Quality Mass Seed Production of Carp, Catfish and Prawn

Researchers: Dr. Md. Shaha Ali, Principal Scientific Officer
Dr. Selina Yeasmine, Senior Scientific Officer
Md. Golam Sajed Riar, Scientific Officer
Mohammad Rashed Parvej, Scientific Officer
Nur-A-Raushon, Scientific Officer

Objectives

- To upgrade and produce quality carps and catfishes spawn/fry and disseminate to the farmers, entrepreneurs and hatchery/nursery owners
- To develop live gene bank with quality brood stocks through implementation of effective breeding plan
- To evaluate the growth performance of Crucian carp (*Carassius carassius*) under poly culture system in farmers ponds
- To produce quality seed and improved nursery techniques of PL of freshwater prawn (*M. rogenbergii*)

Achievements

Expt. 1. Development of carps brood from wild sources of river Halda and Jamuna

With a view to produced wild carp broods (previously collected from wild sources), grow-out ponds were stocked with 20% best selected fingerlings under polyculture system. The grow-out management and broods development is in progress and the present status of wild stocks are shown in Table 1.

Table 1. Present status of the wild stocks of river Halda and Jamuna

Species	Halda (Wt. range (g)	Jamuna (Wt. range (g)
Catla	2500-3500	2000-3000
Rohu	1500-2000	1500-2500
Mrigal	1500-2000	1500-2000

Quality seed production of carp and catfish

Following the established methods of breeding management, every year BFRI has been producing quality seeds of carps/catfishes/prawn and distributing to the fish farmers, hatchery owners and nursery owners in different parts of Bangladesh. On the otherhand, the proposed developed wild and improved broods are being used for mass seed production and distributing to the fish farmers/ and nursery operators and also to the hatchery owners for further mass seed production in their own hatcheries. Quality mass seed production of carps, pangas and prawn so far achieved during the year 2016 is shown in Table 2.

Table 2. Quality seed production of carps, pangas and prawn

Species	Breeding period	Production achieved		
		Spawn (kg)	Fry/ Fingerling (nos.)	PL (nos.)
Common Carp	Dec-Feb	10	1,50,000	
Catla (Halda)	May-June	45	-	
BFRI Rohu	May-August	110	1,00,000	
Mrigal (Halda)	May-August	45	-	
Pure line Silver carp	March-July	15	-	
Pure line Bighead	March-July	30	-	
BFRI-GISB (Silver Barb)	March-July	50	100,000	

Thai Pangas (Red meat)	June-August	25	-	
Vietnamese Pangas (White)	June-August	5	150,000	
Prawn(Wild source)	May-August	-	-	5,000
	Total	335	500,000	5,000

Expt. 2. Growth study of Crucian carp (C. carassius) with monosex male tilapia under different stocking density in polyculture system

To study the growth performance of *C. carassius* fingerling under polyculture system a rearing experiments were conducted in earthen ponds. Fingerling of crucian carp were stocked at the rate of 100 & 150 /decimal with monosex male Tilapia 150 & 100/decimal for each replication and with a plan to rear for a period of 20-21 weeks. Commercial feeds (25-30% protein) were used @ 4-5% of the body weight. The fish were sampled in alternate weeks to assess growth performance and adjust the feed ration. The experiment is in progress. After rearing period the fish will be harvested and growth data will be recorded and compiled. The results of this experiment so far achieved are shown in Table 3.

Table 3. Stocking details of Crucian carp (CC) with monosex male Tilapia (MMT) under polyculture system

Treatment	Crucian Carp (CC)		Monosex Male Tilapia (MMT)	
	(15-05-2016) Initial Wt. (g)	(15-08-2016) Present status (g)	(15-05-2016) Initial Wt. (g)	15-08-2016) Present status (g)
T_1 (CC 100+ MMT 150)	13.00±1.20	69.20±5.60	2.29±0.59	43.29±4.59
T_2 (CC 150 + MMT 100)	12.67±1.74	48.89±12.71	2.21±0.43	55.21±4.43

Production of quality seed of freshwater prawn (*M. rogenbergii*)

There is a golda hatchery in BFRI, FS hatchery complex. Wild source of prawn broods were collected from Boleswari River, Pirojpur and Brine were collected from Pekua Upazilla of Cox's Bazar. Brood rearing and seed production technique were followed as standard protocol in the golda hatchery. The results so far achieved for the production of *M. rosenbergii* post larvae (PL) in BFRI, FS golda hatchery are shown in Table 4.

Table 4. Details of hatching, stocking and survival rate of *M. rosenbergii* PL production in golda hatchery

Water used	Hatching rate (%)	SD /L	Rearing period (days)	% survival of larvae (days)					PL conversion
				7	14	21	28	30-35	
Brine	92.50	100	30-35	90	82	75	20	08	30-35 days of expt. larvae converted to PL
Comments: After 26-27 days of larvae rearing, most of the larvae died due to sudden fall down of air as well as water temperature.									

Stock Improvement and Dissemination of Commercially Important Tilapia and Climbing Perch Koi through Genetic Selection

Researchers: Dr. A. H. M. Kohinoor, Principal Scientific Officer
Md. Moshiur Rahman, Scientific Officer

Objectives

- To improve the stock of BFRI-GIFT strain using family selection protocol

- To improve the stock of Thai Koi through brood stock replacement technique
- To evaluate the production performance of BFRI GIFT and Thai koi with magur, gulsha and Shing at different stocking densities

Achievements

Expt. 1. Stock improvement of the GIFT strain by family selection in Bangladesh

Due to rapid expansion of tilapia hatcheries, quantity of seed production has increased dramatically but genetic quality of those seeds has deteriorated due to poor brood stock management. On the other hand, most of these hatcheries function in genetic and reproductive isolation (i.e. no introductions or replacement by new stocks) and repeatedly use of the same stock every year just to maximize the fixed target of seed production. As a result, tilapia grow out farmers are not in the position of maximizing the target of production and profit due to using such poor quality seeds. In view of overcoming this situation and mitigating the growing demand for genetically improved tilapia brood stock for quality seed production in the country, Bangladesh Fisheries Research Institute (BFRI) has undertaken a family selection program since 1995 to continue improving genetic quality of the GIFT (Genetically Improved Farmed Tilapia) strain. Since then, this work has been conducted in collaboration with WorldFish, Penang, Malaysia. The aim of the present study was to evaluate growth performance of the GIFT strain after six generations of genetic selection for increased body weight at BFRI. The founder stock comprised of 30 families having 300 individuals of the GIFT strain was introduced from Malaysia through WorldFish in March 2005. The stock was reared in 100 m2? hapa for three months, and then individually tagged using Passive Integrated Transponder (PIT) tags at the mean weight between 30 and 40g. After tagging, Tagging being done???, which tag? all the fish were communally grown out in pond until harvest. Breeding value for body weight was estimated using SAS and ASREML statistical packages. The best (highest) breeding values of brooders (40 females and 40 males) from the founder stock were then selected to produce progeny of the first generation (Fx1) in 2007. From each family 20 female and 20 male fingerlings were randomly sampled and PIT tagged. A total of 2,000 tagged fish from 40 families were stocked in a pond (1000 m2) for continuation of the selection program. The same protocol was followed in subsequent generations in 2008, 2009, 2010, 2011, 2012, 2013, 2014 and 2015. In addition, surplus fish after tagging were also reared together with progeny of the founder stock in earthen ponds for growth evaluation. General linear model analysis indicated that the selected fish had 7.17, 13.60, 23.21, 30.30, 35.38, 39.25%, 43.19%, 49.03 and 52.82% greater harvest weight than that of the founder population in F1, F2, F3, F4, F5, F6, F7, F8 & F9 generations, respectively (Fig. 1). The continued stock improvement of GIFT strain by family selection in every generation at BFRI, enable the institute to supply improved germplasm every year to over 280 tilapia hatcheries for high quality seed production in the country. This attempt was greatly contributing to sustainable increase of tilapia production in Bangladesh.

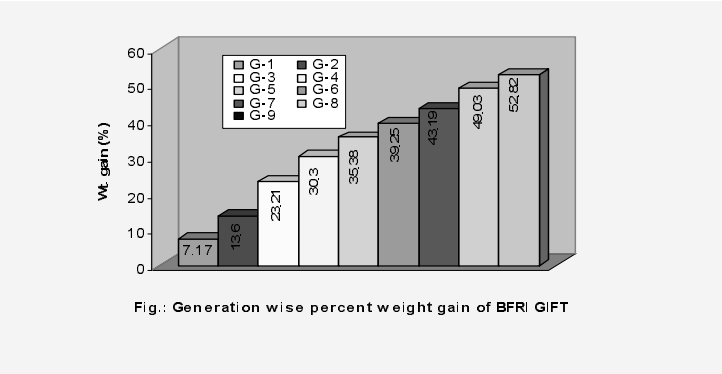


Fig.: Generation wise percent weight gain of BFRI GIFT

Expt. 2. Validation trial of GIFT tilapia with Gulsha and Magur in semi-intensive culture

Validation trial of GIFT with gulsha (*M. cavasius*) and magur (*C. batrachus*) were carried out for five months during February 2015 to June 2016 in six farmers pond at Dohakhola, under Gouripur upazila, Mymensingh. Three stocking densities of magur and gulsha were tested keeping the monosex GIFT Tilapia stocking density similar. Each stocking density of magur and gulsha was considered as

treatment and replicated thrice. Fingerlings of magur and gulsha were stocked at the rate of 5000 & 50000; 10000 & 45000 and 15000 & 40000 in treatments-I, II and III, respectively. In all the treatments mono sex GIFT tilapia were stocked at the rate of 50,000/ha. The same regime of pelleted feed (28% crude protein) was applied in all the treatments. The results showed that monosex GIFT tilapia reached an average weight of 232.40±10.06g in T-1, 246.15 ±9.85g in T-2 and 240.77 ±8.85g in T-3, respectively (Table 1). The average final weights of magur were 160.20 ±7.90g, 151.10 ±7.80 and 145.60±8.60g in treatment-1, treatment-2 and treatment-3, respectively. The poor harvesting weight was observed in treatment-3 whereas, comparatively higher harvesting mean weight was observed in treatment-1. However, it was 20.09 ±3.66, 23.90 ±3.87 and 26.27 ±3.29g in treatments-1, 2 and 3, respectively. After five months rearing, the production obtained were 11808, 13525 and 13225 kg/ha ?months from T-1, T-2 and T-3, respectively. The highest production was obtained from T-2, where monosex were stocked with magur and gulsha at the stocking density 10,000 & 45,000/ha. The lowest production was obtained in T-1 where magur and gulsha were stocked at 5,000 & 50,000/ha. The production level of treatment-1 showed significant difference (P>0.05) with treatment-2 and 3. The contribution of magur and gulsha in total production was 11.86% in treatment-1, while in T-2 and T-3 were 15.67 and 20.72%, respectively. Is BFRI technology is validated through this trial? If so pl . explain with a few words.

Table 1. Harvesting weight and production of fish under different treatments

Treat	Fish sp.	Stock. den./dec	Harvesting Wt. (g)	Survival (%)	Sp. wise prod./dec.	Production (Kg/ha)
T-1	GIFT	200	232.40±10.06	93	42.22	11808[a]
	Magur	20	160.20 ±7.90	70	2.24	
	Gulsha	200	20.09 ±3.66	69	2.77	
T-2	GIFT	200	246.15 ±9.85	95	46.77	13525[b]
	Magur	40	151.10 ±7.80	67	4.02	
	Gulsha	180	23.90 ±3.87	77	3.31	
T-3	GIFT	200	240.77 ±8.85	91	43.82	13225[b]
	Magur	60	145.60±8.60	65	5.68	
	Gulsha	160	26.27 ±3.29	81	3.40	

*Figures in the same row having the same superscripts are not significantly different (P>0.05)

Expt. 3. Stock improvement of Thai Koi through brood stock replacement technique

In another study, stock improvement program of *A. testudineus* was also undertaken through brood stock replacement technique. The largest 400 individuals (200 male and 200 female) of parental generation were selected and stocked in a breeding pond for the production of F_1 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 20 gm 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. The same procedure was followed for the production F2, F3, F4, F5 and F6 generation. Growth performance of improved F1, F2, F3, F4, F5 and F6 generation of Thai Koi (*A. testudineus*) showed 5.9, 11.97, 15.59, 21.58, 25.39 and 28.22% greater harvest weight than that of the offspring of founder generation.

Expt. 4. Validation trial of Koi with Shing and GIFT Tilapia in semi-intensive culture

Validation trial of Koi with Shing and GIFT was carried out for a period of four months from March to June 2016 at Dohakhola village in Gouripur upazilla under Mymensingh in six farmers ponds having an area of 20 decimal each. In all the treatments, the stocking density of Koi was same. But shing and GIFT density were varied with treatment. Design of experiment is shown in Table 2.

