



BREEDING BEHAVIOUR, INDUCED BREEDING AND LARVAL REARING OF ILISH BATA, *LABEO BATA* (HAM.)

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Abstract: Breeding behaviour, induced breeding and larval rearing of a threatened species *Labeo bata* were conducted at the Government Fish Seed Multiplication Farm, Khulna Sadar, Khulna in July 2003. A number of distinctive measures viz., following, nudging, circling, pressing and breeding run were used to release gametes within four chambered nylon *hapa* placed in a concrete cistern. Breeding activity started 5:45-6:45 hrs after the second injection of PG to the females at 26-27 °C water temperature. The relatively sudden increase in female responsiveness and attractiveness to males appear to coincide with the occurrence of ovulation. Females participate in a number of breeding runs over a period of 1 hr, thereafter female activity decreases rapidly. To optimize the effective dose of breeding, the females were first injected at the rate of 0.5, 1.0, 1.5, 2.0 and 2.5 mg PG^{-kg} body weight and the second injection at the rate of 3.0, 4.0, 5.0, 6.0 and 7.0 mg PG^{-kg} body weight. Dose interval between first and second injection was 6 hrs. At the time of first dose to female, males were administered single constant dose of 1.5 mg PG^{-kg} body weight. A total of five experiments were conducted in triplicates and each replication consisted of three males and two females. The best result in terms of breeding was observed at first dose of 1.5 mg PG^{-kg} and second dose of 5.0 mg PG^{-kg} body weight for the females. The fertilization and hatching rates with spawn production of the same dose (1.5+5.0 mg PG^{-kg} body weight) were the best of 83.88±1.6 %, 80.67±7.8 % and 120 g respectively. All spawns were maintained in nursery ponds of 0.004 ha each with a suitable water condition. After 14 days of feeding only mustard oil cake at the rate of 250 g/0.004 ha, the obtained best result was an average length of 24.2 mm and weight of 87 mg with 63% survival for 35 g spawn. The growth and survival rates were inverse in relation to different stocking densities.

Key words: Breeding behaviour, induced breeding, larval rearing, ilish bata, *Labeo bata*

Introduction

Labeo bata (Ham.), commonly known as ‘Bata’ or ‘Bhangan Bata’, is a medium size indigenous carp and enlisted as a threatened species in Bangladesh (Ameen *et al.*, 2000; Hussain *et al.*, 1997). However, it may attain a maximum length of 61 cm (Anon, 1996; Azadi and Naser, 1996a; Rahman 1989). The distinctive identifying feature of the species is the presence of small black spot on 5th and 6th scales on the lateral line (Azadi and Naser, 1996b; Rahman, 1989).

It is a non-migratory fish and remains in one habitat throughout its life (Mathur, 1973; Mathur and Robbins, 1971). Rivers, beels, baors, canals, ponds, inundated floodplains and ditches are its habitat. It is bottom feeder relies on periphyton, detritus, insect larvae, protozoans and algae (Anon, 1996; Mathur, 1973; Mathur and Robbins, 1971). Its feeding intensity is affected by the maturation of the gonads (Chatterji and Siddiqui, 1979).

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It matures during monsoon season (April to July) in Kaptai Lake and attains 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, 1996c) at comparatively lower temperature (26.2-30.3 °C) (Ramakrishnaiah and Banerjee, 1979; Saha *et al.*, 1957).

The species is delicious and therefore a costly fishery item (Azadi and Naser, 1996c). However, little is known about the mechanisms governing breeding behaviour in small indigenous fish like *L. bata* from the Indian subcontinent. Therefore, the present study was undertaken to understand its breeding behaviour in order to develop suitable low cost breeding technique and nursery practice in Khulna from where the fish has seemed to disappear.

Materials and Methods

Brood collection: Fourty female (117-150 g in weight, 131 g on average) and 60 male (96-140 g in weight, 114 g on average) broods were collected from the natural source of Jhenaidah district in July, 2003. Collected broods were transported to the Government Fish Seed Multiplication Farm, Khulna Sadar, Khulna in well aerated tanks.

