)	12.13 <sup>d</sup> ± 0.74	13.60 <sup>c</sup> ± 0.28	14.53 <sup>bc</sup> ± 0.95	16.25 <sup>b</sup> ± 0.92	17.30 <sup>a</sup> ± 0.35	16.43 <sup>a</sup> ± 0.46
Weight gain (%)	471.12 <sup>d</sup> ± 35.31	533.39 <sup>c</sup> ± 3.70	563.88 <sup>bc</sup> ± 29.33	637.00 <sup>b</sup> ± 18.38	685.32 <sup>a</sup> ± 23.60	638.05 <sup>a</sup> ± 26.61
Specific growth rate (SGR % day)	2.91 <sup>d</sup> ± 0.11	3.08 <sup>c</sup> ± 0.01	3.16 <sup>bc</sup> ± 0.08	3.33 <sup>b</sup> ± 0.04	3.44 <sup>a</sup> ± 0.05	3.33 <sup>a</sup> ± 0.06
Food conversion ratio (FCR)	1.76 <sup>a</sup> ± 0.02	1.69 <sup>a</sup> ± 0.01	1.59 <sup>b</sup> ± 0.05	1.50 <sup>b</sup> ± 0.05	1.36 <sup>c</sup> ± 0.03	1.45 <sup>bc</sup> ± 0.04
Protein efficiency ratio (PER)	2.28 <sup>a</sup> ± 0.05	2.32 <sup>a</sup> ± 0.08	2.30 <sup>a</sup> ± 0.05	2.35 <sup>a</sup> ± 0.07	2.55 <sup>a</sup> ± 0.08	2.40 <sup>a</sup> ± 0.10
Apparent net protein utilization (ANPU %)	39.27 <sup>a</sup> ± 0.25	39.55 <sup>a</sup> ± 0.38	39.10 <sup>a</sup> ± 0.35	42.40 <sup>a</sup> ± 0.50	43.85 <sup>a</sup> ± 0.33	43.47 <sup>a</sup> ± 0.28

Growth performances in terms of final body weight, mean weight gain, specific growth rate (SGR, % day) and feed utilization of fish fed the experimental diets were influenced by the levels of protein and energy as lipid (Table 1). Growth rates increased in response to higher dietary protein, but the highest dietary energy level in higher protein diet resulted in reduced weight gain (Table 1.2). On the basis of growth performance and feed utilisation, it may be stated that the diet 5, containing 30% and 18.38 kJ/g protein and gross energy respectively, performed best. This diet presumably contained the most appropriate P/E ratio 16.33 (16.33 mg protein/ kJ of GE) in *Pangasianodon hypophthalmus*. However, the optimum dietary protein to energy ratio (P/E ratio) found for local cat fish, *Pangasianodon hypophthalmus* was 16.33 mg protein/ kJ of GE, for a diet containing crude protein 30%, crude lipid 10% and gross energy 18.38 kJ/g.

### **Expt. 2. Evaluation of selected probiotics in the formulated diets for *Pangasianodon hypophthalmus***

The same aged uniform size fingerlings of *Pangasianodon hypophthalmus* were randomly distributed into groups of 50 fish (averaging  $2.25 \pm 0.05$ g in weight) per 80-L fiberglass tank and three replicate tanks were used for each test diet. Six experimental diets (iso-nitrogenous and iso-energetic) were formulated to contain 32% crude protein and 18.46 kJ g<sup>-1</sup> gross energy for feeding trail-2. Feeds were prepared using locally available fish feed ingredients. The selected five types of probiotics (i) Bactocell (lactic acid producing bacteria, *Pediococcus acidilactici*); (ii) *Bacillus subtilis*; (iii) Levucell (yeast, *Saccharomyces cerevisiae*) (iv) Mixture (*Pediococcus acidilactici* + *Bacillus subtilis* + *Saccharomyces cerevisiae*) and (v) Navio plus (*Bacillus subtilis* + *Bacillus licheniformis* + *Bacillus megaterium* + *Lactobacillus acidophilus* + *Lactobacillus plantarum* + *Saccharomyces cerevisiae*) were added the diets following the recommended dose by the manufacturers. A control diet was prepared with same feed ingredients without mixing probiotic. The fish is being offered the experimental and control diets, 3 times daily at the rate of 10-8% of their body weight and sub-divided into 2-3 equal feeds at 9.00, 13.30 and 18.00 h. Feeding rate will be adjusted based on fortnightly sampling (fish weighing) of fish.

**Table 2.** Mean growth performance and feed utilization of *Pangasianodon hypophthalmus* fed selected probiotics for 8 weeks

Diet no.	1 (Control)	2 (Bactocell)	3 (Bacillus)	4 (Levucell)	5 (Mixture )
Initial body wt. (g)	2.25 <sup>a</sup> ± 0.05	2.30 <sup>a</sup> ± 0.04	2.20 <sup>a</sup> ± 0.05	2.25 <sup>a</sup> ± 0.06	2.20 <sup>a</sup> ± 0.04
Final body wt. (g)	18.75 <sup>b</sup> ± 0.49	22.15 <sup>a</sup> ± 0.48	21.50 <sup>a</sup> ± 0.28	21.00 <sup>a</sup> ± 0.14	21.30 <sup>a</sup> ± 0.28
Weight gain (g)	16.50 <sup>b</sup> ± 0.49	19.95 <sup>a</sup> ± 0.49	19.20 <sup>a</sup> ± 0.28	18.75 <sup>a</sup> ± 0.14	19.10 <sup>a</sup> ± 0.39
Specific growth rate (SGR) (% day)	3.79 <sup>b</sup> ± 0.05	4.13 <sup>a</sup> ± 0.04	3.99 <sup>a</sup> ± 0.03	3.98 <sup>a</sup> ± 0.01	4.06 <sup>a</sup> ± 0.02
Food conversion ratio (FCR)	2.50 <sup>a</sup> ± 0.08	1.70 <sup>b</sup> ± 0.08	1.78 <sup>b</sup> ± 0.03	1.81 <sup>b</sup> ± 0.02	1.90 <sup>b</sup> ± 0.05
Protein efficiency ratio (PER)	1.20 <sup>b</sup> ± 0.05	1.76 <sup>a</sup> ± 0.08	1.70 <sup>a</sup> ± 0.02	1.60 <sup>a</sup> ± 0.01	1.62 <sup>a</sup> ± 0.04
Apparent net protein utilisation (ANPU %)	21.65 <sup>b</sup> ± 0.21	26.80 <sup>a</sup> ± 1.21	25.75 <sup>a</sup> ± 0.47	26.16 <sup>a</sup> ± 0.28	26.55 <sup>a</sup> ± 1.14
Protein digestibility (%)*	87.50	91.90	90.80	90.70	91.05

Growth response parameters are shown in Table 2. The growth rate in terms of mean final body weight, weight gain, percent weight gain of experimental fish fed diet 2 was significantly ( $p < 0.05$ ) highest than the control diet. There was no significant ( $p > 0.05$ ) difference among the growth rate of experimental fish fed diets 3, 4 and 5. Fish fed diets 2-5 showed significantly the higher ( $p < 0.05$ ) SGR while the diet 1 producing the lowest SGR value. Fish fed diets 2-5 showed significantly ( $p < 0.05$ ) superior FCR value than the control diet. The significantly higher ( $p < 0.05$ ) PER values were obtained fish fed diets 2, 3, 4 and 5 but no significantly difference among themselves. The ANPU value in diet 2 was significantly highest ( $p < 0.05$ ) and ANPU value in diet 1 was the lowest. From the results of this feeding trial, it is logical to conclude that feed incorporated with the probiotics (Bactocell, *Bacillus*, Levucell) can be used as a fish feed additives in *Pangasianodon hypophthalmus* culture, to enhance fish health, better feed efficiency and growth performance.

### Investigation and identification of emerging fish diseases and development of their control strategies

**Researchers:** Dr. Nazneen Bagum, SSO  
Dr. Md. Zahirul Haque, SSO  
Md. Shirajum Monir, SO

#### Objectives

- To isolate and identify Shing viruses from recent outbreaks
- To identify the causative agent(s) for emerging fish diseases outbreak
- To observe histological changes in different organs of diseased fish

#### Achievement

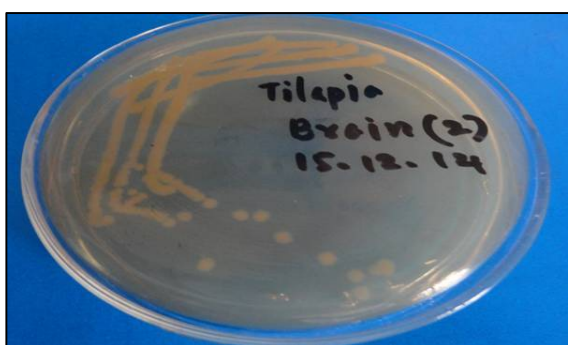
**Prevalence of shing viruses in Mymensingh district:** A total of 144 samples were taken up from 20 independent disease affected Shing farms of five upazillas across the Mymensingh districts of

Bangladesh. The results of PCR analysis to check the presence of viruses among the samples are oriented in Table 1 and Fig. 1. All the 144 samples were subjected for RT-PCR for the presence of virus type-1 and virus type-2. Among the 77 samples, a total of 66 (85.71%) samples were positive for virus type-1 and only 10 (12.98%) samples for virus type-2 and remaining 11 (14.28%) samples were negative for both the viruses.

**Table 1.** Prevalence of virus type-1 and virus type-2 in disease affected shing farms in Mymensingh district of Bangladesh

Identified viruses	Sample collecting sites					Total prevalence (%)
	Mymensingh sadar	Muktagacha	Phulpur	Gouripur	Fulbaria	
virus type-1	10/10	14/16	11/14	19/22	12/15	66/77 (85.71)
virus type-2	2/10	1/16	0/14	3/22	4/15	10/77 (12.98)

**Biochemical tests for bacterial identification:** The bacteria were isolated from the infected eye, brain, liver, spleen and kidney of infected Tilapia. The bacteria were grown on basic agar media of Tryptic Soya Agar (Figs. 1 and 2). After 1 to 2 days of incubation on Tryptic Soya Agar the colonies were found round, convex and entire. Morphological and biochemical tests such as grams stain, motility, oxidase, catalase, indole, O/F were conducted for identify bacteria those isolated from diseased Tilapia.



**Fig. 1.** Bacteria isolated from brain of Tilapia



**Fig. 2.** Bacteria isolated from infected eye of Tilapia

A total of 48 bacterial isolates collected from diseased Tilapia were initially characterized for their morphological, physiological and biochemical properties. The isolates were designated as TE10-TL14. The origin of the isolates is given in Table 1. Among these, 25 isolates were identified as *Streptococcus* spp. since they exhibited the morphological and biochemical properties (Table 2).

**Table 2.** *Streptococcus* spp. isolates with their organ

Isolates	Host (fish name) of the isolates	Organ of isolation
TE10	Tilapia ( <i>O. niloticus</i> )	Eye
TB11		Brain
TS12		Spleen
TK13		Kidney
TL14		Liver

Bacteria of *Streptococcus* spp. were isolated from diseased Tilapia that isolated bacteria showed grams positive coccus. Biochemical tests such as oxidative, catalase and indole were showed negative results whereas oxidative/fermentative was showed fermentative positive. Isolates *Streptococcus* sp<sub>1</sub> and *Streptococcus* sp<sub>2</sub> were showed  $\gamma$ -haemolysis but *Streptococcus* sp<sub>3</sub> was showed  $\alpha$ -haemolysis in blood agar.



## Development of aquaponic techniques in Bangladesh

**Researchers:** Dr. Jubaida Nasreen Akhter, SSO  
Md. Rayhan Hossain, SO

### Objectives

- To optimize stocking density of fish and plant in aquaponic system.
- To estimate nutrient level and electrical conductivity in hydroponics system.

### Achievement

During July 2014 to Jun 2015 few experiments were conducted in Aquaponic Garden at BFRI campus to optimise stocking density of Genetically Improved Farmed Tilapia (GIFT) and *Anabas testudineus* with vegetable in aquaponic system. Fishes were reared in fiberglass tanks with supplementary feed under a shade system. Stocking density of GIFT was maintained at 30, 60 and 90 m<sup>-3</sup> in different treatments and stocking density of *Anabas* sp. was maintained at 90 and 120 m<sup>-3</sup>. Stocked fishes were fed commercial floating feed at the rate of 3% body weight four times daily. Culture period is 60 days from June to July for batch 1 (Table 1), 120 days from September to December for batch 2 (Table 2) and from January to April for batch 3 (Table 3). Under these experiments vegetable or salad were produced simultaneously.

**Table 1.** Growth and production performance of GIFT

Treatment	T <sub>1</sub> R <sub>1</sub>	T <sub>1</sub> R <sub>2</sub>	T <sub>2</sub> R <sub>1</sub>	T <sub>2</sub> R <sub>2</sub>	T <sub>3</sub> R <sub>1</sub>	T <sub>3</sub> R <sub>2</sub>
Stocking density (m <sup>-3</sup> )	30	30	30	30	30	30
Initial wt. (g)	90	90	50	50	160	160
Av. Final wt.(g)	143	123	86	93	218	213
Total production (kg)	3.9	3.2	4.4	5.1	5.6	5.7
Yield kg m <sup>-3</sup>	3.9	3.2	3.0	3.3	5.6	5.7
Wt. gain per day (g)	0.89	0.60	0.61	0.72	0.98	0.89

**Table 2.** Growth and production performance of GIFT

Treatment	T <sub>1</sub> R <sub>1</sub>	T <sub>1</sub> R <sub>2</sub>	T <sub>2</sub> R <sub>1</sub>	T <sub>2</sub> R <sub>2</sub>
Stocking density (m <sup>-3</sup> )	90	90	60	60
Initial wt. (g)	30	30	30	30
Av. Final wt.(g)	68	67	84	85
Total production (kg)	5.4	5.5	4.7	4.6
Yield kg m <sup>-3</sup>	5.4	5.5	4.7	4.6
Wt. gain per day (g)	0.31	0.30	0.45	0.45
Survival (%)	90	91	93	92

**Table 3.** Growth and production performance of *Anabas* sp.

Treatment	T <sub>3</sub> R <sub>1</sub>	T <sub>3</sub> R <sub>2</sub>	T <sub>4</sub> R <sub>1</sub>	T <sub>4</sub> R <sub>2</sub>
Stocking density (m <sup>-3</sup> )	90	90	120	120
Initial wt. (g)	10	10	10	10
Av. Final wt. (g)	52	51	48	46
Total production (kg)	4.9	4.8	9.3	8.9
Yield kg m <sup>-3</sup>	4.9	4.8	5.9	5.7
Wt. gain per day (g)	0.35	0.34	0.31	0.30
Survival (%)	95	95	92	91

## Natural propagation of freshwater mussel in Bangladesh

**Researchers :** Arun Chandra Barman, SO  
 Dr. Mohosena Begum Tanu, SSO  
 Mohammad Ferdous Siddique, SO  
 Md. Abdus Salam, SO  
 Md. Sydur Rahman, SO

### Objectives

- To identify male and female brood of pearl producing mussels for natural propagation.
- To know the Ganado Somatic Index (GSI) of freshwater mussels.
- To know the reproductive behavior of freshwater mussels.

### Achievement

Twenty specimens per month of an adult population of mussel were collected. Before dissection, shell length and height were measured. Shell and tissue were blotted dry, and total and wet weight without the shell was measured with a balance. Average length, height and weight of the sampled mussels were 84 mm, 42 mm and 14 g respectively. Mantle, adductor muscles, gills, labial palps, and siphons were removed, keeping only the visceral mass (gonad, liver, and gastrointestinal tract) and the foot. These tissues were fixed in buffered Bounn's fixatine and processed using histological techniques (Humason 1979). Paraffin sections 7 to 9 µm thick were stained with hematoxylin and eosin.

**Indifferent stage:** This stage is characterized by a total absent of gametes; therefore, it is not possible to distinguish the sex. The connective tissue occupies almost all of the space. In the month of May 22.2% mussels were found in this stage whereas in June percentage was 11.0%.

**Ripe stage:** In the female, most oocytes were free within the follicles, but some oocytes remained attached to the follicle wall. In the male, follicles filled by spermatozoa arranged in characteristic bands. In the month of May 44.4% mussels were found in this stage whereas in June percentage was nil.

**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. Large spaces inside the follicles occurred. In some follicles, only a few residual spermatozoa were present. In the month of May 22.2% mussels were found in this stage whereas in June percentage was 77.8%.

**Spent:** At this stage, some unspawned oocytes and spertoza were observed within follicles. The gametes were being phagocyted by amebocytes. The diameter of at least 100 oocytes, in each of six randomly selected females, was measured with an eyepiece graticule calibrated with a stage micrometer. The measurements were made along the longest axis of the oocyte size was obtained. Individuals with few measurable oocytes and extensive phagocytosis were not considered, following the criteria of Grant and Tyler (1983). In the month of May 22.2% mussels were found in this stage whereas in June percentage was 11.2%. Result of Histological study was given in the Table 1.

**Table 1. Histological study**

Mussel status	Ripe	Spawning	Spent	Indifferent
May	44.4%	22.2%	11.1%	22.2%
June	0.0%	77.8%	11.2%	11.1%

## Refinement of freshwater pearl culture techniques in Bangladesh

**Researchers:** Dr. Mohosena Begum Tanu, SSO  
Arun Chandra Barman, SO  
Sonia Sku, SO  
Nur-A-Raushon, SO

### Objectives

- Determination of suitable culture techniques for maximum pearl production
- Dissemination of technology through on-farm trial and training.
- Refinement of image pearl culture technology

### Achievement

#### *Expt. 1. Optimization of number of tissue slice for maximizing pearl production in different mussel species ( Lamellidens marginalis, Lamellidens corrianus) against net bag hanging method*

Different number of mantle tissue (2, 6, 8, and 10) was inserted and pearl formation was investigated. Operation includes two steps, mantle tissue slice making and transplantation. For slice making, mussel of healthy and strong condition was selected. Mussel was opened and mantle tissue was then separated along pallial line from the mussel. Separated tissue strip was then transferred into a glass board and cut into small splices of 2mmx2mm size. For the mantle tissue transplantation mussels of 1 year age, healthy and strong with broad and distinct growth line and without disease and injury was selected. A piece of mantle was taken with needle in one hand and a wound was created in the mantle tissue of mussels along the horizontal direction with a hook in another hand. At this point tissue slice was transplanted into the bottom of the wound. Similarly the next one was transplanted following the direction from posterior side to center.