Table 2. Stocking density and species combination of fish under different treatments

Treatment	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

The fish were fed with supplementary feed (30% crude protein) at the rate of 5-20% of estimated body weight in all the treatments. After four months of rearing, all ponds were completely harvested, first by seine netting followed by draining out of the ponds. Koi reached at an average final weight of 145 ±6.12, 136±5.85 and 133± 6.11g in treatments-1, 2 and 3, respectively (Table 3). The final weights of Shing were 31 ± 6.89g in treatment-1, 34±5.55g in treatment-2 and 40 ± 6.14g in treatment-3. Relatively, identical growth of monosex GIFT tilapia in terms of weight was attained in all the treatments. It grew to an average weight of 215±8.80, 207±9.10 and 201± 7.40g 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 tilapia were ranged from 83-87, 74-82 and 91-92%, 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 17778 kg/ha were obtained in treatment-1 and the lowest of 16823 kg/ha in treatment-2. There were no significant differences ($P<0.05$) observed among the treatments.

Table 3. Harvesting weight and production of fish under different treatments

Treat.	Fish sp.	Stocking /dec.	Harvesting Wt. (g)	Survival (%)	Sp. wise prod./dec.	Production (Kg/ha)
T-1	Koi	500	145 ±6.12	87	63.08	17620[a]
	Shing	150	31 ± 6.89	74	3.44	
	GIFT	20	215±8.80	92	3.96	
T-2	Koi	500	136±5.85	83	56.44	16823[a]
	Shing	130	34±5.55	77	3.40	
	GIFT	40	207±9.10	90	7.45	
T-3	Koi	500	133± 6.11	85	56.53	17778[a]
	Shing	110	40 ± 6.14	82	3.61	
	GIFT	60	201± 7.40	91	10.97	

*Figures in the same row having the same superscripts are not significantly different ($P>0.05$)

Stock Improvement and Dissemination of Thai Pangas *(Pangasianodon hypophthalmus)*

Researcher: Dr. Md. Shaha Ali, Principal Scientific Officer

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. Production of base population (F_1 generation) of Thai pangas from founder stock using group breeding technique

Breeding of pangas (Batch-1) was initiated in 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 the production of base population (F_1 generation) 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. About 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 feed 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 were being used for the production of F_2 generation in the year 2015. The status of base population of pangas in brood ponds is shown in Table 2.

Table 1. Status of base population (F_1 generation) 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	25.6 0 ± 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

Growth performances of base population (F_1 generation) of Thai pangas in rearing ponds were more or less same in all groups.

Table 2. Status of base population (F_1 generation) of Thai pangas in brood ponds

Group	Pond size (Dec.)	SD (Nos)	Initial		Final	
			Length (cm)	Weight (g)	Length (cm)	Weight (g)
A	25	200	30.25 ± 3.43	525.6 0 ± 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

Growth performances of base population (F_1 generation) of Thai pangas in brood ponds were more or less same in all groups.

Expt. 2. Comparative growth study of improved (F_1 generation) and existing stocks of Thai Pangas (Pangasianodon hypophthalmus) in farmers ponds at Kurigram district, Bangladesh

For evaluation of growth performance of each generation, comparative growth trial were conducted using fingerlings from base population (F_1 generation) groups of improved Thai pangas with existing local stocks of pangas in the farmers field of 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 of rearing the fish were harvested and data were presented in Table 3.

Table 3. Comparative growth performance of improved (F_1 generation) and existing stocks of Thai pangas in farmers ponds at Kurigram district, Bangladesh

Stock	Socking density/ dec.	Initial weight (g)	Final weight (g)	Trial period (day)	Daily weight gain (g)/d	Comments
Improved Pangas (**F_1 generation**)	120	18.56±3.56	884.67±17.89	180	4.81	Improved (F_1 generation) of Thai pangas (BP) showed 10-11% higher growth compared to local Thai stock
Local stock of Pangas	120	20.34±6.45	803.56±18.33	180	4.35	

For the production of F_2 generation of Thai pangas breeding was initiated in July 2015. At the end of the March, 200 pairs of immature brood (above 1.5 kg Male and above 2.5 kg Female) were stocked in BFRI, FS pond complex to mature them for breeding. For the production of F_2 generation at least 60 pairs out of 200 pairs of brood from each groups (A, B, C and D of F_1 generation) 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. For rotational breeding, female from group- A were mated with male from group-D, female from group-B were mated with male from group-A, female from group-C were mated with male from group-B and female from group-D were mated with male from group-C which were produced F_2 generation group A, B, C and D respectively.

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. About 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 4. During all phases of the growing period, the fish were fed 30% protein rich feed 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) will be kept in brood ponds until they will be used for the production of F_3 generation in the year 2018. The present status of F_2 generation of pangas in brood ponds is shown in Table 5. This F_2 generation of improved Thai pangas will be used for the production of F_3 generation during the year 2018. Genetic enhancement of pangas will further be continued generation after generation following the same rotational breeding protocol.

Table 4. Status of F_2 generation 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	16.46 ± 1.33	35.7 8 ± 1.49	38.23 ± 1.27	490.77 ± 6.45
B	20	1500	17.66 ± 3.05	41.03 ± 3.03	42.41 ± 2.34	510.15 ± 8.25
C	20	1500	15.78 ± 1.19	31.28 ± 1.53	37.22 ± 1.92	487.89 ± 2.34
D	20	1500	19.95 ± 2.68	42.38 ± 4.20	43.29 ± 2.63	514.45 ± 5.89

Growth performances of F_2 generation of Thai pangas in rearing ponds were more or less same in all groups.

Table 5. Present status of F_2 generation of Thai pangas in brood ponds

Group	Pond size (Dec.)	SD (Nos)	Initial		Present Status (April 2016)	
			Length (cm)	Weight (g)	Length (cm)	Weight (g)
A	25	200	38.23 ± 1.27	490.77 ± 6.45	45.49 ± 3.94	1,055.40 ± 39.26
B	25	200	42.41 ± 2.34	510.15 ± 8.25	49.23 ± 9.56	902.20 ± 28.38
C	25	200	37.22 ± 1.92	487.89 ± 2.34	46.77 ± 2.55	990.76 ± 54.23
D	25	200	43.29 ± 2.63	514.45 ± 5.89	48.29 ± 7.77	1127.34 ± 35.33

Growth performances of F_2 generation of Thai pangas in brood ponds were more or less same in all groups. These broods are being rearing in brood ponds until breeding will be initiated for the production of F_3 generation during the year 2018.

Expt. 4. Comparative growth study of improved F_2 generation and existing stocks of Thai Pangas (Pangasianodon hypophthalmus) in farmers ponds at Mymensingh

For the evaluation of growth performance of each generation, comparative growth trial were conducted using fingerlings of improved F_2 generation of Thai pangas with existing local stocks of pangas in the farmers field at Mymensingh district. The stocking density was maintained 120 fingerlings/decimal and the fish were feed with 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 6.

Table 6. Comparative growth performance of improved F_2 generation and existing stocks of Thai pangas in farmers ponds at Mymensingh

Stock	Socking density/ dec.	Initial wt. (g)	Final wt. (g)	Trial (day)	Daily wt. gain (g)/d	Comments
Improved Pangas (**F_1 generation**)	120	15.33±1.16	954.93±29.44	180	5.22	Improved F_2 generation of Thai pangas showed 14-15% higher growth compared to local Thai stock
Local Stock	120	16.02±6.45	824.22±22.21	180	4.49	

Development of Induced Breeding and Culture Techniques for Mekong Giant Catfish, *Pangasianodon gigas*

Researchers: Dr. Md. Khalilur Rahman, Chief Scientific Officer
Dr. Jubaida Nasreen Akhter, Principal Scientific Officer
Md. Rayhan Hossain, Scientific Officer

Objectives

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

Achievements

Rearing managements

Sixteen fishes were stocked in pond no 1 and a total of 10 fish were checked on 18 March 2016. Female *Pangasianodon 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. There were no physical abnormality were observed (Table 1).

Table 1. Observing gonadal maturation of *Pangasianodon gigas* on 18 March 2016

Sl	Sex	Length (m)	Weight (kg)	Comments
1	♂	1.58	60	Active, strong & strut
2	♀	1.59	61	Active, strong & strut
3	♀	1.50	57	Active, strong & strut
4	♀	1.52	58	Active, strong & strut
5	♀	1.64	65	Active, strong & strut
6	♀	1.65	65	Active, strong & strut
7	♀	1.50	58	Active, strong & strut
8	♂	1.50	59	Active, strong & strut
9	♂	1.60	62	Active, strong & strut
10	♂	1.64	64	Active, strong & strut

Among 34, a total of 10 fish were checked in pond 2 on 28 April 2016. 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. Neither parasite nor diseases were found in fish body surface rather fish were taint to swim actively and taking feed spontaneously (Table 2). Abdomen was found hard having huge papas both male and female. No milt was found with the male.

Table 2. Observing gonadal development of *Pangasianodon gigas* on 28 March 2016

Sl	Sex	Length (m)	Weight (kg)	Comments
1	♀	1.48	50	Active, strong & strut
2	♂	1.44	42	Active, strong & strut
3	♀	1.46	46	Active, strong & strut
4	♀	1.52	58	Active, strong & strut
5	♀	1.46	48	Active, strong & strut
6	♀	1.36	39	Died due to injury
7	♀	1.44	43	Active, strong & strut
8	♀	1.50	54	Active, strong & strut
9	♂	1.32	34	Active, strong & strut
10	♀	1.30	30	Active, strong & strut

Again gonadal maturation of *Pangasianodon gigas* was in the pond 1 on 01 June 2016 (Table 3). Female *P. gigas* was assessed by observing external features of abdomen and, colour and shape of genital papillae while their male counterparts were assessed by gentle pressing the abdomen to get milt. Bulging belly was observed in female fishes and genital papillae were looking radish. Comparatively large and soft belly was observed with the female. However no milt was found with male.

Table 3. Observing gonadal development of *Pangasianodon gigas* on 01 June 2016

Sl	Sex	Length (m)	Weight (kg)	Comments
1	♂	1.61	62	Active, strong & strut
2	♀	1.68	64	Active, strong & strut
3	♀	1.64	65	Active, strong & strut
4	♀	1.64	64	Active, strong & strut
5	♀	1.63	65	Active, strong & strut

6	♀	1.65	66	Active, strong & strut
7	♀	1.67	67	Active, strong & strut
8	♂	1.68	68	Active, strong & strut
9	♂	1.61	63	Active, strong & strut
10	♂	1.63	65	Active, strong & strut

Inducing breeding trial 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 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. Preparatory dose was 3 mg/kg BWt and was administered at 20:00 hours on 12/06/2015. After 12 hours interval 2nd dose was applied at the rate of 6 mg/kg BWt at 08:00 hours on the following morning (13/06/2015). The male received a single dose at a rate of 3 mg/kg BWt at the time of 2nd dose of the female (Table 4).

Table 4. Details of induced breeding trials on *Pangasianodon gigas*

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

At 16:00 hours (13/6/15) 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 morning (14/06/2015), at 08:00 hours, the injected fishes were checked again and found no change. Then the fishes were treated with $KMnO_4$ to prevent secondary infection on body skin, lip of mouth and fins. Finally, the injected fishes were released in the pond.

Mass Seed Production and Conservation of Endangered Important Fish Species in Bangladesh

Researchers: Md. Moshiur Rahman, Scientific Officer
Jonaira Rashid, Scientific Officer

Objectives

- To optimize the hormone doses for Foli, *Notopterus notopterus*
- To develop appropriate seed production technique of Shal Baim, *Mastacebelus armatus* through artificial propagation
- To optimize the suitable stocking density of mohashol (*Tor putitora*) for mass seed production

Achievements

Expt. 1. Standardization of hormone doses for N. notopterus

Fishes were collected from different areas of Mymensingh during March, 2016. After collection, male and female brood fishes were stocked in the separate ponds of Freshwater Station. Brood fishes were fed daily (2-5%) with locally available pellet feed (28-30% crude protein). Water quality parameters such as Temperature (°C), pH, DO (mg/l) and total ammonia (mg/l) were analyzed at weekly interval. The values of temperature, transparency, dissolved oxygen; pH and total ammonia were 19.6–30.69°C, 40-60 cm, 4.72-7.55 mg/l, 7.21-8.66 and 0.0-0.02 mg/l, respectively. Regular exchanges of underground water were facilitated to attain sexual maturity. Corresponding data representing the effects of PG doses on ovulation rate, fertilization rate, hatching rate and survival rate are shown in Table 1.

Table 1. Performances of different doses of PG on induced breeding of Foli

Treatments	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)	Survival rate (%)
T_1	66.67 ± 6.52	55.16 ± 9.54	58.09 ± 7.56	71.51 ± 6.89
T_2	82.23 ± 8.41	78.83 ± 6.92	86 ± 2.86	79.31 ± 6.32
T_3	73.91 ± 4.32	69.71 ± 8.29	75.24 ± 4.36	70.29 ± 3.74

Expt. 2. Development of induce breeding technique of Shal Baim, Mastacebelus armatus

To optimize seed production technique of *Mastacebelus armatus*, mature fishes (average 200-300g weight) were collected from natural habitat during February, 2016. Brood fishes of both sexes were stocked in cemented tanks (15m^2 areas). Tanks were provided with all facilities including continuous water supply through porous plastic pipes for aeration. The fishes were fed with pellet feed and trash fish (3-5% body wt). As the fish has hiding tendency, pieces of PVC pipe were used as shelter in each tank. Water quality parameters (DO, pH, temperature and Total ammonia etc) of the tanks were monitored at fortnightly intervals. The values of temperature, dissolved oxygen; pH and total ammonia were 21.6 – 26.69°C, 30-40 cm, 3.63-6.31 mg/l, 7.2-8.53 and 0.0-0.03 mg/l, respectively. A regular exchange of underground water was facilitated to attain sexual maturity. Breeding trials were carried out during June–July. Different doses of PG extract were used as inducing agent in the breeding trials T_1, T_2, and T_3 for females. PG doses of 30, 35 and 40 mg/kg body weight of fish. 1st two doses did not show any response in fish but in the 3rd dose precipitated ovulation and successful striping of ovulated eggs observed.

