BREEDING PERFORMANCE AND NURSERY PRACTICES OF *LABEO BATA* (HAMILTON-BUCHANAN, 1822)

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Abstract: A study was conducted to observe the breeding performance and nursery practices of the threatened indigenous fish species *Labeo bata* in Jessore (Longitude: 89°122 003 East; Latitude: 23°102 453 North), Bangladesh during 1998 to 2002. Eight trial doses of PG (Pituitary Gland) used for induced breeding of *L. bata* were 1.0mgPG/kg in the first dose, and 1.0 to 8.0mgPG/kg in second dose for female. On the other hand, five trial doses were administered for the male to identify the suitable single dose (1.5mgPG/kg) only. The best performance was obtained with 5.0mg of PG/kg body weight in the second dose for female of wild and successive three generations. The highest growth (lt.: 19.37mm and wt.: 57.67mg) and survivability (63.33%) of the fish fry was observed by using rice bran and mustard oil cake after 10 days of nursing.

Key words: Breeding performance; Nursery practices; Threatened species; *Labeo bata*.

INTRODUCTION

Khan (2000) has documented about 56 freshwater fish species critically or somewhat endangered in Bangladesh and *Labeo bata*, locally called as ilish bata, bhangon bata or bata, is one of them belonging to the Family Cyprinidae. It is a non-migratory fish and remains in one habitat throughout its life (Mathur, 1973; Mathur and Robbins, 1971). Earlier the fish was widely distributed throughout the rivers, haors, baors, beels, jheels, canals and ponds of Bangladesh (Hussain *et. al.*, 1996; Bhuiyan *et al.*, 1992; Rahman, 1989; Hafizuddin *et al.*, 1989; Hafizuddin, 1985; Islam and Hossain, 1983). The most identifying characteristic of the fish is the presence of small black spot on 5th and 6th scales on the lateral line (Rahman, 1989).

The fish matures during monsoon season (April to July) in Kaptai Lake and attends first maturity at 18.62 cm in total length (Azadi and Naser, 1996a). It breeds in floodplains during rainy season as Indian major carps (Anon, 1996). It spawns from May to July in Kaptai Lake (Azadi and Naser, 1996b) at the range of water temperature 26.2-30.3°C (Ramakrishnaiah and Banerjee, 1979; Saha *et al.*, 1957).

However, little is known about the breeding technique and larval rearing of *L. bata* from the Indian subcontinent. Therefore, the present study was undertaken to understand its breeding performance in order to develop suitable low cost breeding technique and nursery practices in Jessore from where the natural fish species has seemed to disappear.

MATERIALS AND METHODS

Species collection

One thousand fingerlings (lt.: 5-7cm each and total wt.: 1.8kg) of *L. bata* were collected from Kumar river at Jhenaidah district with a local fisherman. The fingerlings were transported in well aerated tanks to Arabpur Fish Farm, Jessore where brood management with suitable pond water and induced breeding were conducted.

Breeding performance

Eight and five trial doses for female and male, respectively were used to find out the breeding performance in respect of pituitary gland extract (Table 1). The female fish was given two doses with the interval of 6hrs. The male was given a single dose of PG, just after the first dose of female.

Brood selection

The fish became matured within a year. The brood fishes were sexed by external characteristics, such as, the bulging abdomen, soft ventral abdominal region, comparatively larger size, felt pectoral spine, smooth pectoral fin and swelling anal rim with reddish colour of females. But in comparison to males the normal abdomen, milt comes out with gentle pressure on the abdomen, smaller size of similar age, serrated pectoral spine, rough pectoral fin and concave anus from exterior was found.

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Hormonal extract preparation and injection

The acetone dried PGs were purchased from the local market. At first PGs were placed on blotting paper so as to remove excess acetone as much as possible followed by weighing with a sensitive scientific balance to pool the desired amount of PG. The pooled amount of PGs were finely crushed by tissue homogenizer and diluted with required amount (fish was injected with no more than 0.3-0.4ml for each brood, so to avoid injury) of distilled water. The solution was centrifuged to settle the tissue residue at the bottom and the resulting supernatant solution was then taken for injection with 3ml syringe. According to Rottmann *et al*. (1991), the concentration of hormone were mixed in recommended dose, multiplied by the approximate weight of individual brood fish, was divided by the desired volume of the injection. In practice, a brood fish was placed in a cloth bag, lying laterally in the water. The upper half of the fish was held above the surface. For intraperitoneal injection, the needle was inserted at the base of a pectoral fin, at an angle of 45^{o} and the mixture was injected at 6am into the body cavity. The second injection for female was administered at 12am to the opposite side of the first injection.