Breeding behaviour: The breeding behaviour was observed in *hapa* (280 x 160 x 45 cm), which was separated in four chambers by clothing that formed a raceway. The *hapa* was placed in a concrete cistern (300 x 180 x 75 cm) where 30 cm water depth was maintained with circulating water at rate of 200 ml^{sec}. A continuous observation in daylight was done from the above and the sides of the cisterns to record the breeding behaviour. Male and female breeders were kept separate until the second dose to the females. After second injection to the females, both female and male breeders were transferred to breeding *hapa* providing water flow and showering. Recording of behavioural observations was started as males showed increased activity and 'interest' to the females' immediately after transfer to female *hapa*. Recording of behaviour was started as soon as there was a noticeable increase in courtship activity for up to 90 min by which time of breeding activities usually ceased.

Induced breeding: Five trial doses to the females were administered to optimize the dose of PG. The females were first injected intraperitoneally at the rate of 0.5, 1.0, 1.5, 2.0 and 2.5 mg PG^{-kg} body weight and the second injection at the rate of 3.0, 4.0, 5.0, 6.0 and 7.0 mg PG^{-kg} body weight. Dose interval between the first and the second injection was 6 hrs. At the time of first dose to females, male fishes were administered single constant dose of 1.5 mg PG^{-kg} body weight. Each experiment was conducted with three replications and each replication was consisted of three males and two females (3:2). The breeders were removed from the *hapa* when the natural breeding completed. Water hardened eggs were transferred to the incubation jar. To determine the rate of fertilization, three samples of eggs were observed under compound microscope after 3 hrs of ovulation. Transparent eggs with cell divisions were considered as fertilized; on the other hand translucent eggs with milky colour were regarded as unfertilized. Three samples were taken to record the hatching rate. One hundred (100) eggs were put in a small floating plastic cylinder (dia.10 cm) with a nylon mesh at the bottom and placed in conical bottom incubation (cement) jar. The hatchery water quality was maintained within suitable range for the breeding activity.

Larval rearing: After eradication of backswimmers by dragged *chatjal* (net made of coarse cloth), raw cow dung (500 g) with Urea (50 g), TSP (30 g) and MP (10 g) spread as manure in each nursery pond of 0.004 ha. The pond was also manured by mustard oil cake (200 g) for the next day followed by cow dung application. The spawn released in the nursery pond after fourth day of first manuring.

After stocking the spawn, pond water increased to a minimum level (about 2 cm) for each day of nursing and mustard oil cake (250 g^{-day}) as food was applied except the day of netting. After one week, *chatjal* was dragged for stretching the spawn. After two weeks of nursing all fries were transferred to the rearing pond.

Some physical and chemical variables of the nursery ponds water were recorded daily and weekly intervals respectively at noon. Water temperature and secchi disc reading were recorded daily at noon. The pH, total alkalinity and total hardness were measured at weekly intervals.

Results

Breeding behaviour: After second injection of female, fish showed changes in behaviour when males were transferred to the female *hapa*. The males became more active and exhibited increased gulping movements of the mouth. Occasionally, male suddenly started rapid swimming directed towards or around the female. The females showed a pronounced increase in gulping and in the amplitude and frequency of gill ventilation movements.

Male behaviour: Male breeding behaviour consisted of the followings- follow, nudge, circle, pressing and breeding run. *Follow*, the male followed the female with the pelvic fins folded just before and during the period of breeding. Following occurred in a series of bouts ranging in duration from a second to a minute. *Nudge*, after following to the female, the male may nudge the body of the female with its snout, usually around the genital pore and sometimes on the head region. The pelvic fins partially folded but the dorsal fin fully extended. Following and nudging was observed occurring simultaneously. *Circle*, the male circled when the female remained stationary or swim slowly. It was observed that the female kept its position horizontal and the male body was tilted up 60-70° from the horizontal position of the female. Circling appeared also at the same time of following and nudging but declined in frequencies after 30 min of the first breeding run. *Pressing*, the male attempted to come along the side of the female. The tail of the male was bent at an angle that both the male and female's ventral region could be touched each other. The male used to push the female upward toward the water surface by its pelvic fin. *Breeding run*, when the female ready to breed, it cooperates the male in a series of breeding runs in which the pair of swimming occurred toward the water surface. During the breeding run both exhibited rapid undulations of the tails. Milt and eggs were released during the last phase of the breeding run i.e., when they come near the surface. Breeding runs were usually repeated over a period of 30-60 min. The maximum number of runs were 10 in 15 min, but in most cases runs occurred at a rate of 4-5 per 15 min. Most of the spawning occurred within the first 30 min.