Table 2. Observation of induced breeding trials of *M. armatus*

Treatment	Mean body weight (g)		Dose of 1st injection (mg/kg^{-1})		Interval of 2nd injection (hr)	Dose of 2nd injection (mg/kg^{-1})		Ovulation period (hr)	Fertilization rate (%)	Hatching period (hr)	Incubation Temp. (°C)	Remarks
	Male	Female	Male	Female		Male	Female					
T_1	250.5 ±9.41	271.9 ±6.23	-	10	06	10	20	-	-	-	-	No ovulation
T_2	190.2 ±10.1	231.8 ±8.43	-	15	06	10	20	-	-	-	-	Partial ovulation
T_3	201.0 ±11.2	231.8 ±7.38	-	20	06	10	20					Complete ovulation.

Expt. 3. Optimization of stocking density of mohashol (Tor putitora) for mass seed production

Brood mohashol fishes were stocked in pond having an area of 20 decimal. The male and female mohashol were reared in separate pond. Stocking density was maintained 2 and 3 female

fishes/decimal under treatment-1 and treatment-2, respectively in two different ponds. The weight of female fishes were 824.6 ± 52.24 in treatment-1 and 909.5 ± 89.35 in treatment-2 whereas in case of male fish weight were 732.5 ± 68.41. Brood fishes were fed with improved supplementary diet containing 28-30% protein at the rate of 2-5% body weight. Water quality parameters such as Temperature (°C), pH, DO (mg/l) and total ammonia (mg/l) were analyzed at weekly interval. The values of temperature, transparency, dissolved oxygen; pH and total ammonia were 15.8 – 21.76°C, 30-50 cm, 5.26-8.21 mg/l, 6.96-8.59 and 0.0-0.01 mg/l, respectively. Deep tubewell water was supplied every day to enrich dissolved oxygen in male and female brood rearing ponds. After rearing, breeding trial was conducted during November-January. During this period in treatment-1, 92.5% female was responded and in treatment-2, 85% female was responded. Among them 89.1% female was normally spawned and 10.9% atretic oocyte was found in treatment-1 while 80.4% female normally spawned and 19.6% atretic oocyte was found in treatment-2. No post spawning mortality was observed.

Table 3. Breeding performance of mohashol in different treatments

Description	Treatment-1	Treatment-2
Female No.	40	60
Responded Female No. (%)	92.5	85
Normally spawned brood (%)	89.1	80.4
Atretic oocyte (%)	10.9	19.6
Not Spawned (%)	3	9
Post spawning Mortality (%)	0	0

Table 4. Spawning performance of mohashol in different treatments

Description	Treatment-1	Treatment-2
Fecundity No. (500-800g Fish)	4275-6460	3,895-5,985
Egg Fertility (%)	83-92	79-88
Hatchability (%)	75-82	70-78

Development of Feeds with Probiotics and Optimization of Feeding Strategies for Important Fish Farming

Researchers: Dr. Md. Zulfikar Ali, Principal Scientific Officer
Dr. Sayeeda Sultana, Senior Scientific Officer
Mritunjoy Paul, Scientific Officer

Objectives

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

Achievements

A series of feeding trials were conducted to develop and optimize of feeds with probiotics and feeding strategies for *Mystus cavasius*. 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 *Mystus cavasius* 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 *Mystus cavasius* for 6 months (feeding trail-3) is also in progress. Details of technical progress of the feeding trials are described below briefly.

Expt. 1. Optimizing dietary protein to energy ratio (P/E ratio) in Mystus cavasius

Six experimental diets were formulated to contain two levels of protein (28 and 32%), 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 13.78 to 16.76 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%. Composition of the experimental diets and their proximate analyses are shown in Table 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 were 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.

Table 1. Formulation and proximate composition of the experimental diets (% dry weight) for *Mystus cavasius*

	Diets number					
Diet no.:	1	2	3	4	5	6
(Protein / Lipid), (%)	(28/5)	(28/10)	(28/15)	(32/5)	(32/10)	(32/15)
Ingredients						
Fishmeal	40.00	40.00	40.00	48.50	48.50	48.50
Mustard Oil Cake	18.00	18.00	18.00	17.00	17.00	17.00
Rich bran (auto)	12.00	12.00	12.00	10.00	10.00	10.00
Starch	25.80	22.30	17.30	20.30	16.80	11.80
Soybean Oil	00	3.50	8.50	00	3.50	8.50
Alpha cellulose	1.50	1.50	1.50	1.50	1.50	1.50
Binder (Carboxymethyl cellulose)	2.00	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin and Minerals Premix	0.20	0.20	0.20	0.20	0.20	0.20
Proximate composition						
Crude Protein	28.10	28.15	28.15	32.08	30.08	35.10
Crude Fat	5.20	10.16	15.17	5.30	10.07	15.06
Ash	9.87	9.87	9.97	11.22	11.22	11.22
Fibre	3.98	3.98	3.98	3.81	3.81	3.81
NFE	45.34	45.34	45.34	44.16	44.16	44.16
GE (kJ g^{-1})	17.08	18.46	20.43	17.76	19.14	21.12
P / GE ratio	16.48	15.25	13.78	16.76	16.76	15.21

NFE = Nitrogen free extractives, calculated as 100 – (% protein + % Lipid + % Ash + % Fibre)
GE = Gross energy content, P / GE ratio = Protein to energy ratio in mg protein/ kJ^{-1} GE

Table 1.1. Mean growth performance and feed utilization of *Mystus cavasius* fed various P/E ratios

	Diets number					
Diet no.:	**1**	**2**	**3**	**4**	**5**	**6**
(Protein / Lipid), (%)	**(28/5)**	**(28/10)**	**(28/15)**	**(32/5)**	**(32/10)**	**(32/15)**
Initial body wt. (g)	0.75^{a} ± 0.04	0.75^{a} ± 0.03	0.77^{a} ± 0.03	0.77^{a} ± 0.02	0.76^{a} ± 0.04	0.76^{a} ± 0.01
Final body wt. (g)	5.23^{d} ± 0.04	5.50^{c} ± 0.07	5.78^{bc} ± 0.04	6.10^{b} ± 0.07	$\underline{7.03^{a}}$ ± 0.11	6.98^{a} ± 0.11
Weight gain (g)	4.48^{d} ± 0.014	4.75^{c} ± 0.04	5.01^{bc} ± 0.01	5.34^{b} ± 0.05	$\underline{6.27^{a}}$ ± 0.06	6.22^{a} ± 0.09

Weight gain (%)	597.65^{d} ± 34.75	633.68^{c} ± 18.24	650.43^{bc} ± 22.97	697.57^{b} ± 12.88	825.39^{a} ± 37.70	817.79^{a} ± 3.13
Specific growth rate (SGR) (% day)	3.24^{d} ± 0.0/8	3.32^{c} ± 0.04	3.36^{bc} ± 0.05	3.46^{b} ± 0.03	3.71^{a} ± 0.07	3.70^{a} ± 0.01
Food conversion ratio (FCR)	1.66^{a} ± 0.02	1.59^{a} ± 0.01	1.49^{b} ± 0.05	1.40^{b} ± 0.05	1.28^{c} ± 0.03	1.35^{bc} ± 0.04
Protein efficiency ratio (PER)	2.30^{a} ± 0.05	2.35^{a} ± 0.08	2.32^{a} ± 0.05	2.38^{a} ± 0.07	2.50^{a} ± 0.08	2.40^{a} ± 0.10
Apparent net protein utilization (ANPU, %)	40.29^{a} ± 0.25	40.50^{a} ± 0.38	40.10^{a} ± 0.35	42.40^{a} ± 0.50	43.80^{a} ± 0.33	43.40^{a} ± 0.28

Note: Values are ± SD of two replicates. Figures in the same row having different superscript are significantly different ($P < 0.05$).

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 2). 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 2). On the basis of growth performance and feed utilisation, it may be stated that the diet 5, containing 32% and 19.14 kJ/g protein and gross energy respectively, performed best. This diet presumably contained the most appropriate P/E ratio 16.76 (16.76 mg protein/ kJ of GE) in *Mystus cavasius*. However, the optimum dietary protein to energy ratio (P/E ratio) found for *Mystus cavasius* was 16.76 mg protein/ kJ of GE, for a diet containing crude protein 32%, crude lipid 10% and gross energy 19.14 kJ/g.

Expt. 2. Evaluation of selected probiotics in the formulated diets for *Mystus cavasius*

The same aged uniform size fingerlings of *Mystus cavasius* were randomly distributed into groups of 50 fish (averaging 1.05 ± 0.05g 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) wiere formulated to contain 32% crude protein and 18.46 kJ g^{-1} 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 + Lactobaciluus acidophilis+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. Composition of the experimental diets and their proximate analyses are shown in Table 2.1. 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 2.1 Formulation and proximate composition of the experimental diets (% dry weight) for Gulsha

Diet no.:	**1 (Control)**	**2 (Bactocell)**	**3 (Bacillus)**	**4 (Levucell)**	**5 (Mixture 3)**
Fishmeal (Indonesia)	33.00	33.00	33.00	33.00	33.00
Meat & bone Meal	15.00	15.00	15.00	15.00	15.00
Mustard Oil Cake	12.00	12.00	12.00	12.00	12.00
Rich bran (auto)	22.00	22.00	22.00	22.00	22.00
Starch	12.75	12.70	12.70	12.70	12.70
Binder (Carboxymethyl cellulose)	2.00	2.00	2.00	2.00	2.00
Alpha Cellulose	3.00	3.00	3.00	3.00	3.00
Vitamin and Minerals Premix	0.20	0.20	0.20	0.20	0.20
Probiotics (Diets 2-5)	-	0.05	0.05	0.05	0.05
Proximate composition					

Crude Protein	32.15	32.15	32.15	32.15	32.15
Crude Fat	10.75	10.75	10.75	10.75	10.75
Ash	13.01	13.01	13.01	13.01	13.01
Fibre	8.58	8.58	8.58	8.58	8.58
NFE	37.40	37.40	37.40	37.40	37.40
GE (kJ g^{-1})	18.46	18.46	18.46	18.46	18.46
P / GE ratio	17.64	17.64	17.64	17.64	17.64

NFE = Nitrogen free extractives, calculated as 100 – (% protein + % Lipid + % Ash + % Fibre)
GE = Gross energy content, P / GE ratio = Protein to energy ratio in mg protein/ kJ^{-1} GE

Table 2.2 Growth increment of *Mystus cavasius* fed selected probiotics for 3 weeks

	Diet number				
Diet no.:	**1** (Control)	**2** (Bactocell)	**3** (Bacillus)	**4** (Levucell)	**5** (Mixture)
Initial body wt. (g)	1.06 ± 0.05	1.05 ± 0.04	1.06 ± 0.03	1.05 ± 0.04	1.07 ± 0.03
Final body wt. (g)	3.94^{b} ± 0.49	5.05^{a} ± 0.48	4.72^{a} ± 0.28	4.90^{a} ± 0.14	4.80^{a} ± 0.28
Weight gain (g)	2.88^{b} ± 0.49	4.00^{a} ± 0.49	3.66^{a} ± 0.28	3.85^{a} ± 0.14	3.73^{a} ± 0.39
Specific growth rate (SGR) (% day)	2.19^{b} ± 0.05	2.62^{a} ± 0.04	2.49^{a} ± 0.03	2.57^{a} ± 0.01	2.50^{a} ± 0.02
Food conversion ratio (FCR)	2.01^{a} ± 0.08	1.59^{b} ± 0.05	1.63^{b} ± 0.03	1.76^{b} ± 0.02	1.81^{b} ± 0.05
Protein efficiency ratio (PER)	1.98^{b} ± 0.05	2.50^{a} ± 0.08	2.45^{a} ± 0.02	2.40^{a} ± 0.01	2.42^{a} ± 0.04
Apparent net protein utilisation (ANPU, %)	35.60^{b} ± 0.21	40.40^{a} ± 1.21	40.32^{a} ± 0.47	40.18^{a} ± 0.28	40.52^{a} ± 1.14

Growth response parameters are shown in Table 2.2. 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 (Table 2.2). From the results of this feeding trial, it is logical to conclude that feed incorporated with the probiotics (Bactocell, *Bacillu*, Levucell) can be used as a fish feed additives in *Mystus cavasius* culture, to enhance fish health, better feed efficiency and growth performance.

The results of the feeding trials of *Mystus cavasius* with probiotics in lab conditions (Bactocell, *Pediococcus acidilactici*; *Bacillus subtilis*; *Levucell*, *Saccharomyces cerevisiae*; a mixture of *Pediococcus* acidilactici + *Bacillus subtilis* + *Saccharomyces cerevisiae*) showed higher growth performance, feed and protein utilization than in fish fed with control diet (without probiotic). A feeding trail on development and optimization of feed and feeding regimes in *Mystus cavasius* in pond conditions is still in progress and it will be completed at the middle of August 2016. After completion of the feeding trial, the technical recommendations will be provided.