Natural spawning in hapa

Both male and female broods were kept in a hapa (placed in a concrete cistern of 300cm x 180cm x 75cm) just after second dose of female with water shower for natural spawning. After spawning the water hardened eggs were collected in a plastic bowl and measured, then placed in a conical bottom cemented incubation jar (r. 50cm; ht. 100cm, cap. 300l) having a water flow (0.2-0.3l/sec) system. The deep tube well water quality parameters were measured after 12hrs settled for hatchery operation (Table 4).

Determination of percentage and required time for each step

One hundred PG treated water hardened eggs were collected (in triplicates) 3hrs after insemination from incubation jars and were observed under compound microscope to determine the fertilization percentage (fertilization rate (%) =number of fertilized eggs/total number of eggs x 100). Transparent eggs with cell divisions were considered as fertilized eggs while the translucent ones with milky colour were considered as unfertilized. Hatching percentage (hatching rate (%) = number of hatched spawn/number of fertilized eggs x 100) were also calculated from triplicates of 500 eggs for each sample in mini incubation jars made of tin (cap. 12l). During the hatching, the water discharge rate increased up to 0.4l/sec. Deformities percentage was also calculated with naked eye. Ovulation and hatching time were counted by eye estimation but yolk sac absorption time was determined by the vertical movement of larvae with feeding of the poultry egg yolk merge.

Nursery practices

Spawn nursing with pond preparation was done from 01 to 25 July, 2001 at Haque Fish Production Center, Narayanpur, Jessore.

Nursery pond preparation

Two weeks before stocking, the pond was limed by $CaCO_3$ at the rate of 750kg/ha. One week before stocking, the pond was manured by cowdung and mustard oil cake (MOC) at the rate of 1000kg and 500kg/ha, respectively. The pond water was finally treated by spraying diesel at the rate of 50l/ha to remove insects and other aquatic pests after 5 days of manuring.

Stocking of spawn

After 2 days of backswimmer eradication, spawn (20g) was released in nine ponds each having an area of 0.004ha with a water depth of 30cm at evening.

Post-stocking management

After stocking, no additional manure was applied but food supplementation were provided by Rice Bran (RB) for D_1, Mustard Oil Cake (MOC) for D_2, and both RB and MOC (1:1) for D_3 at the rate of 150% of the initial weight during stocking from 2^{nd} to 5^{th} days and 7^{th} to 9^{th} days. All feed stuffs were passed through a fine meshed sieve of 400μ to remove the husk and to ensure uniformity in the size of the particles. The prepared feed was sprinkled on the water surface around the shoreline of the pond. At sixth day, chatjal was dragged for checking health condition and the fry was not fed in any ponds. Fresh water was let into raise the water level. The first addition of fresh water was conducted two days after stocking. Fresh water was then added once every two days, increasing the water level by 10cm each time. The number of spawn and fry were counted by taking 1ml and 10ml samples, respectively. The average number of 3 samples was calculated to determine the number of total amount of spawn and fry. The fry of all ponds were completely harvested by dragging chatjal and they were transferred to grow out ponds.

Climatological and physico-chemical parameters

Climatological factors and water quality parameters of nursery ponds were collected during the experimental period of 15 to 25 July, 2001. Data of atmospheric temperature and rainfall were collected from SPARRSO (Space Research and Remote Sensing Organization), Dhaka, Bangladesh. Nursery pond surface water temperature and secchi disk depth reading were recorded daily at 12am and other parameters such as pH, DO, total alkalinity and total hardness were recorded (Lamotte, Aurun, 3/29/94 AM KIT, Code xx 00153, Chestertown, Maryland 21620, USA) at three days interval after stocking the spawn.

Statistical analysis

The data on breeding performance and nursery practices were normalized by arcsine transformation. Statistical analysis of the data for all experiments were done by one way Analysis of Variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT) to determine differences between the means taking at 1% ($P<0.01$) or 5% ($P<0.05$) significance levels (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Breeding performance

The breeding performance of *L. bata* was studied in 1999, 2000, 2001 and 2002 for wild, F_1, F_2 and F_3 generations, respectively. It is mentioned in Table 1 that T_1, T_2, T_3, T_7 and T_8 did not perform (Table 1).