Female behaviour: Prior to breeding, the female did not show changes in behaviour rather than pronounced gulping movements. Before ovulation, female started cooperation to the male in a series of breeding runs. An unovulated female tend to ignore or evade any courtship attention.

Induced breeding: The best result in terms of breeding was observed at first dose of 1.5 mg^{-kg} and second dose of 5.0 mg PG^{-kg} body weight for the females while the doses of the males (1.5 mg PG^{-kg} body weight) was constant. Fertilization rate with the dose of female 1.0+4.0, 1.5+5.0 and 2.0+6.0 mg PG^{-kg} body weight were 56.67±2.1, 83.88±1.6 and 41.56±3.4 % respectively while the spawn production of the same doses were 50, 120 and 35 g respectively (Table 1).

Table 1. Details of the induced breeding of *Labeo bata* using PG extract (time in parenthesis).

Treatment	Doses of PG ^{kg} body weight			Percentage (%) of fertilization (Time between resolving dose and spawning)	Percentage (%) of hatching (Time between spawning and hatching)	Quantity of spawn (g)
	Female		Male			
	First	Second	Single			
T ₁	0.5	3.0	1.5	--	--	N/R
T ₂	1.0	4.0	1.5	56.67±2.1 (5:45-6:30)	51.75±2.3 (17:00-19:00)	50
T ₃	1.5	5.0	1.5	83.88±1.6 (6:00-6:15)	80.67±7.8 (17:30-18:30)	120
T ₄	2.0	6.0	1.5	41.56±3.4 (6:00-6:45)	34.46±4.8 (17:30-19:45)	35
T ₅	2.5	7.0	1.5	--	--	N/R

N/R = Not response; Values are the mean ± SD

Larval rearing: The initial total length of spawn during stocking was 5.7 mm (50 hrs after hatching). The stocking density in Pond -1 (P₁), Pond -2 (P₂) and Pond -3 (P₃) were 12.35 kg^{-ha}, 29.64 kg^{-ha} and 8.65 kg^{-ha} respectively. After 14 days of feeding with mustard oil cake at a rate of 250 g/0.004 ha, they attained an average length of 23.4 mm and weight of 75 mg with 58% survival for 50 g spawn, an average length of 17.7 mm and weight of 63 mg with 37% survival for 120 g spawn and an average length of 24.2 mm and weight of 87 mg with 63% survival for 35 g spawn (Table 2). After that the fry were transferred to stocking ponds.

Table 2. Production, growth attainment and survival of fry of *Labeo bata* after 14 days of nursing under different stocking densities (average value in parenthesis).

Ponds	Stocking density		Final production		Growth attainment		Survival (%)
	Million ^{-ha}	Kg ^{-ha}	Million ^{-ha}	Kg ^{-ha}	Length (mm)	Weight (mg)	
P ₁	7.26	12.35	4.21	315.75	21.1-26.7 (23.4)	67-88 (75)	58
P ₂	17.43	29.64	6.45	406.35	13.6-27.1 (17.7)	42-72 (63)	37
P ₃	5.09	8.65	3.21	279.27	22.5-26.2 (24.2)	76-95 (87)	63

Table 3. Water quality variables in nursery ponds of *Labeo bata* spawn.