Investigation and Identification of Emerging Fish Diseases and Development of their Control Strategies

Researcher(s): Dr. Nazneen Bagum, Senior Scientific Officer
Md. Shirajum Monir, Senior Scientific Officer

Objectives

- To isolate and identify Shing viruses from recent outbreaks
- To identify the causative agent(s) for Vietnamese Koi diseases
- To observe histological changes in different organs of diseased fish
- To develop control strategies to minimize fish mortality

Achievements

Isolate and identify Shing viruses from recent outbreaks

Apparently juvenile healthy Shing (*Heteropneustes fossilis*) were collected from different areas of Mymensingh district and those juvenile Shing were kept in aquariums for preliminary observation of pathogens (virus and bacteria). Before dissecting out the tissues for primary culture, the healthy Shing were starved for four days and maintained overnight in sterile, aerated water containing 1000 IU ml^{-1} penicillin and 1000 µg ml^{-1} streptomycin. Prior to sacrifice, the fishes were tranquilized by plunging in iced water for 5 min, then disinfected in sodium hypochlorite (500 ppm available chlorine) for 5 min, washed in sterile water and swabbed with 70 % ethyl alcohol. The liver, heart and brain tissues were aseptically excised from the fishes and collected in sterile vials containing phosphate buffered saline (PBS, pH 7.2) having 500 IU ml^{-1} penicillin, 500 µg ml^{-1} streptomycin and 1.25 µg ml^{-1} amphotericin B. Subsequently, the tissues were washed thrice in the same medium prior to trypsinisation and observed the pathological changes.

Virus identified from different organs of diseased Shing (H. fossilis)

A total of 54 samples were collected from different infected fish farms of different upazillas of Mymensingh. Different individual organs of the infected Shing were subjected to RT-PCR for identifying tropism of nodavirus and rhabdovirus in Shing fish. The highest nodavirus positive (69%) samples was found in brain of Shing fish that collected from Gouripur upazilla but the highest rhabdovirus positive (40%) samples was in spleen of Shing fish sampled from Muktagacha upazilla of Mymensingh district. However, within the two viruses nodavirus was in the highest number of infection than rhabdoviruses in all samples (Table 1).

Table 1. Virus identified from different organs of diseased Shing (*H. fossilis*)

Sample collection location	n	Percentage of virus isolated from different organs									
		Brain		Eye		Spleen		Kidney		Liver	
		Noda	Rhabdo	Noda	Rhabdo	Noda	Rhabdo	Noda	Rhabdo	Noda	Rhabdo
Mymensingh sadar	17	59%	0	47%	0	6%	12%	6%	11%	0	6%
Muktagacha	10	60%	0	40	0	20%	40%	10%	20%	0	10%
Tarakanda	8	50%	0	25%	0	13%	25%	0	12%	0	0
Gouripur	13	69%	0	53%	0	15%	0	0	0	0	0
Fulpur	6	50%	0	33%	0	17%	0	0	17%	0	0
Total	54	59%	0	43%	0	13%	14%	4%	11%	0	4%

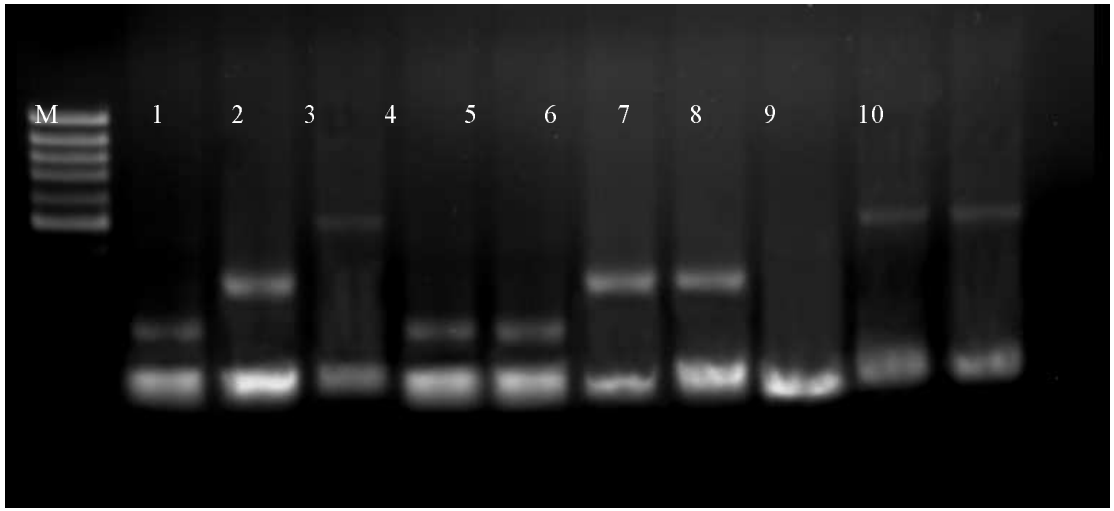


Fig. 1. Agarose gel electrophoresis of PCR amplification products of Shing viruses. Lanes: (M) 100 bp DNA marker; (1, 2 & 3) positive control; (8) negative control, (4, 6 & 9 and 5, 7, & 10) Noda & Rhabdovirus identified from different infected organs of Shing.

Identify the causative agent(s) for Vietnamese Koi diseases

Climbing perch, *Anabas testudineus* (koi) is an important indigenous freshwater fish species of Bangladesh. Though people prefer native koi due to its good taste, the farmers do not prefer it for culture due to its very slow growth rate in compare to that of exotic Thai or Vietnamese koi. But growth rate of Thai koi has been decreased due to genetic erosion and inbreeding depression. Later, Vietnamese koi has been imported in 2012. Growth rate of this koi is reported to be 50-60% higher than that of Thai koi and due to this. Vietnamese koi has got much popularity to the farmers. But recent outbreak of disease and consequent mass mortality of Vietnamese koi is causing serious economic loss to the farmers of the country. Stocking of fish at high density (3000-4000/dec) and subsequent application of high amount of feed increases the organic load deteriorating the water quality of the ponds. Some farmers stocked poor quality fry produced in advance in February through administering higher amount of hormone to the broods which are not completely ready for breeding. Cross bred fries, which are disease vulnerable, have also been stocked by some of the farmers. Indiscriminate application of antibiotics by some farmers is causing destruction of beneficial bacteria and increasing resistance of harmful bacteria to the antibiotics.

Collection of diseased Vietnamese Koi: About 200 diseased Vietnamese Koi (*Anabas testudineus*) were collected from different upazillas such as Sadar, Muktagacha, Phulbaria, Fulpur, Gouripur under Mymensingh district and only Kandua upazilla under Netrokona district for investigating the causative agents of disease.

Clinical signs and post mortem findings

Naturally infected Vietnamese Koi showed loss of appetite, sluggish movement, swimming close to the surface of the water, lethargic, no escape reflex, erratic swimming which was either spiraling or spinning just below the surface of water, haemorrhages on the skin especially in the base of fins and tail, ulcer on body, haemorrhage of the eye, uni- or bilateral exophthalmia, in some cases cloudy change as well as destruction of eye (pop-eye) was observed and severalty infected fish ultimately died 50-60% within 3-15 days in the cultured ponds.

Post mortem findings: Stomach and gut became nearly empty and the peritoneal cavity filled with ascitic fluid. Internally, the infected liver found enlarged than normal, pale in colour and haemorrhagic. In some cases, haemorrhages in intestine; enlarged spleen and nearly black, enlarged and congested kidney were noticed. In most cases, enlarged as well as haemorrhages were observed in the brain of infected fish.

Isolation and Identification of bacteria: Fish sampling and primary isolation of bacteria were done under complete aseptic condition from the kidney, liver, spleen and ascitic fluid and inoculated on Tryptic Soy Broth (TSB) that incubated at 28-37 °C for 24-48 h. A loopfull of incubated broth was streaked on the different laboratory medium: Tryptic Soy Agar (TSA) and 5% sheep blood agar. The isolated bacteria from infected Vietnamese Koi gave pure cultures when grown on TSA media. Phenotypically, the isolated bacteria appeared slightly mucoid, white, small round as pin-point colonies on TSA, displaying either β- or non-haemolysis (γ) after 48 h of incubation at 32 °C on 5% blood agar. They were all Gram-positive cocci, mostly in short or long chains, non-motile. Biochemical profiles of the isolates tested were a positive Voges-Proskauer (VP) reaction while the isolates were negative for oxidase, catalase and indole. Furthermore, these isolated bacteria were able to grow from 22 to 37 °C, but not at 4 and 14 °C. Bacterial growth was not observed for any of the isolated at higher than 4% NaCl except SA-4 isolated. The phenotypic characteristics of isolated bacteria were almost similar to the *Streptococcus agalactiae* type strain. However, differences were found between the growth on temperature and salt tolerance growth test when compared with different isolated (Table 2).

Table 2. Comparison of phenotypic characteristics of the isolated bacteria from Vietnamese Koi (*Anabas testudineus*)

Test	Isolated bacteria			
	***Streptococcus agalactiae* (SA-1)**	***S. agalactiae* (SA-2)**	***S. agalactiae* (SA-3)**	***S. agalactiae* (SA-4)**
Growth on TSA	+	+	+	+
Gram stain	+	+	+	+
Cell morphology	Cocci pairs & short chain	Cocci pairs & short chain	Cocci pairs & short chain	Cocci pairs & short chain
Haemolysis	γ-haemolysis	β-haemolysis	γ-haemolysis	β-haemolysis
Motility	-	–	–	–
Oxidase	-	–	–	–
Catalase	-	–	–	–
VP	+	+	+	+
Indole	-	–	–	–
O/F	F (weak)	F(weak)	F(weak)	F(weak)
Growth on TSB				
4 °C	-	–	–	–
14 °C	-	–	–	–
22 °C	+	+	+	+
28 °C	+	+	+	+
37 °C	+	+	+	+
Growth on TSB				
0.5% Nacl	+	+	+	+
1% Nacl	+	+	+	+
2% Nacl	+	+	+	+
3% Nacl	+	+	+	+
4% Nacl	-	–	-	+
5% Nacl	-	–	–	–

Identification: +, Positive; -, Negative; F, Fermentative

For molecular detection of the isolated bacteria, seven representative isolates SA-1, SA-2, SA-3, SA-4, SA-5, SA-6, SA-7 and SA-8 were chosen to run PCR assay. The *Streptococcus agalactiae* isolates tested product gave 220 bp clear bands. The gene was amplified with using of the universal primers of 16s rRNA. The primers with the following sequence: F1 (Forward), 5'-GAG-TTT-GAT-CAT-GGC-TCA-G-3' and R1 (Reverse), 5'-AAC-AAC-ACG-TGT-TAA-TTA-CTC-3' were designed which gave an amplicon of 220 bp. The molecular technique was used for *S. agalactiae* identification due to PCR technique is certainly more reliable than the traditional biochemicals methods.

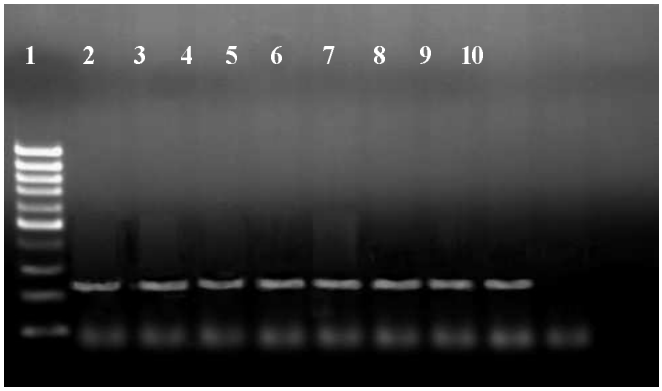


Fig. 2. PCR amplification generated by *S. agalactiae* species specific primer. Lane- 1, 100 bp ladder; Lane 2-9, SA-1; SA-2, SA-3, SA-4, SA-5, SA-6, SA-7, SA-8 isolates, Lane-9, positive control; Lane-10, negative control

Fish were divided into 6 groups with 20 apparently healthy fishes each group. The experiment was conducted in 10-12 L aquarium. The bacterial suspension was prepared according to the McFarland turbidity and ten folds serial dilutions were done to obtain *S. agalactiae* concentration of 3 x 10^3 to 3 x 10^7 cfu/ml. However, after 5 days the fish injected with 10^3, 10^4, 10^5, 10^6 and 10^7 cfu/ml bacteria showed 10, 25, 55, 80, 85% mortality, respectively (Table 3).

Table 3. Total cumulative mortality observed for Vietnamese Koi interperitioneally inoculated with live *Streptococcus agalactiae*

		No. of dead fish after injected *S. agalactiae* (day post inoculation)						
Group	cfu/ml	1	2	3	4	5	Total No. of dead fish	Mortality (%)
1	10^3	0	0	2	0	0	2/20	10
2	10^4	1	2	2	0	1	5/20	25
3	10^5	2	3	2	2	2	11/20	55
4	10^6	3	5	4	2	2	16/20	80
5	10^7	4	6	3	2	2	17/20	85
6	PBS	0	0	0	0	0	0/20	0

The susceptibility rate of all *Streptococcus agalactiae* isolates to cefradine, erythromycin doxycycline was high, while it was moderate or less sensitive to ampicillin, lincomycin, oxytetracycline and gentamycin (Table 4).