The operated mussel having 6, 8, 10 pieces of inserted mantle tissue slice was cultured for 1 years in Net-bag hanging method in 3-4 feet water level of the pond. Stocking density of mussels and fish was 80 mussels/decimal and 30 fish/decimal (catla 6, rui 10, mrigal 10, kalibaush 4) respectively. Research pond was splitted by bana. Organic and inorganic fertilizer was given fortnightly to the pond@3kg cowdung 100gT.S.P and 100g urea per decimal. Liming was done fortnightly @ 500 g dolomite/decimal. The design of the experiment and the result was given in the Tables 1 and 2 respectively:

**Table 1.** Design of the experiment

Culture method	No. of tissue slice	Sp. of mussel to be used for transplantation
Hanging in net bag	6,8,10	<i>Lamellidens marginalis</i> , <i>Lamelliden corrianus</i>

**Table 2.** Pearl productions against mantle tissue slice

No of mantle tissue inserted	No of mussel	Survival rate	Average pearl producing rate/mussel
6	200	71	5 pearl/ mussel
8	200	65	6 pearl/mussel
10	200	68	6 pearl/mussel

**Expt. 2. Optimization of culture techniques for maximizing pearl production in different mussel species (*Lamellidens marginalis*, *Lamellidens corrianus*) against a desirable number of tissue slice**

The operated mussel having 6 pieces of inserted mantle tissue slice was cultured for 1 years in different culture techniques such as. Net-bag hanging method and Grazing method in 3-4 feet water level of the pond. Operation procedure was as same as discussed in experiment-1. Stocking density of mussels (80 mussels/decimal) and fish (30fish/decimal; catla 6, rui 10, mrigal 10, kalibaush 4) was same. Research pond was splitted by bana. Organic and inorganic fertilizer was given fortnightly to the pond@3kg cowdung 100gT.S.P and 100g urea per decimal. Liming was done fortnightly @ 500 g dolomite/decimal. Survival rate of the operated mussel was monitored once in a month. The design of the experiment and the result was given in the Tables 3 and 4 respectively:

**Table 3.** Design of the experiment

Culture method	No. of tissue slice	Sp. of mussel to be used for transplantation
Hanging in net bag	6	<i>Lamellidens marginalis</i> , <i>L. corrianus</i>
Stocking in cage	6	<i>Lamellidens marginalis</i> , <i>L. corrianus</i>
Stocking in open pond (grazing)	6	<i>Lamellidens marginalis</i> , <i>L. corrianus</i>

**Table 4.** Pearl production against culture technique

Name of culture technique	No of mussel	Survival rate (%)	Pearl producing rate (%)	Comments
Net bag hanging	800	80	60	Experiment is going on
Grazing	800	55	57	Experiment is going on

**Expt. 3. Optimization of size of image against pearl culture methods**

Different size and shapes of images will be inserted into the mussel and was cultured in Net-bag hanging method and Grazing method in 3-4 feet water level of pond. Stocking density of mussels (80 mussels/decimal) and fish (30fish/decimal; catla 6, rui 10, mrigal 10, kalibaush 4) was same. Research pond was separated by bana. Liming was done fortnightly @ 500 g dolomite/decimal. The design of the experiment and the result was given in the table 5 and 6 respectively:

**Table 5.** Design of the experiment

Name of culture technique	Length of mussel (cm)	Size of Image	Sp. of mussel to be used for transplantation
Net bag hanging	9-12	3.0 X 1.5 cm <sup>2</sup> 2.5 X 1.50 cm <sup>2</sup> 2.0 X 1.5 cm <sup>2</sup>	<i>Lamellidens marginalis</i> , <i>L. corrianus</i>
Grazing	9-12	3.0 X 1.5 cm <sup>2</sup> 2.5 X 1.5.0 cm <sup>2</sup> 2.0 X 1.5 cm <sup>2</sup>	<i>Lamellidens marginalis</i> , <i>L. corrianus</i>

**Table 6.** Result of Image pearl production

Name of culture technique	No of mussel	Length of mussel	Length of image	Width of image	Survival rate (%)	Mussel containing image
Net bag hanging	328	9 -12 cm	3 cm	1.5 cm	75%	246
Grazing	124	9 -12 cm	3 cm	1.5 cm	50%	62

## Investigation on the access route of toxic drugs and chemicals in fish

**Researchers:** Dr. Md. Khalilur Rahman, CSO  
Selina Yeasmine, SO  
Sonia Sku, SO

### Objectives

- To find out the residues and accumulation level of aquadugs and chemicals in fish, plankton and benthos.
- To categorize the listed drugs and chemicals on the basis of legal approval, registration and beneficial effect.
- To find out the routes and means that the drugs/chemicals gain access into the country and in aquaculture practices.

### Achievements

#### *Determination of residues and accumulation level of aquadugs and chemicals in fish, plankton and benthos*

An experiment was conducted in the pond complex of the Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh. Three laboratory tests with 02 replications in 06 aquarium were also conducted. Eight experimental ponds having an area of one decimal each were used to assess the residues and accumulation level of aquadugs and chemicals in fish, plankton and benthos. The ponds were prepared through sun-drying followed by liming at the rate of 1kg/dec. After five days of drying, the ponds were filled up with underground water from a deep tube well and fertilized with urea at the rate of 100g/dec and TSP at the rate of 50g/dec. After fertilization the pond was left for 7 days to grow plankton in water, which is suitable for fish stocking. Seven days after fertilization, fingerlings of Carps (*Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*) and Pangas (*Pangasius hypophthalmus*) were stocked at the rate of 100 and 120/dec, respectively. In all the ponds, pelleted feed containing 30-35% crude protein was supplied at the rate of 4% of estimated fish biomass twice daily. To assess the accumulation level in fishes different types of aquadugs (Sumethion, Malathion, Rise and Tetracycline) were applied in each pond and sample of fish plankton and benthos were collected. Collected sample were send to the Bangladesh Agricultural Research Institute (BARI) for analysis. Doses of Aquadugs/Chemicals used in experimental ponds and aquarium were different which are shown in Table 1.

**Table 1.** Details of experimental design in ponds and aquarium

Pond trials	Doses	Collection of samples after application of drugs/ antibiotics	
		Water sample	Fish sample
Sumithion	5, 10, 15 & 20 ml/dec	After 1 days from every dose	After 3 days from every dose
Cypermethrin	2, 3, 5 ml/dec	After 1 days	After 3 days from every dose
Tetracycline group (Oxytetracycline & Chlorotetracycline)	1, 3, 5 g/kg fish feed	-	After 14 days from every dose
Malathion	5, 10, 15 & 20 ml/dec	After 1 days from every dose	After 3 days from every dose
<b>Aquarium trials</b>			
Aluminum phosphide 56% (Phostoxin)	2, 3 and 4 tablet/dec/feet	-	After death of all fish

Fish are very sensitive to any kind of toxic materials in the water. The concentrations of Sumethion (Fenitrothion) viz., 05, 10, 15 and 20 ml/dec were applied in carps ponds and samples of carps were tested after 3 days interval. The effects of different concentrations and exposure time of Sumethion (Fenitrothion) on the carps species are presented in Table 2. Sumethion (Fenitrothion) level of residue (ppm) were found in carps were 0.237, 0.261, 0.298 and 0.369 ppm which are higher than the safety level (0.02 mg/kg body wt) recommended by FAO. Among the different doses of Sumethion (Fenitrothion) 20 ml/dec dose showed higher rate accumulation level of residue in fish body. All of fish were found alive in every treatment. On the other hand, there were not found/detected any residue of Sumethion (Fenitrothion) in water. The concentrations of Sumethion (Fenitrothion) viz., 05, 10, 15 and 20 ml/dec were applied in Pangas ponds and samples of pangas were analyzed after 3 days interval. The effects of different concentrations and exposure time of Sumethion (Fenitrothion) in the pangas ponds are presented in Table 3. Sumethion (Fenitrothion) level of residue (ppm) were found in pangas were 0.629, 0.733, 0.664 and 0.805 ppm which are higher than the safety level (0.02 mg/kg body wt) by FAO. Here also 20 ml/dec dose of Sumethion (Fenitrothion) showed higher rate of accumulation level of residue in fish body. All of fish were found alive in every treatment. On the other hand, there were not found/detected any residue in water.

During the treatment period, the behavioural changes and responses of fish were observed to different concentrations of the Sumethion (Fenitrothion). Several abnormal behaviours such as restlessness, sudden quick and continuing movement, swimming to the backside were observed and looking less shiny colour after application of Sumethion (Fenitrothion). Finally fish became very weak, settled at the bottom with the increase of pesticide concentration. Results on residues and accumulation levels of Sumithion in fish and water have been received are presented in Table 2 and 3 respectively.

**Table 2.** Quantity of residue of sumethion estimated from water and carps

Water & fish species	No of samples tested	Detected pesticide	Doses used (ml/dec)	Samples duration in ponds (day)	Level of residue (ppm)	MLR set by FAO	Remarks
Water T <sub>1</sub> (5 ml)	3	Sumethion (Fenitrothion)	5	1	Not detected	0.02 mg/kg body wt.	Not detected
Water T <sub>2</sub> (10 ml)	3		10	1			
Water T <sub>3</sub> (10 ml)	3		15	1			
Water T <sub>4</sub> (10 ml)	3		20	1			
Carps T <sub>1</sub>	3		5	3	0.237		Higher than the safety level
Carps T <sub>2</sub>	3		10	3	0.261		
Carps T <sub>3</sub>	3		15	3	0.298		
Carps T <sub>4</sub>	3		20	3	0.369		

**Table 3.** Quantity of residue of sumethion estimated from water and catfish

Water & fish species (Sample code)	No of samples tested	Detected pesticide	Doses used (ml/dec)	Samples duration in ponds (day)	Level of residue (ppm)	MLR set by FAO	Remarks
Water T <sub>1</sub> (5 ml)	3	Sumethion (Fenitrothion)	5	1	Not detected	0.02 mg/kg body wt.	Not detected
Water T <sub>2</sub> (10 ml)	3		10	1			
Water T <sub>3</sub> (15 ml)	3		15	1			
Water T <sub>4</sub> (20 ml)	3		20	1			
Pangas T <sub>1</sub>	3		5	3	0.629		Higher than the safety level
Pangas T <sub>2</sub>	3		10	3	0.733		
Pangas T <sub>3</sub>	3		15	3	0.664		
Pangas T <sub>4</sub>	3		20	3	0.805		

## Study on food, feeding habit and breeding biology of commercially important cuchia species, *Monopterus cuchia*

**Researchers:** Dr. David Rintu Das, SSO  
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Mst. Sonia Sharmin, SO

### Objectives

- To develop breeding and nursing techniques of *M. cuchia*.
- To develop grow-out culture technique of *M. cuchia* in cisterns.
- To study availability, marketing channel, export and culture potentiality of *M. cuchia* in study areas.

### Achievement

The study on breeding biology and fry rearing of commercially important cuchia species, *Monopterus cuchia* was conducted during July 2014 to June 2015. Brood cuchia (Female Av. Wt. 450 g & Male 240 g) were stocked after hormone treatment with various doses (5 & 2 mg cPG, 10 & 4 mg cPG, 15 & 6 mg cPG and 10 mg cPG+ 200 IU HCG & 5 mg cPG/Kg BW) for artificial breeding in ponds and cemented cisterns. The stocked cuchia were fed with fry of fishes like *Channa punctatus*, *Cyprinus carpio* and *Lepidocephalichthys berdmorei* and earthworm twice daily @ 5% of body weight. Water hyacinths were provided in the pond and cisterns to create suitable and safe shelter. The female received single doses of 10 mg cPG/Kg BW+ 200 IU HCG /kg BW and the male received 5 mg cPG/Kg BW showed positive results and collected more than 3000 cuchia fries. After 27 days of injection, eggs were found at the nest/hole in cistern and pond. After 30 days of injection, larvae were found with yolk-sac absorbing conditions in the nest. It was observed that parent cares around the time of hatching in the nest and then declines gradually as the fries become increasingly independent. When a female cuchia feel annoyed at the nest then female cuchia pickup its larvae in its mouth as well as changing their position. If favorable breeding condition can be ensure about 300-500 fries from a single nest. When the fries are able to take food by itself, then they come out from their nest and take shelter in the root of aquatic plants or hiding under mud. It has been found that fry rearing with 75 fries/m<sup>2</sup> stocking density showed best result in respect of growth ( $5.889 \pm 0.248g$ ) and survival rate (94%) among the treatments (T<sub>2</sub>- 100 and T<sub>3</sub>- 125 fries/m<sup>2</sup>). For grow-out culture of *M. cuchia* in cistern condition, it was found that, 5 juveniles/m<sup>2</sup> of stocking density fed with of earthworm slice gave better growth and survival. From the survey of cuchia it was found that freshwater eels were harvested by hands and hook with different feeds from different natural water bodies. October to December is the peak season for harvesting. It was also found that about 5.15 and 7.00 mt. cuchia were exported monthly from Rangpur and Dinajpur district respectively. The market chain from collector to consumers passes through a number of intermediaries like collectors, local bapari/sellers, arottders, and exporters. Higher transport cost, lack of transport facilities, lack of suitable packaging materials, and high mortality due to stress and exploitation by middlemen are the constraints of eel marketing as a result of low market prices.

## Riverine Station & Sub-station

### **Impact of environmental factors on abundance and distribution of important fishes in the River Meghna (Shatnol-Chor Alexander)**

**Researchers:** Md. Robiul Awal Hossain, SSO  
Md. Mehedi Hasan Pramanik, SO  
Md. Istiaque Haider, SO

#### **Objectives**

- To study the environmental factors in different season;
- To study the abundance and distribution of important riverine fishes in different season; and
- To find out the correlation between environmental factors and abundance and distribution of fishes

#### **Achievement**

**Physico-chemical parameters:** Temperature (Air and water), transparency, dissolved oxygen (DO), free carbon dioxide (CO<sub>2</sub>), total hardness, total alkalinity were determined following APHA (1995). Ammonia and nitrite were estimated using a HACH water test kit (model-FF-2). Conductivity and pH meter were used to determined water pH and specific conductance respectively. Three physical and seven chemical parameters were studied for river spots of Meghna and results are shown in Tables 1 & 2.

**Table 1.** Ranges of physical parameters of water quality in the different sampling points of the river Meghna from July 2014 to June 2015

Sampling stations	Parameters		
	Air temperature	Water temp.	Transparency
Madrasha Ghat (MG)	14.5-32	14-28	22-60
Ananda Bazar (AB)	14.6-32.5	16-28	22-50
Ekhlaspur (Ep)	15-31.5	18-29.5	25-65
Shatnal (Sn)	16-32	21-29	30-60
Harina Ghat (HG)	16-30	19-24.5	41-67
Haim Chor (HC)	17-32	18-29	30-52
Chor Voirabi (CV)	16.5-31.5	16.1-29.5	32-55
Ishanbala (Ib)	20.3-30	18.5-28.5	20-41
Char Jalalpur (CJ)	21-32	19.5-30.5	21-40
Hizla (Hz)	22.5-30	21.1-28	22-40
Kaliganj (Kg)	22-30	20-31	18-31
Alexandar (Axr)	18.5-32	19.5-30.5	7-12
Chor Ludhua (CL)	22.1-32	18-30.5	10-24

**Table 2.** Ranges of chemical parameters of water quality in the different sampling points of the river Meghna from July 2014 to June 2015

Samp. Stns.	Parameters						
	DO (mg/l)	Free CO <sub>2</sub> (mg/l)	pH	Total Hardness	Alkalinity (mg/l)	Conductivity (μS/cm)	Ammonia (NH <sub>3</sub> mg/l)
MG	5.2-5.8	6.5-8.6	7.25-8	58-70	30-55	150-282	0.0-0.03
AB	5.6-6.4	10.6-15.5	7.5-8.5	48-90	50-60	160-280	0.0
Ep	5.5-6.2	8.5-12	7-7.75	39-70	30-54	142-266	0.0



Sn	4.6-6	6.5-12.0	7.25-7.75	38-70	32-55	140-270	0.0
HG	5.7-6.8	5.6-8.4	7.25-8.5	85-92	40-48	258-282	0.04-0.05
HC	5-5.8	8.5-11.5	7.5-8.25	110-182	109-122	220-275	0.0
CV	4.8-5.9	10-15.4	7.25-8.5	100-125	62-128	210-266	0.0
Ib	5.5-6.5	8.2-12.5	7-8	92-120	44-86	221-280	0.0
CJ	4.8-6.8	10-15.2	7.75-8.5	89-118	34-76	225-285	0.0
Hz	5.5-7	8.6-15	7.5-8.5	46-98	30-70	168-200	0.0
Kg	4.4-6.2	12-15.5	7.25-8.5	86-106	40-75	175-280	0.0
Axr	4.2-6.8	12.5-14.5	7.75-8.25	410-811	97-141	381->1000	0.0
CL	4.6-6.5	10.5-12	7.5-8.25	110-407	74-130	394->1000	0.0

Air and water temperature was ranged from 14.5 to 32.00°C and 14 to 31°C in different sampling points. Transparency also varied among the study sites in a wide range which was highest 45-72cm in Harina Ghat (HG) of Chandpur and lowest 5-12cm in Chor Alexander of Laxmipur. All the chemical parameters of river water in different sampling points were in suitable ranges for fishes.