Quantity of water hardened eggs

Ovulation took place naturally within 5:30-6:00hrs after the second injection of female at 27-28°C in all the generations. The present experiment showed that T_5 produced the highest number of water hardened eggs (2410.00-2596.67g) in all generations except the F_2 generation where it was in T_4 (2290.00g) (Table 2). The treatments T_4 and T_6 produced almost same quantity of water hardened eggs irrespective of generations (wild fish in 1999, F_1 in 2000, F_2 in 2001 and F_3 in 2002).

Percentage of fertilization

The doses of PG in male (1.5mg) was significantly affected to fertilize the eggs in *L. bata* for wild, F_1, F_2 and F_3 generations (Table 2). In 1999, the highest percentage of fertilization (87.00%) was found in T_5 followed by T_6 (51.00%) and the lowest (32.00%) was in T_4. The same trend was found in 2000 but the reverse trends were observed in case of T_4 and T_6 in 2001 and 2002. In 2001 and 2002, the fertilization percentage of T_4 was higher than T_6 but T_5 gave the highest percentage of fertilized eggs in both generations.

Percentage of hatching

The hatching of larvae took place between 16:30-18:30hrs after ovulation at 27-28°C in all the generations. The percentage of hatching was significantly affected by the doses of PG in 1999, 2000, 2001 and 2002 (Table 2). In 1999, the highest percentage of hatching (84.00%) was found in T_5 followed by T_6 (47.67%) while the lowest (28.67%) was observed in T_4. In 2000, the highest percentage of hatching (87.33%) was found in T_5 followed by T_6 (56.33%) where the lowest (39.67%) was observed in T_4. In 2001, the percentage of hatching was maximum (85.33%) in T_5 followed by T_4 (76.67%) and minimum in T_6 (72.67%). On the other hand, the treatment T_5 gave the highest percentage of hatching (88.33%) in 2002 followed by T_4 (55.33%) and lowest (43.00%) in T_3.

Percentage of deformities

The percentage of deformities of spawn was significantly affected by the doses of PG of *L. bata* only in F_2 generation and non significant in F_3 generation but other generations (wild and F_1) were highly significant (Table 2). In 2002, the highest percentage of deformities (8.33%) were found in T_4 followed by T_6 (6.33%) while the lowest (5.67 %) was observed in T_5. But 2001 showed the minimum percentage of deformities in respect of rest three generations where highest (3.67%) was found in T_6 followed by T_4 (3.00%) and the lowest in T_5 (1.33%).

Quantity of spawn produced

The yolk sac absorption required from 34:00-38:00hrs after hatching at the water temperature of 27-28°C in all generations. The doses of PG significantly affected the production of spawn in 1999, 2000, 2001 and 2002 (Table 2). In 1999, the highest production of spawn (256.03g) was found in T_5 followed by T_6 (95.14g) while the lowest (58.96g) was observed in T_4. In 2000, the highest production of spawn (281.29g) was found in T_5 followed by T_6 (125.01g) and that was the lowest (69.23g) in T_4. In case of 2001, the number of spawn were maximum (275.04g) in T_5 followed by T_4 (221.92 g) and minimum in T_6 (195.87g). On the other hand, the treatment T_5 gave the highest production (290.10g) in 2002 followed by T_4 (111.32g) and the lowest (75.68g) in T_6.

The results of present study were in agreement with the findings of Hossain *et al.* (2004). They used five trial doses of PG to the female viz., 0.5, 1.0, 1.5, 2.0 and 2.5mg/kg body weight followed by 3.0, 4.0, 5.0, 6.0 and 7.0mg PG/kg body weight at 6hrs interval. They found the best result of PG dose for spawning was 1.5+5.0mg for female instead of 1+5mg in present study. The present experiment revealed that *L. bata* produced the highest number of water hardened eggs in 1999, 2000 and 2002 with PG dose of 1+5mg for female except in 2001 where the dose was 1+4mg. That is, the fish

Table 1: Trial doses of PG for breeding of *Labeo bata*.