Table 4. Antibiotics sensitivity test on *Streptococcus agalactiae* isolated from infected Vietnamese Koi

Antibiotic disc (µg)	***S. agalactiae***
Ampicillin (25)	S
Azithromicin (15)	R
Cefradine (30)	S+++
Chlortetracycine(25)	R
Ciprofloxacin (5)	R
Doxycycline (30)	S++
Erythromycin (15)	S+++
Lincomycin (25)	S+
Tetracycline (30)	R
Oxyteracycline (10)	S
Streptomicin (10)	R
Gentamycin (10)	S

Preventive and control measures of Streptococcosis

For preventing outbreak of this disease, farmers should prepare the ponds by removing the excess detritus deposited to the pond bottom and treating soil with quick lime @ 1kg/dec. At the present level of management, stocking of fish should not be more than 1000/dec. To maintain good water quality, application of organic manure should be completely avoided and very rational feeding practice should be done. Periodic liming should be done with dolomite @ 200-300 kg/ha. If necessary, water of the ponds can be treated with 2-3 ppm chlorine. Fencing with small mesh nylon net surrounding the pond and netting upon the pond should be done as a measure of bio-security.

Erythromycin or doxycycline or cefradine can be incorporated into the feed at 3-4 g/kg feed for 5 to 7 days as well as apply the antibiotic in infected pond water at the dose of 5 g/dec for 5 to 7 days. In previous, streptococcal infections responded to antibiotic treatment but in the presence time, the disease cannot be fully controlled due to abuse of antibiotics at the field level. Therefore, antibiotic treatment is generally ineffective and the need of proper vaccine to develop to fully prevent Streptococcosis.

Development of Cage Culture Technology of High Valued Fishes in the River Ecosystem

Researchers: Dr. AHM Kohinoor, Principal Scientific Officer
Md. Moshiur Rahman, SO

Objectives

- To utilize the open water ecosystem for fish production through cage culture
- To evaluate the production performance of high valued fish in net cages in the river ecosystem

Achievements

Expt. 1. Evaluation of growth and production performance of Shing, H. fossilis in net cages at different stocking densities

The cages were made by locally available cages materials e.g., iron rod, net of suitable mesh size (1.0 cm), plastic floats, bamboo, plastic ropes etc. The area of each floating net cages were be 3.0 m^3. Fingerlings of Shing were stocked in net cages according to the design of experiment during November 2015 for the period of 7 months. Prior to release, fry were subjected to some prophylactic measures to protect them from diseases and ecoto-parasites. They were dipped in a 5-6% salt solution as well as potassium permanganate (5-8%) for 1 to 2 minutes and then released into the cage water. Supplementary pelleted floating feed was applied (containing 30% crude protein) to the fishes @ 6-20 % of body weight twice daily at estimated body weight.

Table 2 shows the physico-chemical parameters of River Brahmaputra water viz., temperature, transparency, pH, dissolved oxygen and total ammonia. The values of temperature, transparency, pH, dissolved oxygen, and total ammonia were 16.9 – 27.60°C, 60 – 98 cm, 7.09 – 8.06, 7.56 – 9.32 mg/l, and 0.00-0.04 mg/l, respectively. The water quality parameters studied during the experimental period were found suitable for fish farming and could not have hampered the normal fish growth.

Table 1. Water quality parameters of River Brahmaputra during experimental period

Parameter	Value
Water Temperature (°C)	16.9 – 27.60
pH	7.09- 8.60
DO (mg/l)	7.56 –9.32
Transparency (cm)	60 – 98
Total alkalinity (mg/l)	150-190
Total ammonia (mg/l)	0.00-0.04

It was observed that the highest average weight was found in treatment-1 where the stocking of Shing was 200/ha. At harvest, the average weights attained by Shing were 30.85 ± 4.21, 27.24± 3.95, 25.62 ± 4.68

and 21.01± 3.96g, in treatments-1, 2, 3 and 4, respectively. The harvesting weight of treatment-1, 2 and 3 was significantly higher ($P<0.05$) than treatment-3. In higher stocking densities, the harvesting weight of Shing was occurred linearly. The survival rate of fish varied between 62 to 75%. Survival rate was higher in lower stocking density. In treatment-1, the highest survival rate was observed. The productions obtained in cages were 4.63±0.58, 4.77±0.33 5.15±0.66 and 4.56±0.23 kg/m^3 from treatments-1, 2, 3 and 4, respectively. The highest and lowest production were obtained from treatment-1 and 4, respectively.,

Table 2. Harvesting wt., Survival, and Production of Shing under different treatments

Treatment	Harvesting Wt.(g)	Survival (%)	Production/m3 (kg)
Treatment-1	30.85 ± 4.21	75	4.63 ± 0.58^a
Treatment-2	27.24± 3.95	70	4.77 ± 0.33^a
Treatment-3	25.62 ± 4.68	67	5.15 ± 0.66^b
Treatment-4	21.01± 3.96	62	4.56 ± 0.23^a

* Dissimilar superscript indicates significant difference at 5% level of probability

Cost and benefit analysis showed that T_1 generated the highest return over a period of seven months of Tk. 256/cage ($3m^3$). The growth performances as well as economic return of shing are not encouraging at all.

Development of Aquaponic Techniques in Bangladesh

Researchers: Dr. Jubaida Nasreen Akhter, Principal Scientific Officer
Md. Rayhan Hossain, Scientific Officer

Objectives

- To optimize stocking density of fish and plant in aquaponic system.
- To estimate nutrient level and electrical conductivity in hydroponics system.
- To identify of nitrifying bacteria in bio-filter of aquaponic system.

Achievements

Optimisation of stocking density for production of Red Tilapia, Gulsha and Vegetable

Few experiments were conducted in Aquaponic Garden at BFRI campus to optimise stocking density of Genetically Improved Farmed Red Tilapia and Gulsha (*Mystus cavasius* with vegetable in aquaponic sysyem. Fishes were reared in fiberglass tanks with supplementary feed under a shade system. Stocking density of Red Tilapia was maintained at 30, 60 and 90 m^{-3} in different treatments and stocking density of Gulsha was maintained at 100, 150 & 200 individual m^{-3}. Stocked fishes were fed commercial floating feed at the rate of daily 80g-100g fish feed m^{-2} of vegetable growing area four times daily. Culture period is 60 days from Dec to Feb for batch 1 (Tables 1 & 3) and 90 days from Feb- May for batch 2 (Tables 2 & 4). Under these experiments vegetable or salad were produced simultaneously. Density of vegetable seedling was maintained at 9, 18 and 27 m^{-2} of growing area according to the nature of plant. Water quality parameters such as NH_3, O_2, pH and water temperature were recorded alternate day (Table 9, 10, 11 & 12).

Table 1. Growth and production performance of Red Tilapia

Treatment	T_1	T_2	T_3
Stocking density (m^{-3})	30	60	90
Initial wt. (g)	40 ±1.2	70±1.2	30±1.1
Av. Final wt.(g)	91.8±3.4	116.5 ±3.5	69.5 ±3.7
ADG (g/day)	0.85 ±0.1	0.79 ±0.1	0.67 ±0.2
Yield kg m^{-3}	2.40	7.00	5.40
Survival (%)	88	97	85

Table 2. Growth and production performance of Red Tilapia

Treatment	T_1	T_2	T_3
Stocking density (m^{-3})	30	60	90
Initial wt. (g)	80 ±1.2	80±1.2	80±1.1
Av. Final wt.(g)	167±10	156 ±13	146 ± 9
ADG (g/day)	0.95	0.84	0.72
Yield kg m^{-3}	4.0	7.3	7.2
Survival (%)	82	77	58

Table 3. Growth and production performance of Gulsha Yield

Treatment	T_1	T_2	T_3
Stocking density (m^{-3})	100	150	200
Initial wt. (g)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Av. Final wt.(g)	2.9±0.3	2.3 ±0.4	2.1 ± 0.2
ADG (g/day)	0.06	0.04	0.03
Yield kg m^{-3}	0.23	0.28	0.31
Survival (%)	74	76	71

Table 4. Growth and production performance of Gulsha Yield

Treatment	T_1	T_2	T_3
Stocking density (m^{-3})	100	150	200
Initial wt. (g)	2 ± 0.3	2 ± 0.4	2 ± 0.3
Av. Final wt.(g)	9.0±1.8	6.8 ±1.2	5.8 ± 0.8
ADG (g/day)	0.08	0.05	0.04
Yield kg m^{-3}	0.65	0.71	0.73
Survival (%)	72	69	62

In case of 1st, 2nd batch Red Tilapia yield was recorded as 2.4 to7.0 kg m^{-3} and 4.0 to 7.3 kg m^{-3} respectively. In case of 1st batch low yield was recorded in T_1 while better production was found in T_2. It was probably due to size of fingerlings as comparatively small size fingerlings were stocked in T_1 and bigger size fingerlings were stocked in T_2. Average daily weight gain by Red Tilapia in 1st, 2nd batch was recorded as 0.67 to 0.85 g day^{-1} and 0.72 to 0.95g day^{-1} respectively. Average daily weight gain was found better in case of large size stocked fish than small size stocked fish for Red Tilapia culture in tanks.

In case of 1st batch yield of lettuce, and tomato was 1.2 to 1.9 kg m^{-2},and 0.8 to 0.9 kg m^{-2} respectively which is satisfactory for aquaponic system. In case of 2nd batch production of water spinach was 3.4 to 4.6 kg m^{-2}. Vegetable production was satisfactory for aquaponic system. In all the experiments ranges of water quality parameters was found suitable for fish culture.

Estimating nutrient level and electrical conductivity in hydroponics system

Nutrient released from faucal matter and leftover food of fishes is important for production of vegetable in hydroponic system as growth of vegetable depend on availability of nutrient in the system. Nutrient is released through bacterial break-down of faucal matter and leftover food. Vegetable takes nutrient from the circulated water of the fish tanks. Low density of nutrient in fish tanks/trays usually results poor growth and production of vegetable. Results of nutrient level in water are presented in Tables 5 & 6.

Table 5. Nutrient level of water and plant in hydroponic tray (Tilapia)

Sl. No	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Cl (ppm)
T_1	35.39	6.43	35.27	15.06	5.00
T_2	25.01	6.33	38.47	14.09	6.00
T_3	26.14	6.50	25.60	9.20	6.00

Table 6. Nutrient level of water and plant in hydroponic tray (Gulsha)

Sl. No	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Cl (ppm)
T_1	34.76	4.633	38.47	13.61	4.00
T_2	24.59	5.648	26.45	8.26	6.00
T_3	27.61	5.849	30.46	6.30	5.00

In case of 1st batch Lettuce and Tomato production trials with Tilapia fish the highest EC levels was found in T_3 (0.36 mS/cm) and the lowest EC level was found in T_1 (0.33 mS/cm). In an aquaponic system, considerably lower level of electrical conductivity (EC) is 0.3-0.6 mS/cm. The highest values of nitrite-nitrogen were found in the treatment T_1 (0.07 ppm) and the lowest values of nitrite-nitrogen were found in the treatment T_3 (0.05 ppm). However, 1st batch Lettuce and Tomato production trials with Gulsha fish tray nutrient level were found lower from Tilapia vegetable tray.

In case of 2st batch water spinach trials with Tilapia fish the highest EC levels was found in T_3 (0.40 mS/cm) and the lowest EC level was found in T_1 (0.39 mS/cm). In an aquaponic system, considerably lower level of electrical conductivity (EC) is 0.3-0.6 mS/cm. Generally lower level of EC like 0.3-0.6 produce good results because in aquaponic system nutrients are generated continuously (Rakocy *et al.*1997). The highest values of nitrite-nitrogen were found in the treatment T_2 (0.14 ppm) and the lowest values of nitrite-nitrogen were found in the treatment T_3 (0.10 ppm). In hydroponic tray, p^H value range form 6.5 to 6.8 which is suitable for aquaponic system.

Identification of nitrifying bacteria in bio-filter of aquaponic system

A very important concern in aquaponic systems is the removal of ammonia, a metabolic waste product excreted through the gills of fish. Ammonia will accumulate and reach toxic levels unless it is removed by the process of nitrification. In nitrification process ammonia is oxidized first to nitrite, which is toxic, and then to nitrate, which is relatively non-toxic. Although plants can absorb ammonia from the water to some degree, nitrates are assimilated more easily, thereby efficiently reducing the toxicity of the water for fish. Ammonia can be converted into other nitrogenous compounds through healthy populations of *Nitrosomonas* bacteria that convert ammonia into nitrites, and *Nitrobacter* bacteria that convert nitrites into nitrates. This is why most aquaponic systems include a biofiltering unit, which helps in facilitating growth of these microorganisms.

Therefore, it is necessary to identify the types and amount of nitrifying bacteria in the biofilter of aquaponic system. Identification of nitrifying bacteria was done in Microbiology Department of Bangladesh Agricultural University following the standard procedures. Total Volatile count was done (Table 7) . In case of bio-filter of red Tilapia culture system highest amount of total bacterial colony in water was in T_3 on the other hand in case of stone highest amount of total bacterial colony was found in T_3 . In qualitative test of bacteria *Staphylococcus, Bacillus subtilis, Nitrosomonas, Nitrobacter* were found in bio–filter which is good for nutrient production.