**Plankton sample collection:** 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 50 $\mu$ . 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) and results are shown in Table 3.

**Table 3.** Mean Quantitative values of plankton with dominating genera in different points of the river Meghna from July 2014 to June 2015

Place	Phyto. (No./L)	Domi. P. (No/L)	Zoo. (No./L)	Domi. Z. (No/L)
MG	146 $\times$ 10 <sup>2</sup>	Ulothrix (91 $\times$ 10 <sup>2</sup> )	33 $\times$ 10 <sup>2</sup>	Brachionus (25 $\times$ 10 <sup>2</sup> )
AB	59 $\times$ 10 <sup>2</sup>	Ulothrix (19 $\times$ 10 <sup>2</sup> )	12 $\times$ 10 <sup>2</sup>	Brachionus (10 $\times$ 10 <sup>2</sup> )
Ep	256 $\times$ 10 <sup>2</sup>	Melosera (105 $\times$ 10 <sup>2</sup> )	4 $\times$ 10 <sup>2</sup>	Nauplius (3 $\times$ 10 <sup>2</sup> )
Sn	29 $\times$ 10 <sup>2</sup>	Ulothrix (17 $\times$ 10 <sup>2</sup> )	8 $\times$ 10 <sup>2</sup>	Brachionus (5 $\times$ 10 <sup>2</sup> )
HG	410 $\times$ 10 <sup>2</sup>	Ulothrix (183 $\times$ 10 <sup>2</sup> )	26 $\times$ 10 <sup>2</sup>	Brachionus (19 $\times$ 10 <sup>2</sup> )
HC	267 $\times$ 10 <sup>2</sup>	Ulothrix (193 $\times$ 10 <sup>2</sup> )	18 $\times$ 10 <sup>2</sup>	Brachionus (14 $\times$ 10 <sup>2</sup> )
CV	305 $\times$ 10 <sup>2</sup>	Ulothrix (235 $\times$ 10 <sup>2</sup> )	13 $\times$ 10 <sup>2</sup>	Brachionus (9 $\times$ 10 <sup>2</sup> )
Ib	293 $\times$ 10 <sup>2</sup>	Ulothrix (185 $\times$ 10 <sup>2</sup> )	30 $\times$ 10 <sup>2</sup>	Brachionus (22 $\times$ 10 <sup>2</sup> )
CJ	239 $\times$ 10 <sup>2</sup>	Ulothrix (98 $\times$ 10 <sup>2</sup> )	35 $\times$ 10 <sup>2</sup>	Brachionus (20 $\times$ 10 <sup>2</sup> )
Hz	443 $\times$ 10 <sup>2</sup>	Ulothrix (305 $\times$ 10 <sup>2</sup> )	27 $\times$ 10 <sup>2</sup>	Brachionus (20 $\times$ 10 <sup>2</sup> )
Kg	389 $\times$ 10 <sup>2</sup>	Ulothrix (278 $\times$ 10 <sup>2</sup> )	26 $\times$ 10 <sup>2</sup>	Brachionus (17 $\times$ 10 <sup>2</sup> )
Axr	196 $\times$ 10 <sup>2</sup>	Ulothrix (112 $\times$ 10 <sup>2</sup> )	6 $\times$ 10 <sup>2</sup>	Nauplius (3 $\times$ 10 <sup>2</sup> )
CL	230 $\times$ 10 <sup>2</sup>	Ulothrix (169 $\times$ 10 <sup>2</sup> )	11 $\times$ 10 <sup>2</sup>	Brachionus (8 $\times$ 10 <sup>2</sup> )

The dominating phytoplankton was *Ulothrix* sp. in all the sampling points ranged from lowest 17  $\times$  10<sup>2</sup> No./L in Shatnol of Chandpur to highest 305  $\times$  10<sup>2</sup> No./L in Hizla of Barisal except *Melosera* sp. (105  $\times$  10<sup>2</sup>) in Eklaspur point of Chandpur. *Brachionus* sp. also was shown as the dominating zooplankton in maximum sampling points ranged from lowest 5  $\times$  10<sup>2</sup> No./L in Shatnol to highest 27  $\times$  10<sup>2</sup> No./L in Madrasha Ghat point except Nauplius in Eklaspur (3  $\times$  10<sup>2</sup> No./L) of Chandpur and Chor Alexander (3  $\times$  10<sup>2</sup> No./L) of Laxmipur.

## Biomonitoring of the rivers Padma, Meghna and Dakatia

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### Objectives

- Physical, chemical and biological assessment of the riverine ecosystem of Padma, Meghna and Dakatia
- Assessment of heavy metal accumulated in soil, water and fishes

### Achievement

Padma, Meghna and their branches and tributaries are intricately associated with the existence of Bangladesh. Fish and fishery resources of these rivers are the main source of livelihood for a number of peoples of the country. The aquatic ecosystem of these rivers is still better than the many rivers of upstream, but situation is gradually declining as the surface runoff coming from the upstream rivers are mixing with this and imposing threats for its longer wellness.

A comprehensive survey was conducted before initiating the main project work and now two years research work has been completed. This study comprises eight sampling points of Padma, Meghna and Dakatia (Table 1). The purpose of this survey was to identify the spots of the river most prone to pollution. Physical, chemical and biological parameters of the selected river spots were studied to understand the status of pollution and deviation from the normal range.

**Table 1.** Sampling locations of Padma, Meghna and Dakatia

Name of the river	Name of spots
Padma	Mowa (Monshigonj), Godagari (Rajshahi), Pakshi (Kustia)
Meghna	Chandpur, Meghnaghat (Narayangonj), Bhoirob (Kishoregonj)
Dakatia	Hazigonj (Chandpur), Puranbazar (Chandpur)

**Physico-chemical parameters:** Ten physical and nine chemical parameters were studied for all three river spots of Padma, Meghna and Dakatia. The range of the values showed seasonal fluctuation and the deviations among the spots also. Water depth was the most deviated physical parameter among the study sites ranged from 8.53±2.40 feet (Hajiganj) to 58.03±35.77 feet (Meghna ghat) . Transperancy also varied among the study sites in a wide range which was highest in Hajiganj 176.33±40.80 cm and lowest in Godagari 20.0±21.35 cm some of the parameters deviated from the normal range indicating the gradual fading of the freshness of rivers. The presence of toxic ammonia and lower amount of dissolve oxygen in some spots gives a glimpse of this idea. However, the situation of these rivers are still better than the Buriganga, Sitalakhya and Balu as the values found in the present study are more suitable than the previous study conducted on the aforesaid rivers.

**Occurrence of benthos in Padma, Meghna and Dakatia rivers:** Among the benthic composition Dakatia was apparently enriched than the Padma and Meghna. In Padma two genera of benthos under two families were observed dominated by the lepidopteridae and chironomidae family respectively. In Meghna about thirteen genera of benthos under six families were found and here bulimidae was the

dominating family immediately followed by the pleuroceridae and lepidopteridae. About twenty two genera of benthos under nine families were found available in three spots of Dakatia river where bulimidae was the dominating family immediately followed by the viviparidae and pleuroceridae. The presence of macro invertebrates indicates the pollution status of the river. From this context it could be assumed that Dakatia is comparatively polluted than Meghna and Padma. Padma is safer than the rest two rivers. The number of effluent releasing mills, factories and industries on the bank of Padma is lesser than Meghna and Dakatia, this was found during the preliminary survey of project. Hence, the present study also confesses with the above statement.

**Occurrence of plankton in Padma, Meghna and Dakatia:** Abundance of plankton in three river systems showed a wide range of variation. More than 40 genera of plankton were identified under 4 families. Among them bacillariophyceae was dominating family in Padma and chlorophyceae was dominating in Meghna and Dakatia respectively. On the contrary, about 12 genera of zooplankton were found under 4 families in Padma, Meghna and Dakatia while rotifer was the dominating family in all three river systems. Average total plankton density (Nos./l) of Padma was  $(5800 \pm 5,483.43)$  higher than the Meghna and Dakatia. In Meghna average total plankton density (Nos./l) was  $(2000 \pm 1116.54)$  and in Dakatia it was  $(5775.0 \pm 8688.06)$ . Phytoplankton largely dominated over zooplankton throughout the study period. The mean contribution of phytoplankton was more than 96% in all three rivers and zooplankton contributed the rest.

**Occurrence of heavy metal in Padma, Meghna and Dakatia:** Among the heavy metals (Pb, Cd, Cr, Cu, Fe, Mn, Zn) the most dominant metal was Fe in both water and sediment followed by Mn. Concentration of all the heavy metals in the sediments and water of rivers were in acceptable limit. Among the heavy metals (Pb, Cd, Cr, Cu, Fe, Mn, Zn) the most dominant metal was Fe in both water and sediment followed by Mn. Concentration of all the heavy metals in the sediments and water of rivers were in acceptable limit. In case of fish concentration of Fe was the highest followed by Zn.

## **Refinement of cage culture technology of monosex tilapia in the River Dakatia, Chandpur**

**Researchers:** Tayfa Ahmed, SO  
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AKM Shafiqul Alam Rubel, SO

### **Objectives**

- To improve the suitable stocking density of monosex tilapia for cage culture
- To determine the effect of current velocity on growth of monosex tilapia in cage culture
- To determine the water quality suitable for cage culture
- To find out the effect of immunizing agent incorporated with supplementary feed on monosex tilapia in cage culture

### **Achievement**

#### ***Growth performance and Disease Infestation of Monosex tilapia in cage culture (3x3x2m) of Dakatia river, Chandpur***

According to the project proposal the refinement of stocking density, stocking size, feeding regime and culture period of cage culture has been done. Now a days out breaking of unknown disease is a problem

for cage culture. For that reason we have conducted an experimental cage culture of 9 cages (3x3x2m) in Dakatia river, Chandpur to know the probable causative agent of the disease. The experiment was conducted for 120 days in Dakatia river, Chandpur with monosex tilapia fingerlings from 15 April 2011 to 12 August 2011. Average 32.26 gm size fingerlings were stocking at the @ 50/m<sup>3</sup> for all cages. Feeding has been administered with pelleted semi-buoyant feed @ 5% of body weight twice, daily. After 120 days, the fishes of all cages attained an average weight of 196.92 gm indicating the moderately satisfactory growth due to poor quality seed. During the culture period of cage culture no disease were observed in the cages of Dakatia river, Chandpur. Survival rate, specific growth rate (SGR), FCR and production of all treatments are shown in Table 1.

**Table 1.** Growth performance and disease infestation of monosex tilapia in cage culture (3x3x2m) of Dakatia river, Chandpur

Parameters	Cage (1-3)	Cage (4-6)	Cage (7-9)	Remarks
Average weight (g) at stocking	32.26	32.26	32.26	
Average weight (g) at harvest	196.58	194.38	199.80	No disease
SGR (%/day)	1.66	1.70	1.70	occurred in the
FCR	2.78	2.81	2.95	culture period
Survival (%)	96.07	96.29	96.63	
Production (kg)	509.91	502.38	520.59	

***Growth performance of probiotics treated nursery reared Monosex tilapia in net cages of Dakatia river, Chandpur***

An experiment was conducted for 120 days in Dakatia river, Chandpur with monosex tilapia fingerlings. The stocking density was 50/m<sup>3</sup> for all treatments. Average weight of 27.77gm no probiotics treated monosex tilapia fingerlings were stocked in T<sub>1</sub> (control). Average weight of 30.33gm and 28.98gm fingerlings with 0.05gm/decimal and 0.08gm/decimal probiotics treated nursery reared fingerlings were stocked in T<sub>2</sub> & T<sub>3</sub> respectively. The probiotics contain 10 types of useful micro-organism at the counted number of 0.1×10<sup>11</sup> cfu/kg. Feeding has been done with pelleted semi-buoyant feed at 5-3% body weight concurrently twice daily. Average weight (gm) of final harvest (120 days), SGR (% day), FCR, Survival rate and production of all treatments are shown in Table 2.

**Table 2.** Growth performance of probiotics treated nursery reared monosex tilapia in net cages of Dakatia river, Chandpur

Parameters	Achievements			Remarks
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	
Probiotics/decimal (0.1×10 <sup>11</sup> cfu/kg)	control	0.05gm	0.08gm	No disease
Av. wt. at stocking (g)	27.77	30.33	28.98	occurred in the
Av. wt. (g) at final harvest (120 days)	117.20	126.18	130.61	culture period
SGR (%/day)	1.70	1.54	1.65	
FCR	3.41	2.90	2.85	
Survival (%)	93.33	90.85	91.52	
Production (kg)	295.00	309.52	322.74	

The water quality parameters were monitored fortnightly in inside and outside of experimental cages of Dakatia river, Chandpur by HACH water test kit (model-FF2). The levels of Dissolve Oxygen (mg/l), Free CO<sub>2</sub> (mg/l), pH, Total Alkalinity (mg/l), Total Hardness (mg/l) were found in suitable ranges in the experimental period in Dakatia river. The presence of Ammonia (mg/l) was also nill in the experimental area of Dakatia river. The suitable water quality parameters of Dakatia river indicating the favorable

environment for cage culture in the river Dakatia. The water quality parameters of Dakatia river, Chandpur are more or less similar in all the year round. The data of some selected water quality parameters of inside and outside of experimental cages are shown in Table 3.

**Table 3.** Average values of water quality parameters of cage culture area of Dakatia river

Parameters	Average value	
	Inside of cages	Outside of cages
Dissolve Oxygen (mg/l)	5.93 ± 0.78	5.65 ± 0.71
Free CO <sub>2</sub> (mg/l)	6.62 ± 1.18	6.78 ± 1.53
pH	7.90 ± 0.38	7.84 ± 0.39
Total Alkalinity (mg/l)	94.50 ± 54.56	93.10 ± 56.64
Total Hardness (mg/l)	102.00 ± 48.44	97.77 ± 48.98
Ammonia (mg/l)	0.00	0.00

According to the project proposal another comparative study will be started on seed quality and their growth performance of different monosex tilapia producing hatchery of three different region of Bangladesh. For that reason, monosex tilapia fry were collected for nursery rearing from three different monosex tilapia producing hatchery of Bangladesh. After the completion of nursery period (av. wt. 25-30g size) the monosex tilapia fingerlings will be stocked in the cages of Dakatia river, Chandpur, for the study.

### **Investigation of tilapia (*Oreochromis niloticus*) disease in cage and other fish culture systems and control strategies**

**Researchers:** Dr. Masud Hossain Khan, CSO  
Flura, SO  
Md. Istiaque Haidar, SO  
Aovijite Bosu, SO  
Md. Ashikur Rahman, SO

#### **Objectives**

- To investigate the cage and other fish culture systems and aqua-ecological conditions
- To identify the causative agent(s) associated with disease outbreaks
- To observe the histological changes in different organs of diseased fish
- To Minimize the fish mortality using better management strategies

#### **Achievement**

**Case-control study:** Case –control study was carried out covering Mymensingh, Jessore, Chandpur and Chittagong region in grow out farm and cages of tilapia. Epidemiological parameters were investigated during the sampling period using a pre-tested questionnaire. During the case- control study, 20 case pond and corresponding 20 control pond were investigated in Mymensingh and Jessore region. In Chandpur region, 10 case cages and corresponding 10 control cages were observed.

In case of ponds, epidemiological characteristics such as high stocking density, fingerlings collected from local hatcheries, uses of substandard feed and pond connected to other water bodies were identified as potential risk for disease outbreak. On the other hand, pond drying, removal of bottom waste, use of disinfectants and liming the pond with standard doses were identified as risk reducing factor for disease outbreak.

**Table 2.** Epidemiology of tilapia cage in Chandpur (cage)

Epidemiological characteristics	Affected cages	Unaffected cages	Comments
Cage size	15×8×5 to 20×10×6.5 m <sup>3</sup>	15×8×5 to 20×10×6.5 m <sup>3</sup>	Not considered risk for disease
Number of cages	50-70	50-65	Not a considered risk for disease
Arrangement of cages	Mostly parallel	Mostly parallel some Zig-zag	More water flow and more hygienic in unaffected zig-zag-cages
Distance between cages	3- 7 inches	4-6 inches	No remarkable differences observed between two groups
Quality of fry	Apparantly healthy	Apparantly healthy	Apparantly healthy fry might contain pathogen in dormant condition
Stocking density	1000-1200/cage	800-1000/cage	Low stock density could be safer for tilapia
Affected culture cycle	Both winter and summer cycle	Both winter and summer cycle	Risk both in cold and hot season
Depth of water	10-12 feet	10-15 feet	Lower depth could be risk for disease
Water flow	Poor	Satisfactory	Insufficient water flow might increase risk of disease
Source of fry	Local hatchery	Local hatchery	No difference found
Cleanliness of cages	Clean Irregularly/Clean monthly	Fortnightly /monthly	Might have little risk for disease
Workers assigned to specific cages	no	no	It increases risk of disease
Huge domestic waste	Pass through cages	Little or no access of domestic waste	Might be a potential risk for disease
Apply antibiotics in feed	Sometimes, when infected	Not applied	Not effective against disease

In case of cages, epidemiological characteristics such as stocking density, water flow and cleanliness of cages and entry of huge domestic waste after opening the sluice gate etc were identified as potential risk for the occurrence of disease. On the other hand, net drying, removal of unused feed from cage bottom, use of probiotics with feed and removal of dead fish from the cages were found to play a significant role in reducing risk of disease outbreak.

**Identification of disease producing agent:** During investigation, 50 fish were captured and examined with naked eye. In case disease outbreak, prevalence of disease (%), fish size, clinical signs, mortality pattern, seasonality etc. were recorded. Ten affected fish were carried to the laboratory for further pathological investigation.