Treatment	Trial doses of PG				Response by PG in different years
	Female		Male		
	1st dose (mg/kg)	2nd dose (mg/kg)	Single dose (mg/kg)	Range of doses (mg/kg)	
T_1	1	1	1.5	0.5, 1.0, 1.5, 2.0 and 2.5	NR
T_2	1	2	1.5		NR
T_3	1	3	1.5		NR
T_4	1	4	1.5		Gd-'99, '00; Br-'01, '02
T_5	1	5	1.5		Bt-'99, '00, '01, '02
T_6	1	6	1.5		Br-'99, '00; Gd-'01,'02
T_7	1	7	1.5		NR
T_8	1	8	1.5		NR

NR =No Response; Gd =Good; Br =Better; Bt =Best

Table 2: Breeding performance of *Labeo bata* in diffrent years following the PG doses (in all experimental treatments, four individuals of both female and male fish were used as brood).

Treatment	Eggs (g)				Fertilization (%)				Hatching (%)			
	1999	2000	2001	2002	1999	2000	2001	2002	1999	2000	2001	2002
T_4 (1+4;1.5)	1635.00	1378.33	2290.00	1593.33	32.00	43.00	79.67	58.33	28.67	39.67	76.67	55.33
T_5 (1+5;1.5)	2410.00	2546.67	2216.67	2596.67	87.00	90.67	88.33	92.33	84.00	87.33	85.33	88.33
T_6 (1+6;1.5)	1576.67	1753.33	2133.33	1388.33	51.00	60.33	76.33	48.67	47.67	56.33	72.67	43.00
LSD	210.85	244.18	--	171.89	9.41	7.75	5.80	10.48	10.29	8.20	6.30	11.41
Level of significance	**	**	NS	**	**	**	**	**	**	**	**	**

Treatment	Deformities (%)				Spawn (g)			
	1999	2000	2001	2002	1999	2000	2001	2002
T_4 (1+4;1.5)	8.33	4.67	3.00	8.33	58.96	69.23	221.92	111.32
T_5 (1+5;1.5)	2.00	1.67	1.33	5.67	256.03	281.29	275.04	290.10
T_6 (1+6;1.5)	5.00	5.00	3.67	6.33	95.14	125.01	195.87	75.68
LSD	4.40	2.26	1.49	--	28.20	33.65	15.89	32.14
Level of significance	**	**	*	NS	**	**	**	**

** Significant at 1% level; * Significant at 5% level; NS = Non significant
1999 for Wild fish; 2000 for F_1 generation; 2001 for F_2 generation and 2002 for F_3 generation
T_5 means, Female: first dose 1mg and second dose 5mg PG/kg body weight; Male: 1.5mg PG/kg body weight i.e., (1+5;1.5).

gave the highest number of water hardened eggs in 2001 with lower dose may be due to influence of rain (rainfall, 22mm; Table 4) and cloudy weather. As a result, the atmospheric temperature (25.6-31.6°C) dropped 1°C from the previous day (26.8-32.6°C) and water temperature decreased 2-3°C (Personal Observation). Similar trend was observed by Ramakrishnaiah and Banerjee (1979) and Saha *et al.* (1957) that water temperature was somewhat low on the days of spawn occurring (26.2-30.3°C; avg.: 28.3°C) as against 27.0-30.8°C (avg.: 28.9°C) on other days and that cloudy days accompanied by thunderstorm and rains influence the minor carp *L. bata* spawning in bundhs. Dwivedi and Reddy (1986) opined that the environmental parameters like temperature, oxygen, pH, water current enhanced the fish breeding and hatching and the spray and shower not only increase the dissolved oxygen, but also keep their environment cool and simulate natural conditions. The hatchery water quality viz., water temperature (27-28°C), pH (7.2-7.8), DO (5.4-6.2 mg/l), total alkalinity (380-410mg/l) and total hardness (450-510mg/l) were suitable for fish seed production in present study (Table 4).

Nursery practices

The nursery management with different diets on *L. bata* spawn was studied from July 15-25, 2001.

Length of fry

There was a significant variation among the treatments (feed) in relation to length of fry (Table 3). The highest length of fry (19.37mm)) was found in D_3 (RB+MOC) followed by D_2 (18.07mm) and the lowest (16.70mm) in D_1.

Weight of fry

The weight of fry was significantly varied with the rate of feed used (Table 3). The highest weight of individual fry (57.67mg) was found in D_3 (RB+MOC) followed by D_2 (50.03 mg) and the lowest (41.60mg) in D_1.