Table 7. Total Volatile Count (TVC) of bacteria in bio-filter

Treatment	TVC (cfu/ml)			
	Water		Stone	
	Red Tilapia	Gulsha	Red Tilapia	Gulsha
T_1	3.5 × 10 7	3.5 × 10 7	3.2 × 10 7	1.2 × 10 7
T_2	4.5 × 10 7	4.2 × 10 7	3.2 × 10 7	2.2 × 10 7
T_3	5.2 × 10 7	4.5 × 10 7	3.7 × 10 7	3.7 × 10 7

Natural Propagation of Freshwater Mussel in Bangladesh

Researcher(s): Arun Chandra Barman, Senior Scientific Officer
Dr. Mohosena Begum Tanu, Principal Scientific Officer
Mohammad Ferdous Siddique, Senior Scientific Officer
Md. Sydur Rahman, Scientific Officer

Objectives

- To know the Gonadal histology of Freshwater mussel *Lamellidens marginalis* and *L. corrianus*
- To know the Condition Factor (CF) of freshwater mussels *Lamellidens marginalis* and *L. corrianus*
- To know the reproductive behavior of freshwater mussels *Lamellidens marginalis* and *L. corrianus*

Achievements

Twenty specimens per month of an adult population of mussel were collected. Average length, height and weight of the sampled mussels were given in table.

Table 1. Various biometric characteristics of analyzed sample (n=20) of *Lamellidens marginalis* recorded in different month

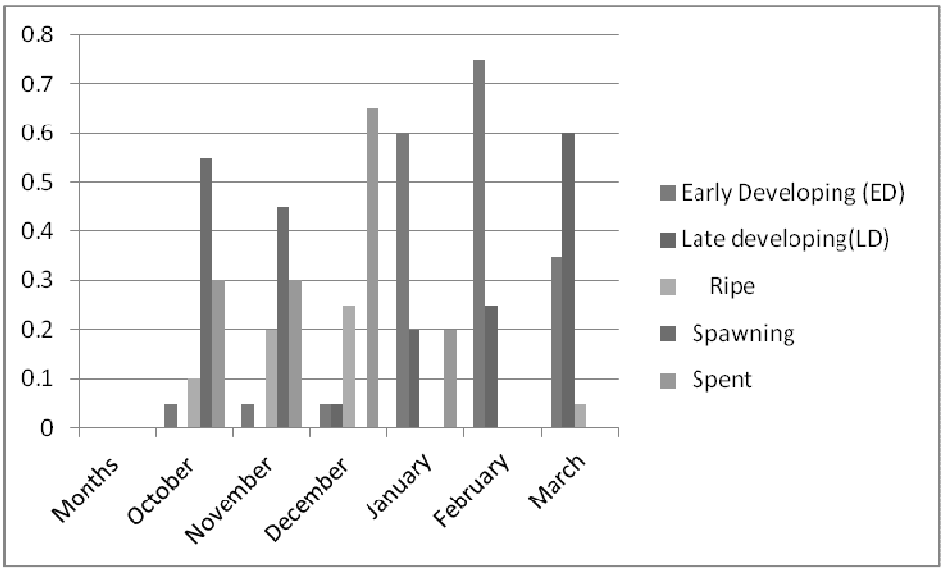
Month	Shell length (SL) (mm) (Mean ±SD)	Shell Height (SH) (mm) (Mean ±SD)	Tissue Weight (TW) (MM) (Mean±SD)
October	64.11±9.47	17.64±4.82	15.21±4.48
November	86.53±8.05	27.26±6.99	14.52±3.86
December	86.50±5.05	45.51±2.90	17.55±3.29
January	88.179±8.22	43.48±5.24	19.17±4.74
February	91.86±6.37	46.53±3.24	19.20±5.05
March	89.52±5.78	45.99±3.78	18.2±4.74

In the month of May and June researchers found four gametogenic stages. The results are as follows:

Indifferent Stage: This stage is characterized by a total absent of gametes.
Ripe stage: In the female, most oocytes were free within the follicles and in male, follicles filled by spermatozoa arranged in characteristic bands.
Spawning: In the female, large spaces inside the follicles and between free oocytes were present and in male a marked decrease in the quantity of spermatozoa was observed.
Spent: At this stage, some unspawned oocytes and spertozoa were observed within follicles.

Table 2. Percentage (%) of different reproductive stages of *L. marginalis* in different month

Months	Early Developing (ED)	Late developing (LD)	Ripe	Spawning	Spent
October	5%	----	10%	55%	30%
November	5%	----	20%	45%	30%
December	5%	5%	25%	---	65%
January	60%	20%	---	---	20%
February	75%	25%	---	---	---
March	35%	60%	5%	---	---



During the study period, gametogenesis first initiate in October as indicated by the presence of early growth stage (5%) and continued until December. Ripe, and spent stages were present all the time during the study period but ripe individuals first appeared in October (10%) and become evident in December (25%). Late developing stage was only found in December. Spawning stage was totally absent in December when the temperature was 22.1. So it is clear that harvesting should be prohibited from October to November for the sustainable management of the stock.

Refinement of Freshwater Pearl Culture Techniques in Bangladesh

Researcher(s): Dr. Mohosena Begum Tanu, Principal Scientific Officer
Arun Chandra Barman, Senior Scientific Officer
Nur-A-Raushon, Scientific Officer

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

Achievements

Expt. 1. Optimization of number of tissue slice for maximizing pearl production in different mussel species (Lamellidens marginalis, Lamellidens corrianus) aganist 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 spices of 2 mmx2 mm 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 year 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 30fish/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. Survival rate of the operated mussel will be monitored once in a month. Water temperature, pH, plankton growth, and organic matter, NH_4-N, DO and Ca^{2+} was monitored fortnightly. After one year culture, survival rate and pearl production rate of the operated mussel were observed. 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	*Lamellidens marginalis, Lamelliden corrianus*

Table 2. Pearl productions against mantle tissue slice

No of mantle tissue inserted	No of mussel	Survival rate(%)	Average Pearl producing rate/ mussel
6	1441	68	5 pearl/ mussel
8	1200	55	6 pearl/mussel
10	1200	58	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 year 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. Water temperature, pH, plankton growth, organic matter, NH_4-N, DO and Ca^{2+} was monitored fortnightly. After one year culture, survival rate and pearl production rate of the operated mussel were observed. 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	*Lamellidens marginalis, L. corrianus*
Stocking in cage	6	*Lamellidens marginalis, L. corrianus*
Stocking in open pond (grazing)	6	*Lamellidens marginalis, L. corrianus*

Table 4. Pearl production against culture technique

Name of culture technique	No. of mussel	Survival rate (%)	Pearl producing rate/mussel
Net bag hanging	1294	62	50
Grazing	1740	75	57

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. Organic and inorganic fertilizer was given fortnightly to the pond. @3kg cow dung 100g T.S.P and 100g urea per decimal. Liming was done fortnightly @ 500 g dolomite/decimal. Water temperature, pH, plankton growth, organic matter, NH_4-N, DO and Ca^{2+} parameters were monitored fortnightly. Survival rate of the operated mussel was monitored once in a month. After one year culture, survival rate and mussel having image were observed. The design of the experiment and the result was given in the Tables 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^2 2.5 X 1.5 cm^2 2.0 X 1.5 cm^2	*Lamellidens marginalis, L. corrianus*
Grazing	9-12	3.0 X 1.5 cm^2 2.5 X 1.5 cm^2 2.0 X 1.5 cm^2	*Lamellidens marginalis, L. corrianus*

Table 6. Result of Image pearl production

Name of culture technique	No. of Operated mussel	Length of mussel (Average)	Length of image	Width of image	Survival rate (%)	Image pearl production	Image Jewelry
Net bag hanging	350	9 -12 cm	3 cm	1.5 cm	50%	341	15
Grazing	230	9 -12 cm	3 cm	1.5 cm	72%		

Early Development of Brood of Thai Pangas *Pangasianodon hypophthalmus* using Green House Concept

Researchers: Dr. David Rintu Das, Senior Scientific Officer
Mst. Sonia Sharmin, Scientific Officer

Objectives

- To accelerate maturation of broods of Thai pangas, *Pangasianodon hypophthalmus*
- To improve quality of broods of Thai pangas, *P. hypophthalmus* between January to February

Achievements

Expt. 1. Enhancement of development of ovary of Pangasianodon hypophthalmus using 'green house' concept

The experiment was conducted following design and procedure as given in Table 1. The study was conducted in four equal earthen ponds of 0.1 ha each on Floodplain Sub-Station, Santahar, Bogra. The selected ponds were dried, bottom soil will be excavated to increase depth and the dykes were repaired properly. For this purpose, two ponds were fully covered with transparent polyethylene sheet fastened in frame, made of bamboo that was treated as "green house pond" (GP) (Fig. 1). The other two ponds were kept open and were treated as open pond (OP) as control. After excavation soil of the ponds were treated with lime (CaO) @ 250 kg/ha.

Fig. 1. A green house pond modal which was fully covered with transparent polyethylene sheet

After then ponds were filled up with water up to a depth of more than 1.0 meter. Ponds water was treated with dolomite @ 20 ppm and fertilizer with urea @ 2.5 ppm and TSP @ 1.00 ppm. After growing sufficient plankton, all the four ponds were equally stocked with adult & healthy *Pangasianodon hypophthalmus* @ 990 Nos/ha (density 12kg/decimal) in October. The ratio of stocked broods (female: male) were 3:1. The size of male pangus was 1.5-2 kg and that of female was 2.5-3 kg (Table 1). The stocked brood fishes were fed with commercial pellet feed (protein, 35%) supplemented with vitamin premix.

Cod liver oil were added at 1-2 ml/kg feed to hasty eggs for quite maturation. Feed were supplied @ 5-3% of total body weight twice daily. Duration of brood fish culture was 5 months (Table 1). To keep the pH and others water quality parameters in suitable range, water of all ponds were treated with dolomite @ 10-12 kg/ha fortnightly. After one month of stocking, the stocked pangas were observed frequently by netting to check any development of gonad. Partial water of the ponds were exchanged in every 7 days intervals after stocking of fish but it was not more than 10% at a time. Temperature, pH, alkalinity, dissolved oxygen and hardness of water were checked at three days interval following standard methods (AHPA, 1992).

After stocking of fish, gonadal development of brood pangas were checked monthly observation by external features of sexual maturity. Gravid females were identified by their swollen, distended, soft abdomen with reddish and swollen vent. Mature males were identified by their reddish genital opening and oozing of milt with gentle pressure on the abdomen. For this study, about 5-6 fishes were examined randomly, particularly during the winter season (January- February). To confirm the progress of gonadal maturity of brood the important aspects: the size of the gonad in length and weight and fecundity were determined. Fecundity was estimated by gravimetric method. Induced breeding that's with the early matured was conducted in the month of February. The experiment was conducted following design and procedure as given in Table 1.

Table 1. Design of the experiment

Treatments	Replications	Stocking ratio (Female: Male)	Stocking density (no./decimal)	Culture period (Month)
T_1 (GP)	2	4:2	6	5
T_2 (OP)	2	4:2	6	5

❖ GP= Green house pond, OP= Open pond

Gonadal development of brood pangas were checked monthly observation by gonad of fishes and external features of sexual maturity. In the green house ponds the stocked non gravid female pangas started to be gravid after stocking and gravid females & males were found at the end of culture period. All females & males become berried in last February but 65% females & males become ready 90% for induced breeding (Fig. 4). But, no pangas become berried in the open ponds throughout the culture period. Gravid females were identified by their swollen, distended, soft abdomen with reddish, swollen vent as well as dissecting gonad. Mature males were identified by their reddish genital opening and oozing of milt with gentle pressure on the abdomen. For this study, total 3 fishes were examined randomly during the experimental period (October-February). It was evident from the results (Table 2) that gonad weight increased slowly from October to February with the gonadal development of greenhouse reared broods.

Table 2. Total body length, weight and gonad weight at successive months of greenhouse reared female, *Pangasianodon hypophthalmus*

Month	No. of fish examined	Total length (cm)	Total weight (kg)	Gonad weight (g)
October	3	63.4 ±1.74 (Initial)	2.8 ±3.01 (Initial)	156±0.26 (Initial)

November	3	65.5±1.40	3.2±0.49	225±0.04
December	3	67.5±0.38	3.6±0.19	303±0.30
January	3	66.3±2.04	3.7±0.54	351±1.01
February	3	67.1±0.59	3.9±0.31	376±0.86

To get fry from brood fishes induced breeding programme was started first on 4th March, 2016 in the hatchery complex of the Sub-Station, Santahar. The female broods were 4.0-4.5 kg and the males were 3.0-3.5 kg in weight during hormone injection. Hormone injection for female 1st dose: 1.5mg PG+ 200 IU HCG/ kg BW, 2nd dose: 12mg PG/kg BW and for male 2 mg PG/BW (Fig. 3).