**Clinical sign of diseased tilapia:** During investigation, In case of pond, following clinical signs were observed as loss of appetite, spine displacement, darkening of skin and scale loss. In case of cage culture system-spinning, eye protrusion, erratic swimming and hemorrhages at the base of fins and in the opercula.

**Seasonality & Diease Occurrence:** In case of pond, mainly August to December and also May to July. The morbidity and mortality rate varied with season, location, farm design, species, culture system, management practice, etc. In cage culture systems, mainly winter season (Oct- Dec)

**Fish mortality:** Most of the farms in this year at Tarakanda areas, it was found severally outbreak of tilapia disease. Farmers reported that tilapia morbidity and mortality was observed up to 80-90% within 7-20 days from August to October 2014. However, interestingly the mortality was much lower (20-30%) in the remaining areas of Mymensingh. Most of the farmers reported that tilapia morbidity and mortality in cages ranged between 22-30%. Disease mainly occurs in the month of October to December. A few cage operators mentioned that average 8047 (16%) piece of tilapia died due to disease during October in their farm. The highest mortality was found 442 tilapia/day with an average of 270 fish. During the month of November the number of dead fish was 7059 (17%) and highest mortality was found 387/day, with the average of 235 fish/day

**Bacteriology:** In order to isolate and identify potential bacterial causative agent, affected tissues were inoculated onto Tryptone Soya Agar (TSA) and finally isolated as pure culture for diagnosis. Primary diagnostic tests such as Gram staining, Motility test Indole test, O-F test, Catalase test, Oxidase test etc were done in BFRI laboratory which suggested the bacteria as *Streptococcus spp.* For further authentication of the causative agent (pathogen); Spleen, kidney and brain samples from affected fish were preserved in 80% ethanol and sent to MSD Animal Health Laboratory, Singapore. MSD confirmed the pathogen as *Streptococcus agalactiae* using molecular technique (PCR).

### Development of mass seed production technique of *Pangasius pangasius*

**Researchers:** Mrs. Akhery Nima, SO  
Khondoker Rashidul Hasan, SO  
AKM Shafiqul Alam Rubel, SO  
B. M. Shahinur Rahman, SO

#### Objectives

- Optimization of induced breeding technique of *P. pangasius*
- Study of indiscriminate killing of pangas seed in riverine habitat.

#### Achievement

**Brood rearing:** Old broods as well as new broods of *P. pangasius* are being reared under intensive feeding case in different ponds for their gonadal development. Broods are being reared with commercial semi-buoyant feed @ 3-4% of their body weight daily. Periodic checking of health and disease of broods was done as a routine work. Average weight of pangas brood was 2.5-3.0 kg.

**Induced breeding trial:** For attempts have been made to breed *P. pangasius*. The breeding trials on *P. pangasius* were conducted by using selected spawners during June and August. Carp pituitary gland extract (cPGE) was used as inducing agent. A total of 6 pairs of spawners were injected. Total amounts of cPGE were split into two doses. One third of total cPGE was injected at 1<sup>st</sup> injection and two third at 2<sup>nd</sup> injection. During the study period 06 female spawners released eggs easily after the 2<sup>nd</sup> injection. Eggs were fertilized with milt following dry fertilization method. Fertilized eggs did not hatch out after 24 hours at 29-30<sup>0</sup>c due to poor fertilization of eggs, milt quality/no. of viable sperms in the milt, water quality etc.

**Study on indiscriminate killing of pangas:** Indiscriminate killing of immature pangas from the Meghna and other river is a burning issue now a day. Therefore a study was conducted under this project to asses the impact of indiscriminate killing of pangas seed. Data was collected through *In Situ* observation in

monthly on the much availability of pangas fry/fingerlings and indiscriminate killing of pangas by different gear from some pre-selected points of upper and lower Meghna river *Viz.* Chandpur Sadar, Haimchor, Chor Voirabi, Ramgati (Laxmipur), Hatia, Barishal, Monpura (Bhola), Sureswar (Shoriatpur). Survey was conducted to collect data about the peak season of pangas killing, method of fishing, no of fishers involved in pangas fishing etc. Three types of fishing net (current jal, behundi jal, chorghera jal) and two types of fishing trap (pangas chai) & borshi (hookline) are identified. Among these pangas chai are too much harmful to indiscriminate killing of pangas. Average CPUE of pangas chai in peak season (April-June) 45 kg/ haul. Average length and weight of the pangas fry in the month of November-May was 12-23 cm and 28-76 gm, respectively. Highest killing pressure of pangas fry occurred in November and May of lower Meghna and in May of upper Meghna (Table 1).

**Table 1.** Monthly avg. CPUE (kg/haul) of Pangas Chai from different places

Month	Barisal	Monpura	Hatia	Haimchar	Charvoirabi	Sureswar	Chandpur	Ramgati
November	70	91	65	28	30	38	40	42
February	28	27	30	30	33	28	31	33
March	35	40	36	19	24	18	22	14
April	32	36	37	28	38	36	32	26
May	81	101	100	-	48	58	-	38

In view of its growth, increasing demand in the market more study need to be continued for the indiscriminate killing of *P. pangasius* in riverine habitat and its breeding technology in order to produce mass seed of native pangas.

### Present status of limnology and natural breeding ground of carps in Kaptai Lake

**Researchers:** A.K.M. Saiful Islam, SSO  
Kazi Belal Uddin, SO  
S. Sanjib Basak, SO

#### Objectives

- To know the present status of natural breeding ground of carps in Kaptai Lake
- To identify the specific breeding locations through collecting egg/spawn
- To know the physico-chemical and biological parameters of different breeding ground
- To provide scope for management decision of lake ecosystem

#### Achievement

The growth of fish and other aquatic organisms strongly depends on the water quality. Monthly data and mean values of water quality parameters over the study period are presented in Tables 1 and Table 2. The result of the water quality analysis indicated the suitable ranges for fishes in study areas of Kaptai Lake. The water quality parameters remained more or less similar. In the present study we investigated some physical and chemical factors of water from breeding ground of Kaptai Lake. The air and water temperature of experimental areas of Kaptai Lake were found to vary from 23 to 31°C and 22 to 30.5°C, respectively. These water parameters supposed to be suitable for growth of fishes. Dissolved oxygen and free CO<sub>2</sub> in the experimental sites ranged between 5.61 to 7.34 mg/L and 2.13 to 3.99 mg/L, respectively. In this study, dissolved oxygen was found suitable for fish throughout the study period. pH and total



alkalinity of different areas varied from 6.75 to 7.85 and 53.56 to 70 mg/L respectively. Transparency and water depth ranged from 0.95 to 2.85 and 7.1 to 18.4 m respectively during the study period.

**Table 1.** Water quality parameters as obtained from the Kasalong channel during the study period

Parameters	Natural breeding ground of Kaptai Lake								
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Mean±SD
Air temp. (°C)	23	24	25	27	26	28	29	30	26.5±2.45 (23-30)
Water temp. (°C)	22	23	24	25	25	27	29	29	25.5±2.62 (22-29)
DO (mg/l)	6.34	5.93	5.75	5.61	6.01	6.33	6.03	5.8	5.98±0.26 (5.61-6.34)
CO <sub>2</sub> (mg/l)	2.13	2.56	3.94	2.64	2.91	2.84	3.58	3.3	2.99±0.6 (2.13-3.96)
pH	7.42	7.54	7.7	7.5	7.78	7.7	7.8	7.5	7.62±0.14 (7.42-7.80)
Total alkalinity (mg/l)	54.28	57.25	67.2	70	69	66.3	56.08	54.24	62.17±6.53 (54.24-70)
Transparency (m)	2.12	2.11	2.13	2.12	2.18	2	2	1.95	2.08±0.08 (1.95-2.18)
Water depth (m)	15	13.4	10.7	8.6	7.2	12.8	16.3	18.4	12.8±3.82 (7.2-18.4)

**Table 2.** Water quality parameters as obtained from the Barkal channel during the study period

Parameters	Natural breeding ground of Kaptai Lake								
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Mean±SD
Air temp. (°C)	25	26	26	28	29	29	30	31	28±2.14 (25-31)
Water temp. (°C)	24	25	27	27	28	27	29	30	27.13±1.96 (24-30)
DO (mg/l)	7.34	6.93	6.52	6.97	6.12	6.29	6.82	5.68	6.58±0.54 (5.68-7.34)
CO <sub>2</sub> (mg/l)	2.95	2.36	2.66	3.48	3.99	3.66	2.96	2.69	3.09±0.56 (2.36-3.99)
pH	7.35	7.25	6.75	6.95	7.85	6.69	7.55	7.34	7.22±0.4 (6.75-7.85)
Total alkalinity (mg/l)	65.6	57.5	59.64	54.4	60.5	58.7	53.56	58.66	58.57±3.74 (53.56-65.6)
Transparency (m)	1.95	1.98	2.19	2.18	2.85	2.19	1.89	0.95	2.02±0.53 (0.95-2.85)
Water depth (m)	11.4	9.8	8.5	7.9	7.1	12.7	15.6	18.1	11.39±3.89 (7.1-18.1)

## Refinement of creeks aquaculture technology of Kaptai Lake

**Researchers:** A.K.M. Saiful Islam, SSO  
Md. Abul Bashar, SO  
S. Sanjib Basak, SO

### Objectives

- To refine culture technology for sustainable fish production in creeks

- To identify problems regarding fish culture in creeks with their solution

## Achievement

**Preparation of the creeks:** Preparation of the creeks was done by repairing mouth of the creeks (making dam or construction of pen or both). After two weeks, dirty materials were removed from the creeks bottom and lime ( $\text{CaCO}_3$ ) applied at the rate of  $250 \text{ kg ha}^{-1}$  after preparing the creeks. Then all concerned creeks were fertilized with compost (mixture of chopped and sun dried green plants-88%, cow dung-10%, urea-1% and lime-1%)  $1,250.0 \text{ kg ha}^{-1}$ , urea  $37.5 \text{ kg ha}^{-1}$  and TSP  $25.0 \text{ kg ha}^{-1}$ . Then the creeks were left 10 days to promote algal development.

In the study, we found that weight of Catla in  $T_3R_1$  ( $590.8 \pm 30.60\text{g}$ ) was the highest in the experiment. On the other hand, weight of Rui in  $T_1R_2$  ( $490.4 \pm 27.05\text{g}$ ) and weight of Mrigal in  $T_3R_2$  ( $482 \pm 16.54\text{g}$ ) were the highest in 300 days experiment. Highest specific growth rate (SGR) of Catla, Rui and Mrigal were found in  $T_3R_1$  (1.03),  $T_1R_2$  (1.09) and  $T_3R_2$  (1.05) respectively. Survival rate of Rui in  $T_2R_2$  was higher than  $T_1R_2$  and  $T_3R_2$ . In general growth performance of Catla was comparatively higher than Rui and Mrigal. Among the three treatments,  $T_3R_1$  was the best stocking density considering the highest growth of the Catla in the creeks and  $T_1R_2$  was the best stocking density considering the highest growth of the Rui in the creeks. Highest net production was found in  $T_1R_2$  creeks ( $5760 \text{ kg/ha}$ ).  $T_1R_1$  and  $T_1R_2$  creeks yielded  $5555 \text{ kg/ha}$  and  $5760 \text{ kg/ha}$  increased production, respectively with higher economic return over  $T_1C_1$  treatment ( $3381 \text{ kg/ha}$ ) (Table 1).

**Table 1.** Average survival, gross production, net production and production of  $T_1R_1$ ,  $T_1R_2$  and  $T_1C_1$  creeks under polyculture management

Parameters	$T_1R_1$	$T_1R_2$	$T_1C_1$
Survival average (%)	67	68	65
Gross production (kg/ha)	5678	5878	3505
Net production (kg/ha)	5555	5760	3381
Production of all species combined (no./ha)	11597	11692	11284

Physicochemical parameter were studied for a period of one year from July 2014 to June 2015. The highest average air and water temperature ( $26.3 \pm 2.40 \text{ }^\circ\text{C}$  and  $25.1 \pm 2.35 \text{ }^\circ\text{C}$ ) were recored in  $T_3R_1$  and  $T_1C_1$  whereas the lowest average air and water temperature ( $24.5 \pm 2.2 \text{ }^\circ\text{C}$  and  $24.1 \pm 2.34 \text{ }^\circ\text{C}$ ) were recored in  $T_2R_1$  and  $T_1R_2$ . The  $P^H$  of water was found to alkaline in nature ( $6.3 \pm 1.24$  to  $8.5 \pm 0.3$ ) in nine creeks. The maximum average free  $\text{CO}_2$  value ( $8 \pm 1.22 \text{ mg/l}$ ) was recorded in  $T_2R_2$  and minimum ( $5 \pm 0.66 \text{ mg/l}$ ) in  $T_3R_2$ . The value of average total alkalinity was found to fluctute from  $51 \pm 7.44 \text{ mg/l}$  in  $T_3C_3$  to  $63.4 \pm 6.35 \text{ mg/l}$  in  $T_3R_2$ . Total hardness of lake water varied from  $54 \pm 6.23$  in  $T_3C_3$  to  $64.2 \pm 6.58$  in  $T_3R_1$  with regular trends in fluctuation. Mean secchi disc reading varied from  $0.3 \pm 0.32 \text{ m}$  in  $T_1C_1$  to  $0.5 \pm 0.36 \text{ m}$  in  $T_2R_1$ . Dissolved oxygen was found to vary from  $5.45 \pm 0.38 \text{ mg/l}$  in  $T_2R_2$  to  $7 \pm 0.33 \text{ mg/l}$  in  $T_3R_1$ .

**Participatory approach at different stages of technology development:** A group of entrepreneurs living within the immediate vicinity of the creek, who have property right (beneficiaries), were assigned for overall management of the project. At least ten to fifteen beneficiaries were considered for a group selection including a team leader. Entrepreneurs were responsible for guarding, cleaning shoreline of creeks, administering feed as per requirement, taking part of fish harvesting.

## Carp brood development and growth performance evaluation of BFRI produced fingerling and local fingerlings

**Researchers:** Syed Lutfor Rahman, SSO  
Mohammed Asraful Haque, SSO  
Ahmad Fazley Rabby, SO

### Objectives

- To produce quality carp seed at RSS, Khepupara hatchery from BFRI reared brood.
- To compare the growth performance of carp by using BFRI produced fingerlings and local fingerlings at farmer's level

### Achievements

**Comparison the growth performance between improved and local brood fingerling:** Growth performance of BFRI improved fingerlings and local fingerlings of Rohu (*Labeo rohita*), Catla (*Catla catla*), Mrigal (*Cirrhinus cirrhosus*) and Rajputi (*Barbonymus gonionotus*) were evaluated. Fingerlings were stocked in two ponds of RSS with same size, equal density. Feeding management also same. Fingerlings were stocked at the rate of 5000/ha. Local fingerlings were collected from the farmer's nursery pond. Culture period was six months (Table 1). The final average length and weight were found for BFRI improved species of *Labeo rohita*, *Catla catla*, *Cirrhinus cirrhosus* and *Barbonymus gonionotus* 22.68±0.54 cm, 23.11±0.81 cm, 23.86±1.11 cm, 16.48±0.85 cm and 277.67± 7.87 g, 342.4±7.8 g, 227.6±7.50 g, 70.6±5.81 g local's one were 21.8±0.57cm, 21.96±0.87, 22.32±1.50, 15.51±0.92 and 271.04±7.52, 337.28±7.82, 225.57±6.8, 67.46 ± 5.12 respectively. BFRI improved fingerlings of *Labeo rohita*, *Catla catla*, *Cirrhinus cirrhosus* and *Barbonymus gonionotus* showed 8.70%, 7.20%, 5.10% 5.68% and 8.79%, 7.10%, 5.27%, 5.01% more growth than that of local fingerlings in respect to length and weight respectively.

**Table 1.** Growth evaluation in length of improved BFRI Rohu (*Labeo rohita*), Catla (*Catla catla*), Mrigal (*Cirrhinus cirrhosus*) and Rajputi (*Barbonymus gonionotus*) with local private hatchery fingerlings

Sources of seeds	BFRI improved Rui	Local nursery's Rui	BFRI improved Catla	Local nursery's Catla	BFRI improved Mrigal	Local nursery's Mrigal	BFRI improved Raj punti	Local nursery's Raj punti
Initial average length (cm)	5.28±0.15	5.18±0.21	5.35±0.39	5.17±0.48	6.5±0.43	6.17±0.46	6.34±0.29	6.10±0.31
Final average length (cm)	22.68±0.54	21.8±0.57	23.11±0.81	21.96±0.87	23.86±1.11	22.32±1.50	16.48±0.85	15.51±0.92
Growth (cm)	17.4	16.62	17.76	16.79	17.36	16.22	10.14	9.41
Growth (%)	329.55	320.85	331.96	324.76	267.08	262.07	159.94	154.26
Difference (%)	8.70%		7.20%		5.10%		5.68%	

**Distribution and dissemination of improved carp breeds to fish farmers:** Collected improved species of carps were reared in RSS ponds and produced brood fish. Improved spawn of Catla, Rohu, Mrigal and Rajputi were produced in RSS hatchery. Maximum spawn was disseminated to local nursery farmers. Due to shortage of nursery and rearing ponds, small scale improved fingerlings were produced in RSS ponds. In this year total 5 kg spawn and 80,000 fingerlings were produced in RSS, Khepupara. Improved spawn, fingerlings were disseminated to the local farmers in this region.

## **Brackishwater Station**

### **Improvement of management practice for increasing production of shrimp (*Penaeus monodon*) in extensive system**

**Researchers:** Mollah N S Mamun Siddiky, SO  
Md. Shariful Islam, SO  
Md. Mizanur Rahman Washim, SO

#### **Objectives**

- To assess the ecological status of shrimp (*P. monodon*) *ghers* and adjacent water bodies.
- To improve the productivity of shrimp (*P. monodon*) *ghers*.