Production of fry

The effect of feed on the production of fry was significantly different (Table 3). The production of fry was the highest (0.445kg/0.004ha) in D_3 (RB+MOC) followed by D_2 (0.327 kg/0.004ha) and the lowest (0.232kg/0.004ha) in D_1.

Survivability of fry

The survivability of *L. bata* fry was significantly affected by different feed preparations used (Table 3). The highest survival rate of fry (63.33%) was observed when rice bran and mustard oil cake (D_3) was used followed by (53.67 %) only mustard oil cake (D_2), while it was the lowest (45.67%) when only rice bran was used (D_1).

Table 3: Growth, production and survivability of *Labeo bata* in nursery practices (2001).

Treatment	Length (mm)	Weight (mg)	Production (kg/0.004 ha)	Survivability (%)
D_1	16.70	41.60	0.232	45.67
D_2	18.07	50.03	0.327	53.67
D_3	19.37	57.67	0.445	63.33
LSD	1.35	9.89	0.14	11.46
Level of significance	**	**	**	**

** Significant at 1% level
Initial length and weight of *Labeo bata* spawn were 5.6mm and 1.6420361mg, respectively after 38hrs of hatching.
D_1 = Rice bran (RB);
D_2 = Mustard oil cake (MOC);
D_3 = Rice bran and mustard oil cake.

Table 4: Climatological factors and water quality parameters of hatchery water and nursery ponds of *Labeo bata* fry.

Treatment	Atmospheric Temperature (°C)	Water Temperature (°C)	Rainfall (mm)	Secchi depth (cm)	pH	DO (mg/l)	Total alkalinity (mg/l)	Total hardness (mg/l)
Hatchery	25.6-31.6 at July 12, 2001	27-28	22 at July 12, 2001	more than 30	7.2-7.8	5.4-6.2	380-410	450-510
Nursery Pond -1 (D_1)	(25.6-33.5) avg. 29.5 from July 15-25, 2001	28.5-32.0	(16-61) avg. 42.82 from 15-25 July, 2001	42-62	7.5-8.1	3.4-6.2	180-245	150-195
Nursery Pond -2 (D_2)		29.5-32.5		37-61	7.2-8.0	3.2-60	200-257	160-200
Nursery Pond -3 (D_3)		28.5-31.5		45-65	7.4-8.3	3.7-6.6	200-265	160-206

Table 3 showed that the nursery practices with three different food stuffs gave the highest length (19.37mm), weight (57.67mg), production (0.445kg/0.004ha) and survivability (63.33%) when used RB and MOC together at rate 30g/day (150% of spawn body weight) in two installments: second to fifth day and from seventh to ninth day of stocking. But after 10 days nursing of *L. boggut* spawn total length was obtained 16.993mm (Selvaraj *et al.*, 1972) which was almost similar when rice bran was used in the present study. The growth and survival rate of carp hatchlings depend to a large extent on the easy availability of planktonic food organisms in general and particularly on zooplankters. Mumtazuddin *et al.* (1982) reported that the tender fry because of their easy availability and digestibility than the phytoplankters prefers zooplankters. Thus, the ponds showing higher growth and survival rate coincided with the abundance of planktonic food organisms of the pond in the present study. According to Bhuiyan (1964), *L. bata* fry are almost surface feeder that was also coincided with the present observation.

Table 4 indicate that the different nursery pond water quality viz., water temperature (28.5-32.5°C), transparency (37-65cm), pH (7.2-8.3), DO (3.2-6.6mg/l), total alkalinity (180-265mg/l) and total hardness (150-206mg/l) were suitable for fish fry nursery-rearing pond (Mumtazuddin and Khaleque, 1987).

CONCLUSION

The successful induced breeding of the endangered *Labeo bata* have been reported for the first time in Bangladesh. The best dose for female (both wild and successive generations) was 1.0mg and 5.0mg PG/kg body weight for the first and second injection, respectively. The highest growth (lt.: 19.37mm and wt.: 57.67mg), production (0.445kg/0.004ha) and survivability (63.33%) was achieved with mixed diet (rice bran and mustard oil cake; 1:1). Though the study was considered only the PG doses for breeding technique and different diet for spawn nursing yet, in depth long term investigation is necessary to identify the physiological status of male and female brood, diet provide to them, environmental relations, fertility of ponds and other related factors involved to fish breeding.

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