After hormone admistration eggs were released by stripping method. About 60% eggs were come out easily from female brood when stripped (Fig. 2). Fecundity of greenhouse reared broods was 3.2 lakh in 375g of gonad in the end of February. Ambient water temperature of bottle hatchery was 25.6^0 C where hatching rate was found 70%. At the first time 40,000-50,000 fries have been produced from greenhouse reared broods. It is indicated from the primary findings that it will be possible to produce berried pangas within February if stocking can be done with properly matured pangas fish in October using greenhouse concept. In greenhouse, it was observed that 65 % female broods became fully matured gravid and males were 90 % in the end of February. But in open ponds female and males became fully matured within April.

Fig. 2. Hatchling and Fingerlings of pangas using greenhouse concept.

Development of Induced Breeding and Culture Techniques of *Monopterus cuchia*

Researchers: Dr. David Rintu Das, Senior Scientific Officer
Mst. Sonia Sharmin, Scientific Officer

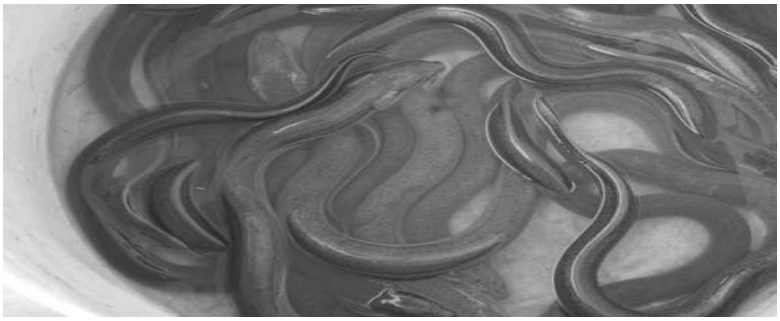
Objectives

- To develop breeding technique of *M. cuchia* through hormone administration.
- To develop nursery technique of *M. cuchia* .
- To develop grow-out technique of *M. cuchia* .

Achievements

Expt. 1. Determination of suitable dosages of hormone for induced breeding of Monopterus cuchia

The experiment was conducted following the design as given in Table 1. The same design of experiment was implemented both in ponds and cistern conditions. For the experiment, healthy matured male and female broods were collected from natural sources of Bogra region during March-April/15 and acclimatized for 3-4 days in the cemented cisterns.



The mature broods of almost same size were selected based on visual examination of secondary sexual characteristics *i.e.*, abdomen and genital opening. Then the broods (Female Av. Wt. 450 g & Male 240 g) were administered with hormone in deep muscle at the dorsal side of the fishes at different doses of cPG (Table 1).

Table 1. Use of different hormone and dosages on *M. cuchia* for induced breeding in pond and cistern

Treatment	Ratio	Stocking density (no/decimal)	Dosages (Female)	Dosages (Male)	Result
T_1	Male: Female (1:1)	40	10 mg cPG/Kg BW+ 200 IU HCG	5 mg cPG/Kg BW	Positive
T_2			12 mg cPG/KgBW + 300 IU HCG	6 mg cPG/Kg BW + 150 IU HCG	
T_3			0.2 ml Ovaprim /Kg BW	0.1 ml Ovaprim /Kg BW	
T_4			0.4 ml Ovaprim /Kg BW	0.2 ml Ovaprim /Kg BW	
T_1			10 mg cPG/Kg BW+ 200 IU HCG	5 mg cPG/Kg BW	Positive
T_2			12 mg cPG/Kg BW + 300 IU HCG	6 mg cPG/Kg BW + 150 IU HCG	
T_3			0.2 ml Ovaprim /Kg BW	0.1 ml Ovaprim /Kg BW	
T_4			0.4 ml Ovaprim /Kg BW	0.2 ml Ovaprim /Kg BW	

After hormone administration, the fishes were transferred to earthen ponds and cemented cisterns for breeding. 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. During breeding period, cuchia makes hole in the soil at the periphery of pond and lay eggs there. First observations were made after 15 days of stocking to find out nests and presence of hatchlings in the nests. Next, close

observation was made every day to observe the development stages of hatchlings. After 35 days of injection, larvae were found with yolk-sac absorbing conditions in cistern and pond (Fig. 1).

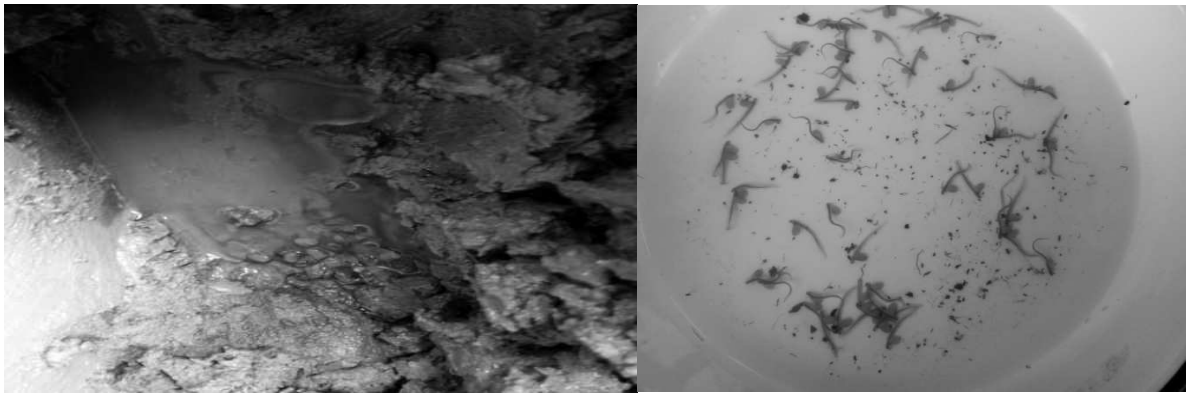


Fig. 1. Larvae of Cuchia were found with yolk-sac absorbing conditions in both system

Positive results were observed where the female received single doses of 10 mg cPG/Kg BW+ 200 IU HCG /kg BW and the male received 5.0 mg cPG/Kg BW.

Expt. 2. Development of nursery technique of M. cuchia in different stocking densities in tray

An experiment was carried out to know the effects of different stocking densities on growth and survival of *M. cuchia* with 15 days old fry. The density experiment was conducted for 60 days in six trays having an individual area of 0.75 m^2 and depth of water was from 10-12cm. Tray floor was covered by clay soil. Water depth was kept at about in 0.15 m. Water hyacinth was supplied into the tray to make suitable environment for *M. cuchia* fry.

The fries were stocked in tray with different stocking densities. Density of T_1-200 fry/ m^2, T_2- 250 fry/ m^2 and T_3- 300 fry/m^2 were followed during experimental period. Earthworm pest & Zooplankton @ 50-20% body weight were supplied as food in each tray twice a day. Waste water from the trays was removed by siphoning and clean water was replaced. Water shower was placed above the tray that allows continuous flow of water. Initial length (cm) and weight (g) were 7.87 ± 0.44 cm and 0.746 ± 0.03 g in each treatment. After 60 days of rearing, the final length of fry in each treatment was 14.52 ± 0.77, 13.10 ± 0.38, and 11.51 ± 0.54 cm for T_1, T_2 and T_3 respectively. While the final weight were 7.110 ± 0.10, 6.750 ± 0.03and 0, 5.017 ± 0.03 g for T_1, T_2 and T_3, respectively. T_1 was observed best survival rate 74 (%).

Table 2. Growth performance of *M. cuchia* fry with different stocking densities reared in tray.

Growth parameters	**T_1**	**T_2**	**T_3**
Initial L (cm)	7.87 ± 0.44	7.87 ± 0.44	7.87 ± 0.44
Initial wt (g)	0.746 ± 0.03	0.746 ± 0.03	0.746 ± 0.03
Final L (cm)	14.52 ± 0.77	13.10 ± 0.38	11.51 ± 0.54
Final wt (g)	7.110 ± 0.10	6.750 ± 0.03	5.017 ± 0.03
Survival rate (%)	74	65	60

Expt. 3. Development of grow-out culture technique of M. cuchia in different stocking densities in ponds and cisterns

The objective of the experiment was to optimize an appropriate stocking density for the rearing of the mud eel fry in pond and cemented cisterns. Six cisterns and ponds having a dimension of 2.47 x 1.5 x .75 m and 10 decimale (each) were prepared for grow-out culture of *M. cuchia* at the Floodplain Sub-Station, Santahar during the experimental period. Three stocking densities viz. T_1= 3/m^2, T_2= 4/m^2 & T_3= 5/m^2 were tested for 6 months with 60 days old fingerlings. Earthworm slice, Tadpole & Fry of carpio @ 20-5% BWt. were supplied as feed in each cistern and pond twice a day. Waste water from the cisterns was removed by siphoning and clean water was replaced. Water shower were placed above the cisterns that allowed continuous flow of water. Data on water quality and growth performance were recorded in 07 days intervals. Effect of stocking densities on the growth of the *M. cuchia* fry reared in the cemented cisterns indicated that the growth of the *M. cuchia* fry varied with different stocking densities. Treatment-1 (3 fry/ m^2) showed the best results in terms of growth and survival rate in (Table 3). The lowest final weight and rate were found in tretament-3 where stocking density was 5 fries/m^2.

Table 3. Growth performance of of *M. cuchia* fry with different stocking densities reared in pond and Cistern.

Growth parameters	Treatment-1	Treatment-2	Treatment-3
Initial L (cm)	15.3 ± 0.032	15.3 ± 0.032	15.3 ± 0.032
Initial wt (g)	7.110 ± 0.10	7.110 ± 0.10	7.110 ± 0.10
Final L (cm)	41.9 ± 0.231	38.6 ± 0.531	34.5 ± 0.516
Final wt (g)	43.889 ± 0.248	39.764 ± 0.450	38.857 ± 0.491
Survival rate (%)	82	71	69

Adoption of Suitable Culture Technologies of Some Commercially Important Fish Species in the Northern Region of Bangladesh

Researcher(s): Dr. Khondaker Rashidul Hasan, Senior Scientific Officer
Maliha Hossain Mou, Scientific Officer
Saokat Ahamed, Scientific Officer

Objectives

- To adopt the polyculture techniques of short-cycle species in the seasonal water bodies;
- To assess the water quality parameters of cultural water bodies;
- To estimation of cost benefit analysis of culture technologies; and
- To disseminate these polyculture techniques in different aqua-ecological zones in the northern part of the country

Achievements

Polyculture of shing under different stocking densities in the framers ponds

The experiment was conducted in farmer's ponds of Rangpur and Niphamari area. Six (06) seasonal ponds were selected for this experiment. The area of ponds ranges between 10 and 15 decimal each. The on-farm ponds were selected with the concerning of relevant Senior Upazilla Fishery Officer (SUFO/UFO). The selected ponds were prepared by drained and drying. Aquatic weeds were removed from the ponds manually and harmful and unwanted fish species removed by using rotenone 25-35 g dec^{-1} ft^{-1} if necessary and liming @1 kg dec^{-1}. After 5 days of liming, cow-dung 6 kg dec^{-1}, urea 100 g dec^{-1} and TSP 75 g dec^{-1} were applied at initial stage during pond preparation. The short-cycle fishes like as *H. fossilis*, *C. batrachus*, GIFT and *B. gonionotus* were selected for adaptive trial in those listed ponds. About 7-10 cm fingerlings of those fishes were stocked as per experimental design (Table 1) during early April 2016. Fish were fed commercially available fish feed 10-6% BW day^{-1} (containing 30- 35% protein). Length, weight data and water quality parameters *viz.*, temperature, pH, DO, CO_2, NH_3 etc. were collected fortnightly. After harvesting, the results are presented in Tables 2 and 3.

Table 1. The experimental design of polyculture of shing

Treatments	Species composition	Stock. density (nos dec^{-1})	Initial length (cm)	Initial weight (g)	Feeding
T_1	Shing	500	8.21	2.9	8-6%
	Magur	50	4.00	2.03	
	GIFT	10	4.36	4.99	
	Shorpunti	05	8.5	5.01	
T_2	Shing	600	8.22	2.9	
	Magur	50	4.05	2.01	
	GIFT	10	4.36	4.99	
	Shorpunti	05	8.4	4.99	
T_3	Shing	700	8.22	3.0	
	Magur	50	4.0	1.99	
	GIFT	10	4.37	5.00	
	Shorpunti	05	8.5	4.99	

Table 2. Water quality parameters observed in different experimental ponds

Water quality parameters	T_1	T_2	T_3
Temperature (^oC)	27.8±2.8	28.4±2.9	27.9± 2.3
D.O (mg l^{-1})	5.6±0.5	5.5±0.4	5.8±0.7
Water pH	7.3±0.2	7.5±0.4	7.5± 0.3
Transparency (cm)	27.4± 2.8	27.6± 2.5	27.6± 2.4
NH_3 (mg l^{-1})	0.17 ± 0.03^a	0.21 ± 0.03^b	0.21 ± 0.04^b

Except ammonia (NH_3) all water quality parameters had no significant differences among treatments ($P>0.05$). In the present experiment, the recorded water temperature and DO were ranged from 33.0°C to 24.6°C and 7.34 to 4.5 mg l^{-1}, respectively in different treatments. Though mean water temperature and DO did not vary significantly ($P>0.05$), however, the overall pH of water (8.30-6.80) and transparency (33.0-22.1cm) in different treatments were within the acceptable range for the fish culture (Table 2). Although the values of Ammonia (NH_3) in T_2 (0.21) and T_3 (0.21) showed significantly ($P<0.05$) higher value over T_1, however T_2 and T_3 did not vary significantly when compared using ANOVA (Table 2).