#### **Achievements**

**Intervention for increasing production of shrimp in extensive system.**

**Pond management:** Growth of aquatic weeds was controlled by eradicating manually. Water of the *ghers* was exchanged as done by the farmer in the previous year. No feed was applied for rearing of shrimp.

**Physicochemical characteristics of water:** The water quality characteristics *viz.*, temperature, transparency, pH, salinity, alkalinity and dissolved oxygen of three selected *ghers* are being determined monthly following standard methods and will be continued up to final harvest. The collected physicochemical parameters up to 233 days of rearing are presented in Table 1.

**Table 1.** Water quality characteristics of the selected *ghers* up to 188 days of culture

<b>Parameters</b>	<b>Gher 1</b>	<b>Gher 2</b>	<b>Gher 3</b>
Temperature (°C)	26-33	27-34	29-33
Salinity (ppt)	12-19	13-20	14-22
Depth (cm)	45-60	52-68	50-60
Transparency (cm)	27-46	36-40	33-45
pH	7.4-8.4	7.5-8.1	7.7-8.5
Alkalinity (mg/l)	146-154	152-160	174-238
Dissolved oxygen (mg/l)	4.1-4.91	5.44-6.53	4.06- 5.34

**Biological characteristics of water:** The quality and quantity of phytoplankton and zooplankton of the selected *ghers* are being estimated monthly following standard methods and presented in Table 2.

**Table 2.** Biological characteristics of the selected *ghers* up to 188 days of culture

<b>Plankton</b>	<b>Gher 1</b>	<b>Gher 2</b>	<b>Gher 3</b>
Phytoplankton (No./L)	2.7x 10 <sup>2</sup> -3.1 x 10 <sup>2</sup>	0.9 x 10 <sup>2</sup> -1.5 x 10 <sup>2</sup>	3.2 x 10 <sup>2</sup> -3.4 x 10 <sup>2</sup>
Zooplankton (No./L)	0.3 x 10 <sup>2</sup> -0.5 x 10 <sup>2</sup>	0.4 x 10 <sup>2</sup> -0.5 x 10 <sup>2</sup>	0.5 x 10 <sup>2</sup> -0.7x 10 <sup>2</sup>

Most common phytoplanktons are *Scenedesmus*, *Cyclotella*, *Coscinodiscus*, *Spirulina* and *Synedra* and that of zooplanktons are rotifers, nauplius larvae, cladocerans and copepods.

**Harvest of Shrimp:** After 70 days of 1st stocking, selected harvesting of shrimps are being done in every new and full moon period by trap method. Amount of shrimp harvested till 10 October 2015 has been shown in Table 3. Sale proceeds and income will be assessed after final harvest at the end of the year.

**Table 3.** Harvest of shrimp from different *ghers* up to 10 October 2015

Particulars	Gher 1	Gher 2	Gher 3
ABW (g) at harvest	22-25	18-24	20-25
Harvest (kg/pond)	65+49.5+85+70+20+50. 5=340	8.2+9.6+21.3+17.1+51.8+94 .7=202.7	28.1+19.6+16.7+84.3+17.7 +26.4=192.8
Harvest (kg/ha)	680.00	511.86	318.67

### **Diversification of culture practice for optimizing production of the shrimp (*Penaeus monodon*) culture system in the coastal *ghers***

**Researchers:** Syed Lutfor Rahman, CSO  
Shamsun Nahar, DD  
Md. Mizanur Rahman Washim, SO

#### **Objectives**

- To study the ecology and production feasibility of different cropping patterns in *Penaeus monodon* culture system in the coastal *ghers*.
- To study the impact of introduction of different fin fishes for increasing production from the coastal *ghers*.
- To maximize production capacity and profitability from the coastal *ghers*.

#### **Achievements**

**Feasibility of double cropping with short culture period for increasing production of shrimp (*Penaeus monodon*) at different stocking densities**

**Experimental design:** The experiment was conducted in twelve on-station ponds of 0.1 ha each following the design as given in Table 1.

**Table 1.** Design of the experiment

Treatments	Stocking (No/m <sup>2</sup> )	Culture period	Crop(s)	Replication (No/m <sup>2</sup> )
T <sub>1</sub>	3	Short cycle (60 days)	Double	2
T <sub>2</sub>		Long cycle (120 days)	Single	2
T <sub>3</sub>	5	Short cycle (60 days)	Double	2
T <sub>4</sub>		Long cycle (120 days)	Single	2
T <sub>5</sub>	7	Short cycle (60 days)	Double	2
T <sub>6</sub>		Long cycle (120 days)	Single	2

The ponds were prepared by drying, liming (Quick lime: dolomite 1:1) @ 250 kg/ha of soil and then filled with the tidal water up to a depth of 1m. Water was treated with chlorine @ 20 ppm to disinfect water and kill all animalcules. Fermented molasses were applied to the pond water to develop colour of water to

prevent penetration of sunlight and then fertilized with urea and TSP @ 25 and 30 kg/ha, respectively for quick development of colour of water and production of plankton. After production of sufficient plankton required quantity of PCR tested PL was acclimatized with the pond water and stocked to the in-pond nursery made of nylon net fastened in bamboo frame. In the nursery the stocked PL were fed with CP nursery feed. After 3rd week of nursery rearing, the juveniles were released to the whole pond by up-folding the nylon net of the nursery enclosure. In the grow-out ponds, the shrimp were fed with CP feed depending on the biomass of shrimp. Growth of fishes was monitored at weekly interval and feeds were adjusted accordingly.

The water of the ponds was treated with dolomite @ 15 ppm on monthly basis and fertilized with inorganic fertilizer whenever necessary. After 45 days of culture period of 1<sup>st</sup> crop, all the culture ponds both short and long cycle affected by White spot disease (WSSV) and total culture system disrupted. Again PL was released for the 2<sup>nd</sup> time to the pond following all the previous activities of pond preparation properly. In the time of 2<sup>nd</sup> stocking there was unavailability of PCR tested and quality seed, so the stocking delayed by about 30 days. By this period, phytoplankton production was reduced and excessive unwanted aquatic weeds grew in the ponds. Having no other means, lastly we stocked specific pathogen free (SPF) PL.

**Table 2.** Growth performance of shrimp upto 45 days of culture in different treatments

Treatments	Stocking (No/m <sup>2</sup> )	Culture period (days)	Crop(s)	Replications	ABW (g)
T <sub>1</sub>	3	60	Double	R <sub>1</sub>	12.24
				R <sub>2</sub>	12.54
T <sub>2</sub>		120	Single	R <sub>1</sub>	14.37
				R <sub>2</sub>	15.60
T <sub>3</sub>	5	60	Double	R <sub>1</sub>	12.53
				R <sub>2</sub>	10.70
T <sub>4</sub>		120	Single	R <sub>1</sub>	9.82
				R <sub>2</sub>	10.88
T <sub>5</sub>	7	60	Double	R <sub>1</sub>	10.00
				R <sub>2</sub>	10.19
T <sub>6</sub>		120	Single	R <sub>1</sub>	11.46
				R <sub>2</sub>	12.15

After 60 days in the 2<sup>nd</sup> culture all the shrimp of short cycle culture period has been harvested by dewatering and long culture cycle (120 days) was continued. But suddenly after 67 days of culture the shrimp of long cycle culture pond except 5Nos/m<sup>2</sup> were affected by White spot disease (WSSV) and culture system disrupted again.

All water quality variables except dissolved oxygen (DO) and salinity were congenial for culture of shrimp in all stocking ponds in both short cycles and long cycle culture systems. Salinity (ppt) level was drastically fluctuated during the culture period. The growth and production performances of shrimp in different ponds are summarized in Table 3.

**Table 3.** Growth performance of shrimp up to 60 days of culture in different treatments

Treatments	Stocking (No/m <sup>2</sup> )	Culture (days)	Crop(s)	Replications	ABW (g)	Survival (%)	Production (Kg/ha)
T1	3	60	Double	R1	18.72	77.50	312.60
				R2	17.48	70.12	364.51

T2		120	Single	R1	18.53	Outbreak of disease after 67 days of culture	
				R2	17.89		
T3	5	60	Double	R1	15.60	73.87	534.75
				R2	14.95	65.28	497.32
T4		120	Single	R1	16.43	Outbreak of disease after 67 days of culture	
				R2	15.95		
T5	7	60	Double	R1	13.54	74.34	729.59
				R2	13.21	76.30	687.46
T6		120	Single	R1	14.76	Outbreak of disease after 67 days of culture	
				R2	15.87		

\*In the long cycle (120 days) culture of 5 Nos/m<sup>2</sup> density, average growth of shrimp was 35.71g, survival was 72% and production of shrimp was 1285.56 kg/ha.

In the 2<sup>nd</sup> crop of short (60 days) culture, average growth of shrimp was 18.10g, 15.27g and 13.37g and production of shrimp was 338.55 kg/ha, 516.03 kg/ha and 708.52 kg/ha at 3, 5 and 7 Nos/m<sup>2</sup> density, respectively. Due to occurrence of viral disease in shrimp, production of all ponds were not assessed and compared both economically and ecologically.

## Development of technique for breeding and larval rearing of mud crab, *Scylla olivacea*

**Researchers:** Dr. Md. Latiful Islam, SSO  
Mst. Subrina Khatun, SO  
Mollah.N.S. Mamun Siddiky, SO  
Umme Habiba, SO

### Objectives

- To develop brood of mud crab, *Scylla olivacea* in captivity.
- To develop larval rearing techniques of mud crab, *Scylla olivacea*.

### Achievements

#### *Expt. 1. Impact of salinity on the production of berried female of mud crab, Scylla olivacea*

The experiment was conducted in the Brackishwater Station hatchery complex of BFRI. The broodstock maturation experiment was designed with three different levels of salinities viz, 25 ppt (T1), 30 ppt (T2) and 35 ppt (T3). Each of the treatment had 3 replications. The experiment was conducted in 9 cemented tanks having an inner area of 8.0 m<sup>2</sup> (5.0 m × 1.6 m × 1.0 m) of each. The tanks were prepared through drying, cleaning and providing a sand bed of 10-12cm thickness. The sand bed covered the half portion of the bottom area of each cistern. The tanks were half filled with required saline water according to the design and aerated through sand bed filter as recirculation system. Gravid female crabs (250-300g) were purchased either from the depot or locally collected from the Brackishwater pond complex. The collected broods were carried into the hatchery, acclimatized with salinity and temperature accordingly for about 1 hour to one day depending on salinity variations. The broods were measured (total length and carapace width), marked with water proof permanent marker, and ablated the eyestalk. Then the broods were stocked in each cistern @ 2 crabs/m<sup>2</sup>. The crabs were fed with chopped tilapia up to satiation twice daily. Uneaten feeds were removed prior to next feeding. The crabs were monitored in each day and checked for spawning. About 75% of the water was changed with same salinity water after one month of intervals.

Water quality parameters like, temperature, pH, dissolved oxygen and ammonia were monitored daily following standard methods. Once a female had completed spawning, was gently collected from the tank and biological parameters were monitored accordingly. The brood stock management experiment was started from December, 2014 and maintained for year round as a continuous procedure.

Results of water quality variables at brood stock management are shown in Table 1. Water temperature (18.0 °C – 31.0 °C), pH (7.6 – 7.9), dissolved oxygen (4.84 mg/l – 9.0 mg/l) and ammonia (0.5 mg/l – 1.0 mg/l) was similar in all the treatments and was within the acceptable ranges for crustacean broodstock management.

**Table 1.** Water quality parameters of different salinity levels

Parameters	25 ppt (T1)	30 ppt (T2)	35 ppt (T3)
Temperature (°C)	18-31	18-31	19-31
pH	7.6-7.9	7.6-7.8	7.5-7.8
Dissolved oxygen (mg/l)	5-9	4.84-9.0	4.97-8.9
Ammonia (mg/l)	0.5-1.0	0.5-1.0	0.5-1.0

Production and performance of berried female under different salinity level is presented in Table 2. From the Table, it could be seen that highest number (11 Nos) of crab spawned in T2 which contains the salinity level of 30 ppt. The highest spawning success of 68.75% and fertilization rate of 88.65% was achieved from the same salinity level treatment (T2) followed by 25 ppt (T1) salinity level 18.75% and 85.76% respectively for the same. The spawning success was nil for the 35 ppt (T3) salinity level. Result of this experiment revealed that, 30 ppt salinity is the best suit level than rest of the tested salinity levels for better performance of broodstock maturation of the mud crab.

**Table 2.** Performance of berried female production under different salinity levels

Particulars	Treatments		
	25 ppt (T1)	30 ppt (T2)	35 ppt (T3)
Ave. body weight (g)	234± 4.30	267±3.89	270±3.87
Ave Carapace width (cm)	11.0±0.35	10.8±0.49	11.2±0.51
Total Number of brood reared	16	16	16
Total No. spawned	3	11	0
Spawning success (%)	18.75	68.75	-----
Incubation (days)	12	12	-----
Fertilization rate (%)	85.76	88.65	-----

**Expt. 2. Impact of green water and different feeding regime on the development of larvae of mud crab, *Scylla olivacea***

Newly hatched zoea of *S. olivacea* was collected within an hour by scooping with a beaker and kept in a plastic bowl with same saline water. Larvae were stocked to the rearing tanks prepared with disinfected 30 ppt salinity water. Feed was supplied four times daily following the experimental design. Before each feeding, dead larvae and uneaten feed was siphoned out from the tank bottom. At least 30% of water was exchanged daily to maintain congenial water quality. Temperature of water was maintained at a level of 27-31°C by using thermostat water heater during low temperature period. The water quality variables such as, temperature, pH, dissolved oxygen and ammonia was recorded daily. Survival rate and larval development was assessed through sampling of larvae and monitoring under microscope in daily basis.



**Table 3.** Survival rate (%) of larvae at different larval stages reared in 30 ppt salinity and green water system

Larval stages	Survival (%)
Zoea-1 (Z1)	33.33
Zoea-2 (Z2)	29.16
Zoea-3 (Z3)	12.50
Zoea-4 (Z4)	0.38
Zoea-5 (Z5)	0.075
Megalopa (M)	Nil

Survival of larvae is presented in Table 3. An initial mass mortality was observed. At the end of Z1 stage, the survival was only 33.33%. The mortality of larvae from Z1 to Z2 stage was slower, while the mortality increased drastically from Z2 to Z3 stages and onwards. At the end of the experiment, only 0.75% of the larvae reached to the Z5 stage. None of the larvae reached to the megalopa stage in this experiment.

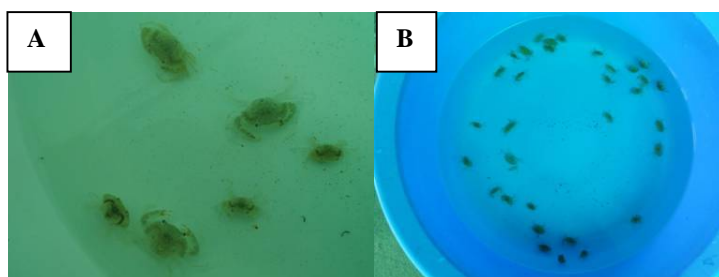
### **Expt. 3. Effect of feeding schedules on survival and growth of mud crab, *Scylla olivacea* larvae**

The second experiment on larvae rearing was conducted on July 8, 2015. A total number of 200,000 larvae (Z1) were stocked in 7 Fibre Glass Tank's. Due to shortage of rotifer, the larvae were initially fed with 5 ind/ml of rotifers instead of 15 ind/ml. In addition, *Artemia* was added at 0.5 ind/ml and artificial diet at 1 g/ton per day (divided into 3-4 rations). Sediments in the tank bottom were siphoned out regularly. About 30% of the water was changed every 5 days. Two to three-days old *Artemia* were supplied as feed to the larvae starting zoea 5 stages. Overall progress is presented in Table 4.

**Table 4.** Survival rates of larvae stocked in fiberglass tanks

Tank	Zoea used for stocking	Z5 count	SR % Z1-Z5	Megalopa count	SR% Z5-M	SR% M-CI	SR% Z1-CI
FGT-1	70,000	5,000	7%	1,250	25%	40%	0.20%
FGT-2	50,000	6,000	12%	2,100	35%	29%	0.50%
FGT-3	40,000	500					
FGT-4	40,000	200					
	200,000						Ave. 0.30%

Similar to the first larvae rearing experiment, the initial mortality of larvae in this experiment was also found from day 1 to 5. Two tanks were discarded due to delayed in molting and metamorphosis to Z5 stage as because of low temperature during continuous monsoon rain. Larvae of the rest two tanks (Z5 stage) started to metamorphosis to the megalopa stage on day 15 after hatching. Megalopa started to moult to crab instar on day 20 of culture and 650 crab instars (C1) were achieved on day 21. This was 0.30% of the stocked number of those two tanks. From the C1 (crablet 1) stage, only 67 number survived to C3 stage on day 45 of rearing and were transferred in the nursery for grow-out (Plate 1).



## Development of breeding, seed production and culture technology of green back mullet *Chelon subviridis* (Val. 1836)

**Researchers :** Syed Lutfor Rahman, CSO  
Rumana Yasmin, SO  
Md. Shariful Islam, SO

### Objectives

- To evaluate the efficacy of different hormones for the breeding of *C. subviridis*.
- To develop sustainable nursery management and culture technology of *C. subviridis*
- To evaluate the economic feasibility of production of *C. subviridis*.

### Achievements

#### *Expt. 1. Optimizing salinity level of water for breeding C. subviridis*

Three different concentrations of salinity viz., 20ppt, 25ppt and 30ppt was tried each with three replicates to optimize required level of salinity for breeding of green back mullet. Only in 30 ppt salinity, green back mullet responded for breeding and in 20 and 25 ppt, it didn't. The spawning period, fertility rate, hatching period, hatching rate and survival of green back mullet were 30-35 hrs, 97%, 21-25 hrs, 98% and 11 days respectively.

**Table 1.** Optimizing salinity level of water for breeding *C. subviridis*

Salinity (ppt)	Response	Spawning period (hrs)	Fertility rate (%)	Hatching period (hrs)	Hatching rate (%)	Survival (days)
20	No	-	-	-	-	-
25	No	-	-	-	-	-
30	No	30.0±5.0	97.0±2.0	21.0±4.0	98.0±1.0	11±5

#### *Expt. 2. Determination of quality and doses of different hormones for breeding of C. subviridis.*

Three types of hormones at different doses was tried for the experiments are given below:

**Table 2.** Determination of quality and doses of different hormones for breeding of *C. subviridis*

Types of hormones	Doses		
Carp pituitary extract(PG)(mg/l)	3	4	5
HCG(IU)	2500	3000	3500
GnRHa(Ovupin)(mg/L)	15	20	25

**Table 3.** Result of different hormones doses for breeding of *C. subviridis*

Ovupin (mg/kg)	Response	Spawning period (hrs)	Fertility rate (%)	Hatching period (hrs)	Hatching rate (%)	Survival (days)
15	No	-	-	-	-	-
20	Yes	34.0±2.0	80.0±5.0	23.0±3.0	82.0±4.0	7 months
25	Yes	30.0±2.0	96.0±2.0	20.0±2.0	95.0±3.0	7 months

In 20mg/kg and 25mg/kg Ovupin hormone dose, green back mullet responded for breeding and in 15 mg/kg it didn't. In 20mg/kg Ovupin hormone dose, the spawning period, fertility rate, hatching period, hatching rate and survival of green back mullet were 34-36 hrs, 80%, 23-26 hrs, 82% and 6 months approximately. In 25 mg/kg Ovupin hormone dose, the spawning period, fertility rate, hatching period, hatching rate and survival of green back mullet were 30-32 hrs, 96%, 20-22 hrs, 95% and 3 months approximately and 25 mg/kg Ovupin hormone dose showed the best result.

**Expt. 3. Production of *C. subviridis* in monoculture management at different stocking densities**

For the study, three stocking densities viz. 6, 9 and 12 Nos/m<sup>2</sup> each with two replications were tried where best growth was found in 6 Nos/m<sup>2</sup> which was 10.89 g than 9 Nos/m<sup>2</sup> was 3.9g and 12Nos/m<sup>2</sup> was 4.83g. Production of *C. subviridis* was highest 57-62 kg/1000 m<sup>2</sup> in lowest density (6 No/m<sup>2</sup>) and lowest production 30-37 kg/1000 m<sup>2</sup> in 9 No/m<sup>2</sup> density.

**Table 3.** Production of *C. subviridis* in monoculture management at different stocking densities

Stocking density (No/m <sup>2</sup> )	Average body weight (g)				Production (Kg/1000 m <sup>2</sup> )	
	R1		R2		R1	R2
	Initial	Final	Initial	Final		
6	0.26±0.05	16.7±2.5	0.26±0.05	13.6±2.0	62.0±5.0	57.0±5.0
9	0.26±0.05	8.5±3	0.26±0.05	5.5±2.3	37.0±4.0	30.0±4.5
12	0.26±0.05	10.1±2.7	0.26±0.05	9.0±2.0	50.0±5.0	44.0±5.5

Temperature, morning DO and salinity of water during study period were 28-34°C, 1.9-4.3 and 10-15 ppt and almost same in all ponds. Transparency and pH of water of all the ponds was congenial for nursery rearing and varied from 23-55 cm and 7.5-8.5 respectively. Alkalinity was almost same (120-210 mg/l) in all ponds.



## Shrimp Research Station

### **Investigation into soil-water characteristics of shrimps farms under existing culture practices**

**Researchers:** Dr. Khan Kamal Uddin Ahmed, CSO  
Md. Ariful Islam, SO  
Md. Motiur Rahman, SO

#### **Objectives**

- To observe and evaluate the effects of different soil-water parameters on the production of shrimp under semi-intensive culture system
- To identify the different groups of phytoplankton and zooplankton as well as their seasonal variation
- To observe the effects of these physico-chemical and biological parameters (plankton) on shrimp production

#### **Achievement**

**Water quality parameters:** The recorded mean water quality parameters in all experimental gher (Semi-intensive) throughout the experimental period are shown in Table 1. The water temperature ranged from  $27\pm 3.96^{\circ}\text{C}$ ,  $24.7\pm 5.59^{\circ}\text{C}$  and  $26\pm 5.63^{\circ}\text{C}$  in the experimental gher of Mongla, Bagerhat sadar upazila and Khulna, respectively. The pH of water at the experimental gher of Mongla, Bagerhat sadar upazila and Khulna varied from  $8.2\pm 0.26$ ,  $8.1\pm 0.56$  and  $8.0\pm 0.50$  respectively. Dissolved oxygen was recorded within a range of  $5.85\pm 2.17$  mg/l,  $8.1\pm 0.56$  mg/l,  $8.0\pm 0.50$  mg/l and ammonia was recorded 0~0.2, 0~0.1 and 0 mg/l at Mongla, Bagerhat sadar upazila and Khulna respectively. The maximum salinity was recorded in Mongla  $9.8\pm 4.32$  whereas the minimum salinity was observed at Bagerhat sadar  $2.33\pm 2.08$  and Khulna  $8.75\pm 2.63$  during the experimental period. The alkalinity was observed as  $167\pm 21$ ,  $147\pm 15.59$  and  $154\pm 37.31$  at Mongla, Bagerhat sadar upazila and Khulna respectively. Iron was present as  $0.15\pm 0.15$  in Mongla whereas at Bagerhat sadar and Khulna the presence of Iron was found 0 (zero).

**Table 1.** Water quality parameters of different gher sites under semi-intensive culture system during september /14 to May/15

Parameters	Mongla (T <sub>1</sub> )	Bagerhat Sadar (T <sub>2</sub> )	Khulna (T <sub>3</sub> )
Temperature (°C)	27±3.96	24.7±5.59	26±5.63
pH	8.2±0.26	8.1±0.56	8.0±0.50
DO (mg/l)	5.85±2.17	7.00±1.55	6.0±0.73
Salinity (ppt)	9.8±4.32	2.33±2.08	8.75±2.63
Alkalinity (mg/l)	167±21	147±15.59	154±37.31
Ammonia (mg/l)	0.1±0.1	0.05±0.05	0.0±0.0
Iron	0.15±0.15	0	0

\* Average value of collected 18<sup>th</sup> samples from September/14 to May/15

**Soil characteristics:** The recorded average soil parameters in all experimental gher throughout the experimental period are shown in Table 2. The value of organic matter was found as  $2.88\pm 2.00$  %,  $3.07\pm 1.60$  % and  $3.23\pm 1.98$  % in Mongla, Bagerhat sadar and Khulna respectively. The mean value of pH was recorded  $7.7\pm 0.36$ ,  $7.6\pm 0.40$  and  $7.76\pm 0.38$  in Mongla, Bagerhat sadar and Khulna respectively. The

average value of soil salinity was maximum in Mongla ( $8.83 \pm 5.42$  ds/m) compared to Bagerhat ( $5.83 \pm 4.87$  ds/m) and Khulna ( $6.92 \pm 3.43$  ds/m). The average value of phosphorus was found the highest in Khulna ( $17.28 \pm 9.14$   $\mu\text{g/g}$ ) followed by Bagerhat sadar ( $16.39 \pm 7.62$   $\mu\text{g/g}$ ) and Mongla ( $13.41 \pm 5.83$   $\mu\text{g/g}$ ). Average Total nitrogen was  $0.184 \pm 0.11$  %,  $0.135 \pm 0.038$  % and  $0.139 \pm 0.04$  % in Mongla, Bagerhat Sadar and Khulna respectively. The maximum potassium was recorded at Mongla ( $0.814 \pm 0.20$  m.eq./100g) whereas the minimum was observed at Khulna ( $0.602 \pm 0.159$  m.eq./100g) during the experimental period. The presence of sulphur was maximum at Khulna ( $120.38 \pm 43.45$   $\mu\text{g/g}$ ) compared to Mongla ( $105.32 \pm 53.39$   $\mu\text{g/g}$ ) and Bagerhat Sadar upazila ( $89.69 \pm 41$   $\mu\text{g/g}$ ) as well. The presence of zinc was the highest at Bagerhat Sadar ( $9.96 \pm 4.93$ ) than Mongla ( $7.73 \pm 5.05$ ) and Khulna ( $8.86 \pm 5.49$ ).

**Table 2.** Soil characteristics of different gher sites under Semi- Extensive Culture System

Parameters	Mongla (T <sub>1</sub> )	Bagerhat Sadar (T <sub>2</sub> )	Khulna (T <sub>3</sub> )
Org. matt. (%)	$2.88 \pm 2.00$	$3.07 \pm 1.60$	$3.23 \pm 1.98$
pH	$7.7 \pm 0.36$	$7.6 \pm 0.40$	$7.76 \pm 0.38$
Salinity (EC) (ds/m*)	$8.83 \pm 5.4$	$5.83 \pm 4.87$	$6.92 \pm 3.4$
Phosphorus ( $\mu\text{g/g}$ )	$13.41 \pm 5.83$	$16.39 \pm 7.62$	$17.28 \pm 9.14$
Total N <sub>2</sub> (%)	$0.184 \pm 0.11$	$0.135 \pm 0.038$	$0.139 \pm 0.04$
Potassium (m.eq./100g)	$0.814 \pm 0.20$	$0.614 \pm 0.123$	$0.602 \pm 0.159$
Sulphur ( $\mu\text{g/g}$ )	$105.32 \pm 53.39$	$89.69 \pm 41$	$120.38 \pm 43.45$
Zinc ( $\mu\text{g/g}$ )	$7.73 \pm 5.05$	$9.96 \pm 4.93$	$8.86 \pm 5.49$

## Bioaccumulation of hazardous chemicals in shrimp farming system of Bangladesh

**Researchers:** H.M. Rakibul Islam, SO  
Md. Ariful Islam, SO  
Rakhi Das, SO

### Objectives

- To identify available chemicals containing antibiotics which are used in shrimp/prawn farms
- To identify the source of hazardous antibiotics in shrimp/prawn culture system.
- To assess available pesticides residues in rice-prawn/shrimp farming system

### Achievement

#### Detection of hazardous antibiotic Nitrofurans metabolites (semicarbazide) to identify its source:

The experiment was conducted for identification and quantification of banned antibiotic such as Nitrofurans metabolites in shrimp/prawn which are used by the farmers during shrimp/prawn farming.

**Assessment of available pesticide residues in shrimp/prawn farms:** An experiment was conducted for investigation of concentration of Organochlorine insecticides such as DDT, Heptachlor, Dieldrin and Endrin used in rice cum shrimp/prawn ghers. Total six (06) sites *viz.* B'hat Sadar, Kochua, Fakirhat, Mollarhat of Bagerhat District and Dumuria, Fultola of Khulna District were selected for this study from where the shrimp/prawn samples were collected for lab. From these concentrations of pesticides risk assessment was conducted based on local and country-wide Consumption rates for key species (DoF 2013).

## Development of cost effective quality feed using locally available feed ingredients for black tiger shrimp (*Penaeus monodon*)

**Researchers :** Dr. Khan Kamal Uddin Ahmed, CSO  
B.M. Shahinur Rahman, SO  
Rubia Akter, SO

### Objectives

- Formulation of cost effective artificial diets for bagda grow-out using locally available ingredients and
- Determine the efficacy of formulated feed on growth, survival and production of shrimp in earthen ponds/ghers

### Achievement

**Feed formulation:** Different types of collected local feed ingredients for the experiments viz. dhyancha seeds (*Sesbania sp.*), duckweed (*Lemna minor*), Kolmilata (*Ipomoea aquatic*), mustard oilcake, soya bean meal, meat and bone meal were selected for the formulation of grow-out feeds (Table-1). Analysis of selected feed ingredients was done in the Shrimp Feed and Nutrition laboratory of Shrimp Research Station, Bagerhat. Three diets with a protein level of 35% will be formulated using Pearson's square method and was adjusted this level by trial and error method. Essential Amino Acid (EEA) and Essential Fatty Acid (EFA) profiles of the selected ingredients were included in test diets. The protein content and amino acid was readjusted until the fulfillment of protein and amino acid level in the diets. Locally available feed ingredients like rice bran, wheat bran, wheat flour, broken maize as well as vitamin & minerals were also used as common ingredients for the formulation of three diets. Formulated diets were analyzed for proximate composition to check the accuracy of formulation (Table-2). Diets were palletized and dried at room for 2-3 days. The feed were kept in airtight polythene bags and stored at room temperature.

**Table 1.** Feed Formulation (Quantity as %)

Types of Diets (% CP)	Diets for Shrimp (35% CP)			
	Feed-1 (T <sub>1</sub> )	Feed-2 (T <sub>2</sub> )	Feed-3 (T <sub>3</sub> )	Feed-4 (Control)
Protein Con.	12.00	12.00	12.00	Quality Feed (Gold Grower)
Meat & bone meal	15.00	15.00	15.00	
Soybean Meal	10.00	12.00	12.00	
Mustard oil cake	10.00	10.00	12.00	
Rice bran (Auto)	16.00	14.00	12.00	
Dhyancha seed	20.00	5.00	5.00	
Kolmilata	5.00	20.00	5.00	
Duck weed	5.00	5.00	20.00	
Wheat flour (atta)	5.00	5.00	5.00	
Lime stone	1.00	1.00	1.00	
Salt	0.50	0.50	0.50	
Vitamin & min. premix	0.20	0.20	0.20	
Pellet binder	0.30	0.30	0.30	
<b>Total</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	
<b>Cost Tk./Kg</b>	<b>41.00</b>	<b>42.80</b>	<b>43.40</b>	

**Table 2.** Proximate composition of feeds

Parameters	Feed-1	Feed-2	Feed-3	Control
Crude Protein	34.80	34.50	34.48	37.5 (28.53)
Crude Fat	8.40	8.20	8.15	7.0
Ash	12.30	11.78	11.79	3.0
Fibre	5.63	6.09	6.26	-
NFE	30.03	30.77	31.82	-
GE (kJ g <sup>-1</sup> )	16.50	16.55	16.45	-

**Growth trial:** Eight ponds were selected in Shrimp Research Station, Bagerhat. Black tiger shrimp (*P. monodon*) post larvae was collected from the local markets of Bagerhat district. Post larvae of shrimp reared in nursery pond for 21-25 days. Thereafter, stocking done with nursed PL. Feed was supplied twice daily @ 10% of body weight for the first month, 8% for the second month and 5-3% for the rest period. 10% of stocked shrimp is sampled by cast net. Weight of the shrimp was taken using portable balance for growth monitoring, feed adjustment and disease checking. Water quality was also monitored and recorded at weekly intervals.

The nursed shrimp PL of average body weight of 1.5 g stocked at the rate of 120/dec. and reared for a period of 90 days. After 90 days culture, the growth recorded 16.56g, 21.74g, 16.48g and 17.01g for Feed-1 (T<sub>1</sub>), Feed-2 (T<sub>2</sub>), Feed-3 (T<sub>3</sub>), and Feed-4 (T<sub>4</sub>) (Commercial feed) respectively. The highest growth performance of 21.74g obtained from Feed-2 (fish meal 12%, meat & bone meal 15%, soya-bean meal 12%, mustard oil cake 10%, Dhyancha seed 5%, Kolmilata 20%, Duck weed 5%, rice bran 14%, wheat flour 5%, lime stone 1%, Salt 0.5%, vitamin & minerals 0.2%, Pellete binder 0.3%) and the lowest of 16.48g was recorded in grower shrimp supplied with feed-3. Different growth parameters and Survival of shrimp with different feeds are shown in Table 3.

**Table 3.** Growth performance and survival of shrimp using different feeds

Treatments	Initial wt. (g)	Final wt.(g)	SGR* (% days)	Survival (%)
T <sub>1</sub> ( feed-1)	1.5	16.56±0.96	2.67	72.0
<b>T<sub>2</sub> ( feed-2)</b>	<b>1.5</b>	<b>21.74±1.41</b>	<b>2.97</b>	<b>75.0</b>
T <sub>3</sub> (feed-3)	1.5	16.48±1.08	2.66	71.0
T <sub>4</sub> (Control) (Commercial feed)	1.5	17.01±1.37	2.69	69.5

\*\*SGR=Specific Growth Rate

## Impact of probiotics on shrimp (*Penaeus monodon*) production

**Researchers:** Rubia Akter, SO  
B.M. Shahinur Rahaman, SO  
Md. Motiur Rahaman, SO

### Objectives

- To evaluate the impact of probiotics on growth and production of Shrimp (*Peneous monodon*)
- To evaluate the level of Production of Shrimp
- To assess the effect of probiotics on immune responses of shrimp
- To evaluate the probiotic suitability shrimp.

## Achievement

**Bacterial load:** The population of total heterotrophic bacteria (THB) of pond waters and sediments is estimated on monthly intervals as depicted in Table 1. Bacterial floras present in ponds are analyzed and it is found that bacterial load in water and sediment ranged from  $4.8 \times 10^3$ —  $155 \times 10^3$  CFU/ml and  $6.3 \times 10^3$ — $364.0 \times 10^3$  CFU/mg in T<sub>1</sub>,  $5.2 \times 10^3$ — $170 \times 10^3$  CFU/ml and  $7.0 \times 10^3$ — $483 \times 10^3$  CFU/mg in T<sub>2</sub> and  $2.3 \times 10^3$ — $50 \times 10^3$  CFU/ml and  $3.0 \times 10^3$ — $251 \times 10^3$  CFU/mg in T<sub>3</sub>, respectively.