Table 3. Growth performances of *H. fossilis* in different treatments

Morphometric Parameters	T_1	T_2	T_3
Average initial weight (g)	2.9±0.00[a]	2.9±0.01[a]	3.0±0.00[a]
Av. final weight (g)	65.11± 1.11[c]	58.0±2.00 [b]	48.0±0.58 [a]
initial length (cm)	8.21±0.1	8.22±0.02	8.22±0.005
final length (cm)	21.1±0.08[b]	20.6± 0.08[b]	19.1±0.37[a]
Weight gain (g)	62.15±1.15c	55.01±1.99[b]	45.00±0.58[a]
% weight gain	2135.40±12.25[c]	1839.59±60.40[b]	1500.00±33.35[a]
ADG (% day^{-1})	0.415±0.005[c]	0.3667±0.015[b]	0.2967±0.005[a]
HC (g^{-1}cm)	3.08±0.04[c]	2.81± 0.08[b]	2.516±0.03 [a]
SGR (% day^{-1})	2.04± 0.02[c]	1.98±0.02 [b]	1.84± 0.015[a]
FCR	2.63±0.09 [a]	2.84±0.02 [b]	3.08± 0.03[c]
Sruvival (%)	77.93±1.63[b]	76.12±0.62[a]	72.71±0.14[a]
Production (kg ha^{-1})	7593.39± 235.31[b]	7793.82±239.72[b]	6981.91±62.22[a]

The final weight of *H. fossilis* in T_1 (65 g) were showed significantly higher than that of T_2 (58 g) and T_3 (48 g) (Table 3). The mean final length of *H. fossilis* was recorded as 21.1 cm, 20.6 cm and 19.1 cm in T_1, T_2 and T_3 respectively. The mean final weight gain and % weight gain of *H. fossilis* were showed significantly higher in T_1 than T_2 and T_3 respectively (Table 3). The % weight gain of *H. fossilis* in T_1 (2135.40) was highest and significantly varied ($P<0.05$) over T_2 (1839.59) and T_3 (1500.00) (Table 4). Significantly higher ADG & HC ($P<0.05$) was also recorded in T_1 (0.41 & 3.08) followed by T_2 (0.36 & 2.81) and in T_3 (0.29 & 2.51). The SGR in T_1 (2.04) was significantly higher ($P<0.05$) than in T_2 and T_3. Significantly ($P<0.05$) better nutrient utilizations i.e. apparent feed conversion ratio (AFCR) were recorded in T_3 (3.08) followed by T_2 (2.84) and T_1 (2.63) respectively (Table 4). FCR were best for fish in T_1 where lowest number of fingerlings was reared (1,23550 nos ha^{-1}). Highest survival rate was also observed inT_1 (79.57) and the lowest in T_3. (72.56) There was a significant variation ($P<0.05$) in the survival rate of *H. fossilis* in T_1 compared to T_2 and T_3 but there is no differences between T_2 and T_3 (Table 3). The mean productions of *H. fossilis* were 253.79, 264.99 and 244.31 kg treat-1 day-150 in treatments T_1, T_2 and T_3, respectively. The total production of *H. fossilis in* T_2 differed significantly ($P<0.05$) with the T_3 (Table 3).

Optimization of Breeding and Development of Culture Technology of Striped Dwarf Catfish, *Mystus vittatus*

Researcher(s): Dr. Kh. Rashidul Hasan, Senior Scientific Officer
Maliha Hossain Mou, Scientific Officer
Saokat Ahamed, Scientific Officer

Objectives

- To study brood rearing techniques of *M. vittatus* in captive condition
- To study reproductive parameters of *M. vittatus*
- To determine the reproductive response of *M. vittatus* to different doses of natural and synthetic hormone in captive condition
- To study the effect of stocking density and feeds on the growth and survival of the nursery rearing of *M. vittatus* in pond condition; and
- To assess the growth and yield performance under mono and polyculture system of *M. vittatus*

Achievements

Studies of reproductive parameters of M. vittatus

Every month the fish collection process was done for the studies of reproductive parameters like; sex ratio, gonadosomatic index (GSI), absolute and relative fecundity to know the spawning season of *M. vittatus*. A total of 549 tengra fishes were collected during July 2015 to June 2016. The sex ratio and GSI of collected fishes studied monthly; the results are shown in Table 1. Fecundity data estimation is ongoing.

Table 1. Sex ratio and GSI values of *M. vittatus* during July 2015 to June 2016

Sl. No.	Month	Total no. of fish	Male (♂)	Female (♀)	Sex ratio	Av. GSI (%)
1.	July	50	27	23	1.17:1.0	26.11±5.24
2.	August	59	31	28	1.12:1.0	20.77±3.68
3.	September	60	35	25	1.40:1.0	11.61±4.02
4.	October	83	58	25	2.32:1.0	4.46±1.19
5.	November	70	40	30	1.33:1.0	3.93±1.23
6.	December	66	50	16	3.13:1.0	2.51±0.93
7.	January	75	43	32	1.34:1.0	1.94±0.52
8.	February	61	42	19	2.21:1.0	3.02±0.62
9.	March	35	18	17	1.06:1.0	6.65±2.33
10.	April	30	16	14	1.14:1.0	14.70±3.50
11.	May	25	13	12	1.08:1.0	18.78±6.86
12.	June	54	29	25	1.16:1.0	23.03±2.95
Total/Average		549	336	213	1.41:1.0	17.45±3.74

Reproductive response of M. vittatus to different doses of natural and synthetic hormone in captive condition

During this period, PG was administered in broods with different doses. In treatment-1 (T_1), the Females were injected with PG @ 2 mg kg^{-1} during 1st dose. After 6 hour, 2nd dose was injected in females @ 3 mg kg^{-1} and at the same time, males were injected @ 2.5 mg kg^{-1}. In treatment-2 (T_2), females were injected with PG @ 4 mg kg^{-1} during 1st dose. After 6 hour, 2nd dose was injected in females @ 6 mg kg^{-1} and at the same time, males were injected @ 5 mg kg^{-1} . In treatment-3 (T_3), females were injected with PG @ 5 mg kg^{-1} during 1st dose. After 6 hour, 2nd dose was injected in females @ 10 mg kg^{-1} and at the same time, males were injected @7.5 mg kg^{-1}. Immediately after administering the hormones spawners were released into breeding hapa settled in the concrete tanks of the hatchery (capacity: 500 l) containing dechlorinated tap water (temperature: 28-31°C; DO: 5.9-6.5 mg l^{-1}; CO_2: 3.0–4.0 mg l^{-1}; pH: 7.8-8.2). Different doses were used to optimize desired hormone doses to detect ovulation, fertilization, spawning, hatching, survival to yolk sac absorption. After 9 hrs of injection, ovulation was occurred in all cases. Of them, T_3 (PG @ 15 mg kg^{-1}) showed the best breeding performances in terms of egg output rate,

fertilization rate, hatching rate and survivability of hatchling. After absorption of yolk sac (2-3 days), the spawn were transferred into metallic trays and fed on Renu gold (commercial fish feed containing 36% protein) up to 7 days to optimize rearing condition of larvae. The latency period, incubation temperature, spawning remarks, egg output rate, fertilization rate, hatching rate and survivability of hatchling is presented in the Table 2.

Table 2. Spawning response and performances of *M. vittatus* (2♂:1♀ ratio) using PG

Treatments	PG (mg kg^{-1})		Latency period (hrs)	Incubation temperature (°C)	% of egg release	% of fertilization	% of hatching	% survival of 03 days hatchling	Remarks
T_1	2.5	5 (2+3)	10	29-31	-	-	-	-	Poor ovulation, fertilization and hatching rate were observed
T_2	5	10 (4+6)	9	29-31	70	60	85	48	Comparative better ovulation, fertilization and hatching rate were observed
T_3	7.5	15 (5+10)	9	28-31	85	65	92	56	Successful ovulation, higher fertilization and hatching rate were observed

Effect of different feeds on the growth and survival of the nursery rearing of M. vittatus in pond condition

The study was conducted in metallic trays to observe the growth and yield of *M. vittatus* at different feeds. For that about 7 days old fry were stocked in well-prepared metallic trays (2.15×0.93×0.30 m^3) at a stocking density of 8,000 fry dec^{-1}. Three types of feeding were done under three treatments T_1, T_2 and T_3 (Table 3) having three replications each. The experimental design is presented in Table 3.

Table 3. Experimental design of nursery rearing of *M. vittatus*

Treatments	Stocking density (no. $decimal^{-1}$)	Feeding/culture period
T_1	8,000	Locally available commercial feed (containing 35% protein)
T_2		Formulated feed (containing 35% protein)
T_3		Mixture of locally available commercial and formulated feed

After a rearing period of 7-8 weeks, the fingerling were harvested and the growth and production parameters *viz.*, average daily growth (ADG), specific growth rate (SGR), health condition (HC), survival were calculated and compared among the treatments. Water quality parameters like as temperature, pH, and dissolved oxygen (DO) etc. was also studied. Water quality parameters and growth performances of fry for each experimental trays were monitored at fortnightly intervals. The results are presented in Tables 4 and 5.

Table 4. Growth performance of nursery rearing of *M. vittatus*

Parameters	Treatments		
	T_1	T_2	T_3
Stocking density (nos. dec^{-1})	8,000	8,000	8,000
Initial length (cm)	0.85	0.85	0.84
Final length (cm)	3.21±0.62	3.39±0.98	3.46±0.88
Initial weight (g)	0.76	0.75	0.75
Final weight (g)	3.97±0.04	3.68±2.28	4.36±2.74
ADG (g day^{-1})	0.06	0.06	0.07
SGR (% day^{-1})	3.31	3.18	3.52
HC (g cm^{-1})	1.23	1.09	1.26
Survival rate (%)	53.09	61.19	64.04

Table 5. Water quality parameter of nursery rearing of *M. vittatus*

Parameters	T_1	T_2	T_3
Air temperature (^{0}C)	29.5±2.89	28.5±0.58	30.00±2.89
Water temperature (^{0}C)	27.83±2.00	27.3±0.50	28.05±3.97
Dissolved oxygen (mg l^{-1})	7.58±1.73	8.60±0.28	8.5±0.14
pH	7.50±0.22	8.20±0.26	8.28±0.39

Culture and Constraints of Commercial Small Fish Species at Farms Level in Jessore Region

Researchers: A.F.M. Shofiquzzoha, Senior Scientific Officer
Md. Shariful Islam, Scientific Officer
Md. Abdul Halim, Scientific Officer

Objectives

- To know the present status of culture at farm levels
- To understand the growth status and production of different selective small species at farms level
- To improve the culture techniques

Achievements

Pond numbers and size for small fish culture in the study area

Pond size is an important factor for small fish culture because all management measures are planned considering the size of the ponds. The management of the small size pond is easier than the large size pond. In the present study, it was found that the average pond size was 0.3125 ha with a range from 0.07 ha to 0.48 ha (Table 1).

Table 1. Number of respondents with pond areas

Location	No. of respondents	No of ponds used for Small fish culture	Minimum size of ponds (ha)	Maximum size of ponds (ha)	Average area of pond (ha)

Jessore Sadar	15	74	0.10	2.67	19.77
Jhikargacha	8	30	0.48	4.913	12.26
Abhaynagar	8	16	0.07	32.39	6.1
Monirampur	4	5	0.10	0.33	0.93
Total	35	125			

Small fish culture season

From the survey, it was found that both monoculture and poly-culture system was carried out by most of the farmers. In the study area, the small fish culture season was determined from December-February (45.71%), March-May (40.00%), June-August (5.71%) and September-November (8.57%).

In the study areas, fish species were cultured mainly koi, shing, magur, pangus and some Indian Major Carps. They practiced both monoculture and poly-culture system. The small fish cultured by the respondents were recorded about 35% koi, 24% shing, 13% magur, 7% pangus and had even 21% poly culture practiced among the farmers. (Fig. 1).

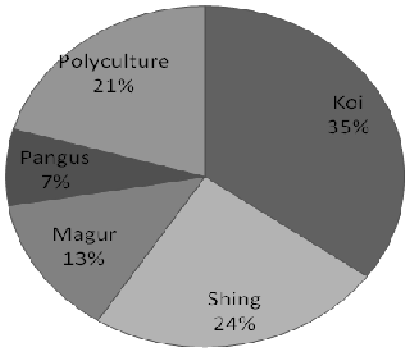


Fig 1. Species are generally cultured in the Jessore region

It has been found that most of the farmers usually culture small fish like koi, shing magur 55%, 32%, 13% respectively in the study areas (Fig. 2.).

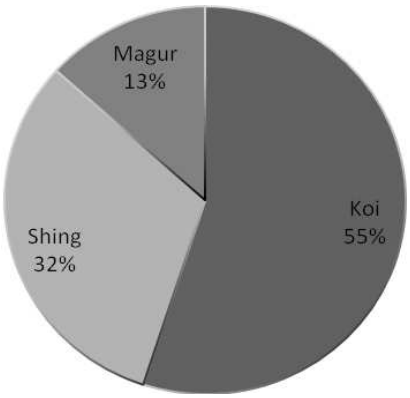


Fig. 2. Small fish species are cultured by farmers