**Table 1.** Quantitative profile of THB in waters and sediments of ponds

Treatments		Bacterial load (CFU/ml and CFU/mg)
T <sub>1</sub>	Water	$2.90 \times 10^4$ - $3.76 \times 10^4$
	Soil	$2.63 \times 10^4$ - $3.15 \times 10^4$
T <sub>2</sub>	Water	$2.80 \times 10^4$ - $3.54 \times 10^4$
	Soil	$2.97 \times 10^4$ - $3.17 \times 10^4$
T <sub>3</sub>	Water	$2.28 \times 10^4$ - $3.11 \times 10^4$
	Soil	$2.31 \times 10^4$ - $3.21 \times 10^4$

**Table 2.** Growth, survival and production (mean  $\pm$  SD) of *Penaeus monodon* in different treatments during the culture period

Particulars	Treatments		
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Stocking density (no./m <sup>2</sup> )	5.98	5.98	5.98
Stocking size (g)	0.025	0.025	0.025
Harvesting size (g)	22.065 $\pm$ 5.52	19.87 $\pm$ 4.98	11.01 $\pm$ 0.86
Survival (%)	73.11%	63.57%	47.67%
FCR	1.83	2.01	2.48
Production (kg/ha)	760	665	205.19

## Investigation into shrimp/prawn diseases and their control strategies

**Researchers :** H.M. Rakibul Islam, SO  
Md. Ariful Islam, SO  
Rakhi Dash, SO

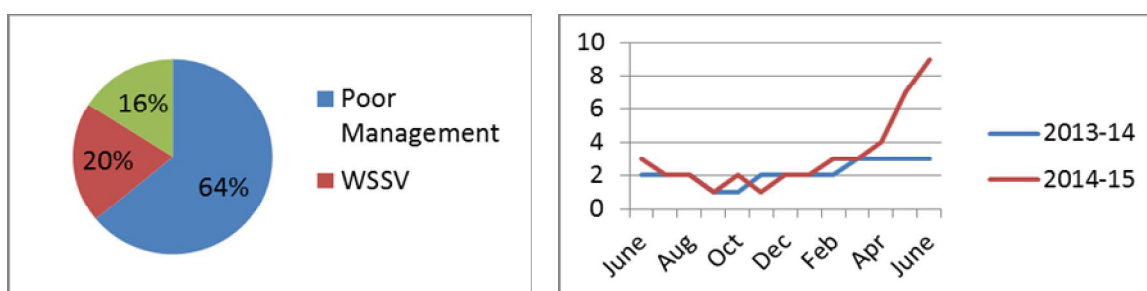
### Objectives

- Investigation of emerging diseases
- Survey of the running hatcheries to overcome the existing problem of PL production
- Assessment of bacterial resistance at different doses of active chlorine.

### Achievements



**Investigation of immersing disease:** In the year 2014-15, investigation of immersing diseases was one of the most important objectives of the research programme. Therefore, under the project 12 ghers were investigated randomly in context to aqua ecology and pathogens (Fig. 1). Among the ghers, 64% of them found to be managed by poor management techniques and hence failed to reach the desired production. Twenty percent of the ghers were found to be infected by white spot syndrome virus. In past few years, the salinity in the river water was very steady and hardly cross the range 2-3 ppt, but in the current year, the river water salinity hikes up to 11 ppt (Fig. 2) due to the dredging and excavation of Ghoshiyakhali channel of the Sundarbans. Therefore, due to heavy rainfall, the shrimp (*Penaeus monodon*) face higher salinity fluctuation which make those more vulnerable to WSSV. Mortality of shrimp in 16% of the investigated ghers were also found due to over stocking which can also be categorized as poor management.



**Brood development and hatchery operation:** Apart from the investigation into emerging disease, another important objectives of the project was to Develop a domestic brood stock by rearing the PL of the successful batch at 2013-14 of the SRS hatchery in order to bring those PL into production cycle. Gravid females from the brood ponds were collected and bring into the prawn hatchery of the Station to start the PL production as another objective of the research project, to fine tune the modifications that were made in the existing SOP (Standard Operating Procedure) for hatchery management, viz., Chlorination of brine and freshwater before mixing, Chlorination of mixed water (12 ppt), ensure 6 hours of contact time to active  $Cl_2$  before aerations, transfer the larvae into a new & dry LRT after three consecutive days, practice antibiotic baths instead of application of antibiotics directly into the LRT, etc. The system was working fine and the first sighting of PL was done at the 18<sup>th</sup> days of rearing symbolizing a healthy environment until the vigorous monsoon rain starts. Due to consistent raining, water temperature gone beyond the control and falls to 26<sup>o</sup> c. Small submersible thermostat of the aquarium were inserted but had minimum impact on the large tanks of 3000 L. Consequently, the production reduced considerably.



## Marine Fisheries & Technology Station

### Development of culture technique and utilization of seaweed

**Researchers:** Dr. Md. Enamul Hoq, CSO  
Md. Asraful Haque, SO  
Md. Shahzad Kuli Khan, SO  
Md. Mohidul islam, SO  
Jakia Hasan, SO

#### Objectives

- Development of seaweed culture technique in Bangladesh.
- Investigate the nutritious value of seaweeds.
- Utilization of seaweeds by producing value added products.

#### Achievements

**Seaweed culture:** Culture experiment site was inshore water of south-eastern part of Chera dweep at Saint Martin Island, Teknaf, Cox's Bazar. Experiment culture of seaweed started on 05 January, 2015 and closed on 05 March, 2015. Coir rope was used as net material for substrate with net size of 2m<sup>2</sup>. Four corners of the nets were tied with rocks placed 25 cm above from the bottom. *Hypnea musciformis*, *Padina tetrastromatica* and *Sargassum oligocystum* were selected for culture experiment. Cut piece of *H. musciformis* and *S. oligocystum* and small whole species of *P. tetrastromatica* were use as seed. Seeds are attach with net by short length string. Three replications were tested for each species. The culture results and hydrological data are shown in Table 1.



**Fig. 1.** Seaweed culture net management, hydrological data collection & production.

**Abundance and distribution study:** During November 2014 to May 2015 different species of seaweed i.e. *Caulerpa racemosa*, *Enteromorpha intestinalis*, *Hypnea musciformis*, *Hypnea sp.*, *Jania rubens*, *Padina tetrastromatica*, *Porphyra sp.*, *Sargassum oligocystum*, *Hydroclathrus clathratus* were collected randomly by hand-picking from the study area at the time of low-tide. Fresh samples were taken into plastic jars and then kept into icebox for laboratory work. In the laboratory, samples were gently brushed under running seawater, rinsed with distilled water, dried with paper tissue and finally preserve by open sun drying. The dried material was powdered manually with the use of a mortar and pestle and blander at room temperature, until the chemical analysis.

**Table 2.** Availability and distribution of seaweed

Sources/ Locaion	Species	Availability						
		Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
St. Martin (Golachipa, Cheradip)	<i>Caulerpa racemosa</i>							
	<i>Caulerpa taxifolia</i>							
	<i>Halimeda tuna</i>							
St. Martin (Cheradip)	<i>Dictyota dichotoma</i>							
St. Martin (Dakshinpara), Bakkhali	<i>Hypnea</i> sp							
St. Martin, Bakkhali	<i>Sargassum</i> sp.							
St. Martin	<i>Padina tetrastromatica</i>							
	<i>Jania</i> sp.							
	<i>Hydroclathrus clathratus</i>							
St. Martin (Golachipa,Cheradip), Bakkhali	<i>Colpomenia sinuosa</i>							

**Proximate/Nutritional analysis study:** To assess the proximate composition and micronutrients of seaweeds some selected seaweed species were analyzed (Tables 3 and 4).

**Table 3.** Proximate composition of cultured & some selected seaweed of Bangladesh

Species/Parameters (%)	Moisture	Ash	Crude protein	Crude lipid	Crude fiber	Carbohydrate
<i>Hypnea musciformis</i>	24.31	9.76	13.73	0.34	5.60	46.26
<i>Hypnea</i> sp.	17.45	3.96	22.31	0.78	4.10	51.40
<i>Jania rubens</i>	8.58	16.27	5.70	0.41	5.90	63.14
<i>Padina tetrastromatica</i>	15.68	27.95	12.29	0.98	6.80	36.30
<i>Sargassum oligocystum</i>	21.09	12.94	8.19	0.83	5.20	51.75
<i>Caulerpa racemosa</i>	16.36	9.90	22.25	2.65	4.80	44.04

**Table 4.** Micronutrients contents in selected seaweeds

Species	Type	Ca (ppm)	K (ppm)	Na (ppm)	Fe (ppm)	Zn (ppm)
<i>Hypnea musciformis</i>	Red	14,075.78	3,083.07	11,036.83	1,428.54	45.49
<i>Jania rubens</i>	Red	228,892.09	7,109.24	16,104.21	468.99	Not Detected
<i>Padina tetrastromatica</i>	Brown	27,941.24	4,136.38	10,472.24	2,871.47	4.45
<i>Sargassum oligocystum</i>	Brown	22,807.39	6,084.18	14,449.90	2,101.32	17.14
<i>Caulerpa racemosa</i>	Green	20,204.13	2,585.10	10,663.87	1,333.86	8.16

**Seaweed product development study:** After washing by clear sea water then seaweeds are washed by running tap water and finally washed by clean freshwater for used as an ingredient in various foods item to enrichment of food product and stored after sun drying at room temperature for further use. To know the impact of seaweed addition in food items, for a test case normal salad and salad with seaweed (other salad items remain constant), seaweed soup, seaweed vegetables were prepared.



## Development of artificial breeding techniques of commercial important marine fin fishes, mullets (*Mugil cephalus* and *Liza* spp.)

**Researchers :** Ehsanul Karim, Scientific Officer  
Jakia Hasan, Scientific Officer  
Md. Mozammel Haque, Scientific  
Dr. Md. Enamul Hoq, CSO

### Objectives

- Brood development, domestication and artificial propagation of mullets ( *Mugil & Liza* sp.) in captivity
- Development of live feed culture technique for larval rearing of mullets

### Achievements

#### *Expt. 1. Brood development, domestication and artificial propagation of mullets*

**Selection of study area:** The experiment was conducted from August, 2014 to February, 2015 in Niribili Fish Farm, Rejukhal, Cox's Bazar. Two (60 decimal sized) brood rearing ponds, equal in depth, configuration and pattern including water supply facilities and also well organized inlet (connected to coastal areas) and outlet system to maintain saline water level were used. The water depth was maintained at a maximum of 1.4 m.

**Brood development:** Adult/sub-adults of mullets, *M. cephalus* were collected from the wild and stocked in the two ponds in Niribili Fish Farm in the month of August 2014. The fish were fed with commercially available floating feed twice daily @ 2-2.5% of their body weight for their rearing stage and added Vit-E (Selvitdex) in the month of October-November for gonadal maturation. The salinity was maintained between of 15-20 ppt in ponds for their growth stage and 25 ppt for brood development.

**Hatchery facility development:** Hatchery facilities such as broodstock conditioning tank and subsequent spawning, incubation, larval rearing and live feed mass culture unit were developed for induced breeding trials in the Niribili Fish Farm, Reju Khal, Cox'sBazar. The breeding trials were started in the November, 2014 and continued till February, 2015. The design of various types of holding tanks is shown in Table 1.



Fig. 1: Saline water introduction



Fig. 2: Blower setup



Fig. 3: UV-filter setup

#### *Expt. 2. Breeding trial of Mugil cephalus*

**Hormonal treatment and spawning:** Experimental trial on induced breeding was conducted during winter. Fishes were captured by seine net and transported to the holding tanks by plastic drums with anesthesia dose (2 ml/10 l water). Each tank has been provided with continuous water aeration. After transportation of broods, they were treated with Furacin (50 ppm), and females and males were separated.

After that, oocytes were sampled following Live Ovarian Biopsy (LOB) method. Injections were initiated within 48 hrs after transportation and acclimatization. Interval between injections varied from 24 to 36 hrs. Carp pituitary gland for 1<sup>st</sup> dose and LRH A<sub>2</sub> (2<sup>nd</sup> dose) with the combinations of Domperidone and Calcium injections were injected in varied dose. HCG also used for the 1<sup>st</sup> dose in male and both male/female in the second dose. In case of both female and male, hormone was injected in deep muscle at base of the dorsal fin.

Natural spawning in tanks with two un-injected males was also performed. Spawning behavior was observed visually. In case of fecundity study, the spent females were dissected and eggs retaining in the abdomen were counted for the measurement of fecundity volumetrically. Released eggs were continuously monitored by using ocular and stage micrometer to estimate eggs diameter. Fertilized eggs were kept in the spawning tank for incubation after treated with 0.5ml/l Streptomycin solution and observation continued through microscope until the starting of cell division.

The fishes started pairing just before they spawned; males were observed a little bit more active than female in the time of mating. The first release of a small number of eggs stimulated the male to release spermatozoa. Interval 1<sup>st</sup> and 2<sup>nd</sup> dose of injections was maintained 24 hrs and also in case of 3<sup>rd</sup> dose. The following results were found from the three breeding attempts described in Table 1.

**Table 1.** First breeding trial of *Mugil cephalus* in the 1<sup>st</sup> week of January, 2015

Sex	Total body wt (kg)	Total length (cm)	Injection dose (unit/fish)			LP (hrs)	D <sub>1</sub> (μ)	D <sub>2</sub> (μ)	TSE (eggs/gm bwt) /MC
			Priming (mg/kg)	Resolving 1 (after 24 hrs)	Resolving 2 (after 24 hrs)				
Female	1.05	41	30 cPG	LRH A <sub>2</sub> 100μg + 0.3ml Dom.+ 0.5 ml Ca- inj.	LRH A <sub>2</sub> 50μg + 0.3ml Dom.+ 0.2 ml Ca- inj	48	565	650	745
Female	1.30	44	40 cPG	LRH A <sub>2</sub> 150μg + 0.3ml Dom.+ 0.5 ml Ca- inj	LRH A <sub>2</sub> 50μg + 0.3ml Dom.+ 0.2 ml Ca- inj	48	580	665	736
Female	1.40	46	45 cPG	LRH A <sub>2</sub> 150μg + 0.3ml Dom.+ 0.5 ml Ca- inj	LRH A <sub>2</sub> 50μg + 0.3ml Dom.+ 0.2 ml Ca- inj	48	560	655	821
Male	0.95	38	-	5000 IU HCG	-	36			Less milt
Male	0.92	37	-	5000 IU HCG	-	36			Less milt
Male	1.02	40	-	No dose	-	-			N/R
Male	1.06	41	-	No dose	-	-			N/R
Male	0.87	34	-	5000 IU HCG	-	36			Less milt

LP = Latency period, D<sub>1</sub>= Mean ova diameter before priming dose, D<sub>2</sub> = Mean ova diameter after spawning, TSE = Total spawned eggs in case of female, MC = Mt conditions in case of male projected by positive sign, LRH A<sub>2</sub>= Leutinizing Releaseing Hormone, Dom.= Domperidone (Dopamine), Ca- inj.= Calcium Injection

In order to assess successful spawning, two female with two injected male and two un-injected male were kept in tank for one night. Eight from ten injected females responded with ovulation and spawning (four spawned in tank and rest were stripped) as spawning rate close 66 %. Rest of oocytes from female stripped produced 34% of fertilization. From this experiment, in most cases fertilization has been failed due to poor quality as well as quantity of milts or due to lack of good males, but one spawning produced more than 60% of fertilization which was calculated as the total number of fertilized eggs divided by the total sampled number (n=100) of eggs. Two females from 10 did not respond to multiple injections and developed atresia. Hydration was observed within 6–12 hrs after the injection of effective dose and spawning within 6–8 hrs after beginning of hydration. Initial diameter of oocytes in all the females varied within a range 550–600 μm (except of one case) showing no significant difference between females with positive and negative response. However, responded females had coalesced oil globule before injections and those which did not respond had partially or not fused oil droplets. The brief hypophysation results and diameter of eggs and oil globules are shown below in Table 2.



**Table 2.** Hypophyztion of *Mugil cephalus*

Weight of fish (kg)	Length of fish (cm)	Initial diameter of oocytes ( $\mu\text{m}$ )	Total dose of hormones per fish				Number of injections	Response ++/+/(-)
			PG (mg)	LRH ( $\mu\text{g}$ )	Domperidone ( $\mu\text{g}$ )	HCG (IU)		
1.05	41	565	30	100	0.3		3	+
1.30	44	580	40	150	0.3		3	+
1.40	46	560	45	150	0.3		3	++
1.85	53	587	60	300	0.3		3	+
1.65	49	563	50	-	-	30000	2	+
1.45	47	570	45	-	-	25000	2	(-)
1.70	51	570	55	250	0.3		3	+
1.25	42	560	40	100	0.3		2	+
1.48	45	575	45	150	0.3		3	+
1.67	49	570	50	-	-	30000	2	(-)

++ spawned with fertilized eggs, + spawned, -/(-) did not spawned & atresia checked

In the first breeding trial of the experiment, only one fertilization was taken place and the eggs took 44-48 hrs after spawning to hatch out. All eggs spawned, both fertilized and not, had single oil globule. Its diameter varied within a range 650–680  $\mu\text{m}$ , and oil globules had 250–280  $\mu\text{m}$  (Table 8). Fecundity of those females were determined as 735–900 eggs per g of body weight. After fertilization cell division started within an hour but the fertilized eggs were settled down before starting further segmentation and finally huge mortality occurred and then the dead cell were floating throughout the tank. Rapid fluctuation of temperature and poor quality as well as quantity of milts due to lack of good males were the main reason of this mortality (Table 3).

**Table 3.** Stages of breeding development *Mugil cephalus*

Age (hrs.) after fertilization	Stage of development	Temperature of water ( $^{\circ}\text{C}$ )
1.00	Two cells division	21.5
2.00	Cell division continued	22.2
6.00	Beginning of segmentation, mortality started	24.2
12.00	Heavy mortality	25.8

### **Expt. 3. Live feed culture technique for marine fish and shell fish larval rearing**

**Phytoplankton culture (*Nannocloropsis oculata*):** The culture period of *Nannocloropsis oculata* is 7days. Here we maintain indoor culture system.

**Zooplankton culture (Rotifer):** We need 20 liter *Nano* for batch-culture system of Rotifer. First the tanks (20 liter capacity) are half filled with *Nano* at a density of 13-14 X 10<sup>6</sup> cells/ml and inoculated with Rotifers at a density of 100 individuals/ml. The salinity of the water is 25 ppt and the temperature maintained at 30 $^{\circ}\text{C}$ . The first day active baker's yeast administered two times a day at a quantity of 0.25g/10<sup>6</sup> rotifers. The next day the tanks are completely with *Nano* at the same density and the same quantity of baker's yeast per million rotifers is added twice a day.



## Improvement of dried fish production system suitable for large-scale entrepreneurs and producers

**Researchers:** Ehsanul Karim, SO  
Md. Mozammel Hoque, SO  
Dr. Shfiqur Rahman, SSO

### Objectives

- Improvement of large scale fish drying techniques
- Standardization of procedure and materials for packaging to increase shelf-life of dried fish in storing and marketing

### Achievements

#### *Improvement of large scale fish drying techniques*

To produce hygienic dry fish in large scale, a model of improved traditional fish drying was developed by Marine Fisheries & Technology Station, BFRI, Cox's Bazar.

Total dry fish processing unit was covered with fine mesh net to protect infestation. The size and capacity of fish drying is variable. Dryer structure were box type with a length of 100 feet and 30 feet width (available drying space 15,000 sq. feet). The drying structure was built 4 feet above ground (+ 6 feet fish drying facilities). Fish dryer construction materials was bamboo pole, bamboo stick, nylon net (mesh size-0.8 cm), fine mesh net, rope and ladder. Bamboo poles were fixed at 3 feet interval. Dryer roof and peripheral fence covered with nylon net (mesh size- 0.8 cm) and dryer bottom covered with fine meshed net. Bottom surface area was used for drying of pomfret and small fishes. Bamboo rows were used for Bombay duck and ribbon fishes. Fish drying capacity was estimated as 3,000 kg dried fish per lot. Fish drying period was 3 days for one lot and one day breaking for cleaning. Fish drying capacity per month was  $(3,000 \text{ kg} \times 6 \text{ lot}) = 18,000 \text{ kg}$  dried fish per month.



**Fig 1.** Construction improved traditional fish drying facilities suitable for large scale entrepreneurs.

A comparative analysis of proximate composition and micro-nutrient of dried fish products from different entrepreneurs was carried out (Tables 1 & 2).

**Table 1.** Proximate composition dried fish products from a small dry fish trader of Cox’s Bazar

Entrepreneurs	Dry fishes	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)	Crude fiber (%)
Sunny Dry Foods	Lotia	16.78	56.55	9.43	16.18	-
	Churi	15.91	64.27	2.37	14.14	-
	Phaissa	14.37	64.50	8.04	13.08	-
	Ichery	14.96	54.16	13.08	17.81	-
Shah Amanat Traders	Lotia	22.90	49.11	15.87	11.95	-
	Churi	16.61	59.54	12.27	10.87	-
	Ichery	23.10	57.06	3.26	16.50	-
Hossain & Brothers	Lotia	19.98	50.41	19.41	10.04	-
	Churi	20.31	59.69	3.27	7.94	1.45
	Phaissa	21.54	52.12	10.59	15.15	-

**Table 2.** Proximate composition dried fish products from a small dry fish trader of Cox’s Bazar

Entrepreneurs	Dry fishes	Ca (ppm)	Fe (ppm)	Zn (ppm)
Sunny Dry Foods	Lotia	14,476.08	36.43	25.34
	Churi	27,242.84	10.50	14.49
	Phaissa	18,713.36	18.71	34.92
	Ichery	21,416.42	47.16	18.08
Shah Amanat Traders	Loittya	14,476.08	36.43	25.34
	Churi	24,945.59	10.27	13.86
	Ichery	22,391.42	45.11	16.74
Hossain & Brothers	Loittya	13,638.04	31.55	26.67
	Churi	25,682.41	9.34	17.26
	Phaissa	16,339.64	19.53	31.72

**Standardization of procedure and materials for packaging to increase shelf-life of dried fish**

Dried samples were packaged in vacuum sealed polythene bags with printed labeling. Before packing, headless technique for churi (ribbon fish) and lotia (bombay duck) was followed for higher shelf-life of dried fish. Dried fishes of 200 gm, 500 gm and 1,000 gm were packed for marketing in case of churi and lotia. Dried rup chanda (pomfret) was packed in celluloid in respect of cost minimize.





## Status of the existing marine fisheries products and investigation on the use of chemicals or pesticides in the products

**Researchers:** Dr. Shafiqur Rahman, SSO  
Ehsanul Karim, SO  
Dr. Md. Enamul Hoq, CSO

### Objectives

- Qualitative and shelf life study of the commercially important marine fisheries products.
- Improvement of available commercially important marine fisheries products.
- Investigation of pesticides and heavy metals in marine fisheries products.

### Achievements

Drying is the most common and widely used fish preservation technique in the coast. During drying process considerable weight loss occurred in fishes and weight loss varies from fish to fish (Table 1).

**Table 1.** Day to day weight loss (g) during drying of the common marine dried fishes

	Fresh Fish Day 1 wt. (g)	Day 2 wt. (g)	Final Day 3 wt. (g)
Loitya	1,045	252	175
Churi	1,082	510	341
Phaisa	1,009	509	376
Ichiri	1,005	239	237
Poa	1,005	691	464

The use of pesticides appears to be widespread, during both drying and storage. Pesticide application during drying is commonest with large fish when high levels of atmospheric moisture slow down the drying process. Last year we studied the heavy metal conc. of local dried fishes. This year we evaluate the dried fishes available in the local market, imported from neighboring country (Table 2).

**Table 2.** Heavy metal content in imported dried fish products

Imported dried fish products	Heavy metals (ppm)				
	As	Cr	Cu	Pb	Zn
Loitya ( <i>Harpadon nehereus</i> )	2.2	ND	0.78	0.17	9.11
Churi ( <i>Trichiurus</i> spp.)	2.8	ND	1.42	ND	7.46

Other ready to eat fish products from marine fish get popularity in the country. The most common ready to eat fish product is Belacan, produced from Acetes/Mysid shrimps and small Anchovy.

**Table 3.** Proximate composition of Belacan from Anchovy (*Engraulis encrasicolus*)

Product	Proximate composition (%)					
	Moisture	Protein	Lipid	Ash	Fiber	Carbohydrate
Belacan ( <i>Engraulis encrasicolus</i> )	14.89	60.09	15.73	9.35	0.40	0.62

## Factors causing emerging shrimp diseases and development of their health management strategies

**Researchers:** Dr. Shafiqur Rahman, SSO  
Zakia Hasan, SO

### Objectives

- To look into the factors affecting the emerging shrimp disease in Cox's Bazar.
- Assessment of the present status and seasonal variation of WSSV infection in wild brood, hatchery produced PL and farm rearing shrimp using Nested PCR technique.
- Diagnosis of bacterial diseases of shrimps causes severe damages in PL production and farming system.

### Achievement

#### *Shrimp farming in relation to disease outbreak*

Most of the gher (farms) were found to be practiced open systems in the study sites where some were unmanageably large, irregular shapes, and uneven bottom, inadequate water supply and drainage system. Majority of them were not able to drain out of water completely or not allowed to remain dry before stocking with Bagda, *Penaeus monodon* during preparation of farm either due to locations of farm i.e. farms are situated at low lying areas where water management is effected through tidal means and nature of the culture patterns involved of these areas i.e. bagda alternation with salt or rice alternates with shrimp. However, in Moheshkhali area most of the farmers were found to stock *P. monodon* for shrimp culture during April-May which continue up to November-December after that farmer use the land for salt cultivation but similarly they use deeper parts of the respective farm for cultivating *P. monodon* with wild fin fish as well with tilapia that continue up to the next season. *P. monodon* alternation with salt was also observed rest of the three Upzilla's but it was not found to practice at the respective shrimp farming cluster during the study period.

Farms were found to be stocked with hatchery produced shrimp post larvae as well as selective stocking of shrimp post larvae (PL) and fish, otherwise still allowed to trap natural shrimp seed and fish carried by tidal water and allowed to grow without any form of or little management i.e. use of lime and fertilizer, separate sluices for flushing and drainage, nursing of PL in nursery ponds and use of screens in sluice gates to prevent predators where prevailed the structures as well farm owner's long term engagement in shrimp farming practices. The individual shrimp farmers preferred to stock the farm with more shrimp PL (572 PL/dec) than did the other categories. The group farmers holding the largest amount of farming area stocked only 253 PL/dec. Overall the average stocking density in the study was 353 PL/dec. However, the average and range of stocking of *P. monodon* at different Upzilla's shrimp farming cluster were 198 (8-314; Sadar), 59 (50-67; Teknaf), 62 (37-109) and 90 (47-70) PL/dec (Table 1).

In the present study a wide variation in the salinity range 36-0 ppt from one place to another place was observed. Salinity level was little bit less in Teknaf shrimp farming cluster however, rest of the study sites it was almost varied within a low range which is suitable for *P. monodon* but during June 2015 it was reached almost zero in all respective study sites at the different upzilla's of Cox's Bazar. But in semi-intensive farm in such case add salt/brine for keeping salinity level at minimum level. On the other hand, for all the study areas temperature range was found almost similar and was varying between 28.2-32.3<sup>0</sup>C. The transparency of the present study was 28 to 72.7 cm. Lower photosynthetic activity due the presence of algal bloom on the surface of water body in the studied gher of the studied period might be the cause of increasing the secchi disc reading up to 72.7 cm. The DO content of water in shrimp ponds is

influenced by quantity of flushing of freshwater, tidal flow, temperature, salinity, algal growth and organic matter decomposition. The dissolved oxygen in all the ghers in the present study was ranging between 3.3 to 7.6 ppm. The sufficient DO in all ghers might be the resultant outcome of the water due to frequent entrance and exit tidal water. Ammonia is the principal end product of protein catabolism of organisms and it is excreted through gills. It is also formed by decay of organic matter. Under farm conditions, the ammonia level should be less than 0.01 ppm. In the present study, the level of unionized ammonia was well above this mark (0.02-0.38 ppm). Total alkalinity (66-135; 105-204, 111-159 and 102-162 at Cox's Bazar sadar, Teknaf, Mohesh-khali and Chakaria, respectively) was found to be varies from site to site. Low alkalinity in freshwater or in the low salinity area will affect the survival rate and molting of shrimp.

**Table 1.** Water quality parameters in shrimp farms under study areas of Cox's Bazar

Parameters	Sadar Cox's Bazar	Teknaf	Mohesh- khali	Chakaria	Semi-intensive farm, Cox's Bazar
Water depth (m)	0.93±0.17 0.68-1.15	0.90±0.14 0.67-1.10	0.53±0.18 0.34-0.87	0.85±0.17 0.61-1.10	1.05±0.11 0.95-1.20
Salinity (ppt)	21.8±8.0 0-35	21.25±4.1 0-30	24±11.4 0-36	22.88±10.41 0-35	18±14.44 5-31
Transparency (cm)	33.2±5.85 28-40	53.92±10.63 42-72.7	33±11.03 20-48	40.5±9.71 30-58	52.5±17.82 35-72
pH	8.5±0.41 8.2-9.1	8.19±0.12 8.0-8.5	8.58±0.35 8.1-9.2	8.33±0.34 8.0-8.8	8.15±0.26 7.9-8.5
Temperature ( <sup>o</sup> c)	30.8±1.41 29.1-32.5	30.31±1.27 28.2-32.3	31.13±0.82 30.2-32.3	31.56±0.59 31.04-32.4	31.75±0.87 30.6-32.7
Dissolved Oxygen (mg/l)	5.65±1.64 3.6-7.3	5.07±.71 3.3-6.0	6.05±1.6 3.7-7.6	5.69±1.2 4.2-7.3	5.58±2.21 3.8-8.8
Ammonia (mg/l)	0.132±0.08 0.03-0.23	0.08±0.02 0.06-0.11	0.15±0.09 0.04-0.28	0.13±0.10 0.04-0.34	0.09±0.06 0.02-0.17
Total alkalinity (mg/l)	107.4±12.58 66-135	149.57±42.58 105-204	127±18.26 111-159	123.43±21.52 102-162	97.5±12.61 84-111

#### ***Detection of WSSV in tiger shrimp brood stocks, nauplii and post larvae***

Tiger shrimp brood (a tip of pleopod, after spawning), nauplii, juvenile were collected from a hatchery (one mother-one tank). Tiger shrimp DNA was extracted according to the protocol of IQ 2000 WSSV detection and prevention system. For WSSV detection nested PCR method was used following the PCR protocol of IQ 2000 WSSV detection and prevention system. The result are given in Table 2.

**Table 2.** PCR test for WSSV detection in one mother on tanks broods, nauplii as well juvenile samples

Stage	No. of test (in different stage)	Positive	Negative	% of Positive
Shrimp brood	23 (in 1 hatchery)	7	16	30
Nauplii	15 (in 1 hatchery)	5	13	28
Juvenile	16 (in 3 gher/farm)	14	ND	100

## Availability of marine pearl producing bivalves in south-eastern coast of Bangladesh and culture potentialities

**Researchers:** Dr. Md. Enamul Hoq, CSO  
Md. Ataur Rahman, SO

### Objectives

- To investigate the major pearl producing bivalves in the south-east coast of Bangladesh.
- To identify the appropriate species for pearl culture by rearing in pond/cistern.
- Production of pearl through grafting nucleus in suitable oyster species.

### Achievements

#### *Availability of pearl bearing bivalve species*

The survey was carried out in different areas of the Sonadia and Moheshkhali in Cox's Bazar and Kuakata in Patuakhali. The primary criterion for the selection of this area was suitable geographical coverage for wider variety of marine pearl producing bivalves and good numbers of bivalve collectors presence. Specimens were collected by hand picking, dredging by hand and using rake hook from the muddy soft areas especially in Ghotivanga khal, Moheshkhali and rocky attached specimens were separated from the rock by using chisel and hammer. During the study periods a total 7 species of marine bivalves were collected from 6 sampling areas of the Sonadia and Moheshkhali in Cox's Bazar and Kuakata of Patuakhali (Table 1).

**Table 1.** List of marine bivalves as identified during the study period

Order	Family	Scientific name	English name	Local name
Pterioida	Anomiidae	<i>Placuna placenta</i>	Windowpane shell	Kortal
Pterioida	Pteriidae	<i>Pinctada</i> sp.	Pearl oyster	Kostura
Mytiloida	Mytilidae	<i>Perna viridis</i>	Green mussel	Kala zinuk
Veneroida	Veneridae	<i>Meretrix meretrix</i>	Poker-chip clam	Chilen
Pterioida	Pteriidae	<i>Pinctada margaritifera</i>	Pearl oyster	-----
Veneroida	Veneridae	<i>Meretrix lyrata</i>	Hard clam	Sada chilen
Ostreina	Ostreidae	<i>Crassoostrea ariakensis</i>		Kostura

**Environmental parameters of the study area:** During the period of investigation, water parameter was studied along with surrounding environment. The salinity of the sampling site was between 18 to 22 ppt. and highest salinity of 34 ppt was found in Chera dip, St. Martine, Cox's Bazar (Table 2). The pH range from 8 to 8.5 and water depth recorded from 0.2 to 2.0 meter and the bottom structure was observed as muddy, sandy and rocky.

**Table 2.** Environmental parameters of the study area

Sampling site	Location	Salinity (ppt)	pH	Bottom structure	Water depth (m)
Pakdia, Ghotivanga	Moheshkhali	20	8.5	Muddy, sandy	0.2-1.5
BIWTC	Nunier chora	18	8	Rocky, sandy	1.5-2.0

Bahadder brige	Sonadia	20	8.2	Muddy	1.0-1.5
Ghotivanga khal	Moheshkhali	22	8.5	Muddy, rocky	1.0-1.5
Chera dip	St. Martin	34	7.9	Sandy, rocky	0.1-5
Fatrar chor	Kuakata	20	8.1	Sandy	0.2- 1.0

### ***Rearing of bivalve and production of pearl***

Most abundant bivalve, *Placuna placenta* (kortal) were collected from the coastal river of Moheshkhali and stocked in fibre glass tank with saline water at MFTS, Cox's Bazar. The rearing experiment was started on December and continue up to June. A total 38 species (kortal) were sampled during the study period at two months interval. Among them 70% were found to produced pearl inside their body. A total 102 pearls were found inside the *P.*



*placenta* among them highest nos. of pearl (85) were found in the month of April and lowest nos. (7) were found in the month of December. From the above study it was observed that most of the pearl bearing oysters specially in *P. placenta* carried pearl inside their body during the aquarium culture. Although the size of produced pearls were very small, further study may give better understanding on culture potentialities on marine oysters.

**Water quality parameters during rearing of bivalves:** *P. placenta* (kortal) were reared during December to June in the fibre glass culture tank. The salinity varied from 20-26 ppt, however the fibre glass tanks were regularly fed with sea water. The pH value observed slightly alkaline (8.0-8.87). Dissolve oxygen was maintained between (7.70-8.6 ml/L), although regular aeration was provided. During the period of investigation the average temperature ranged from 21.6<sup>o</sup>C to 27.9<sup>o</sup>C in the fibre glass tanks. Average mortality rate observed was 12-22%.



**Fig. 1.** Pearl collected from *Placuna placenta* reared in fibre glass tank

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