Pond	Water depth (cm)	Water temperature (°C)	Transparency (cm)	pH	Total alkalinity (mg ^{-l})	Total hardness (mg ^{-l})
P ₁	80-110	27.9-34.6	33-52	7.3-8.1	280-340	190-230
P ₂	85-115	28.2-35.3	35-59	7.5-8.0	250-360	200-265
P ₃	90-120	27.8-33.4	28-46	7.6-8.0	320-380	170-210

No variations in water quality variables was noticed through out the experimental period (Table 3). Water temperature ranged from 27.8-35.3 °C at noon. Water temperature slightly increased with the increase in fish density of 29.64 kg^{-ha}. Water colour was always greenish with secchi disc reading recorded between 28-59 cm. The chemical variables viz., p^H, total alkalinity and total hardness were ranged from 7.3-8.1, 250-380 mg^{-l} and 170-265 mg^{-l} respectively were measured in weekly intervals.

Discussion

Breeding behaviour: The eggs of *L. bata* are buoyant and non-adhesive. Breeding runs were oriented away from the sides or floor of the tank into open water. Although we had no means of determining precisely just when ovulation occurred or was initiated, we believed that the sudden onset of ovulation occurred at the same time as or very shortly after breeding activity. The first eggs were usually released within 30 min of the onset of male-female courtship. Most breeding occurred within 30 min following the first breeding run, thereafter breeding activity declined sharply and by 60 min after onset only an occasional run occurred.

Observation suggests that a female ready to begin ovulation releases a chemical signal, a pheromone, that attracts and excites the male and once males become sexually active, persistent following and nudging are directed towards the ovulated female. Partridge *et al.* (1976) describe similar observations in their study on goldfish and established that the female goldfish releases an odour that both attracts and excites the male. The goldfish and a variety of other species (Liley, 1982; Liley and Stacey, 1983) release pheromone in the fluids from the ovary at the time of ovulation.

Induced breeding: Out of five treatments of broods two were without response of induced breeding might have been the under ($T_1 = 0.5+3.0$) and over ($T_5 = 2.5+7.0$) doses of PG for female. Mustafa (1997) found better respond in spawning grass carp (*Ctenopharyngodon idella*) treated with PG. PG was used in the present study due to its availability and low cost.

There is no significant report regarding induced breeding of *L. bata*. In this study the best result was obtained at rate of hormone was given 1.5 mg and 5.0 mg PG^{-kg} body weight with an interval of 6 hrs for female and single dose of 1.5 mg PG^{-kg} body weight for male during first dose to female (Table 1). Natural breeding occurred 5:45-6:45 hrs after the second dose to female in all 3 treatments (T_2 , T_3 and T_4). Hatching occurred after 17:00-19:45 hrs of breeding at temperature 26-27 °C. Kowtal (1970) estimated slightly shorter hatching times of 15:50-17:00 hrs for *L. elongatus* from the Mogi Guacu river, south-eastern Brazil, with water temperatures of 24-26 °C. Fertilization of eggs was found to be the highest at 83.88±1.6 %. Almost similar result was observed for fertilization rate in *L. rohita* at 75-90% by Ananda (1973). Hatching percentage was 80.67±78% in the present study but Islam and Choudhury (1976) found lower performance in respect of ovulation (60%), fertilization (76%) and hatching (60%) in *L. rohita*. In the present study the yolk sac absorption took place within 38-46 hrs of hatching. Observation on the absorption of yolk sac was confirmed after horizontal movement of spawn in the incubation jar and feeding became start confirmed by using boiled egg yolk merge. A total of 50 g, 120 g and 35 g of spawn were produced from two female fish in Treatment T_2 , T_3 and T_4 respectively (Table 1).

Larval rearing: As far as known, no information is available on the nursery of spawn and fry of *L. bata*. After 10 days of post larval stages of *L. boggut* total length was 16.99 mm (Selvaraj *et al.*, 1972). Haque *et al.* (1993) found maximum survival at the lowest stocking density with a steady fall in the survival rate of the fingerlings with as increasing stocking rate in case of mrigal (*Cirrhinus mrigala*). Saha *et al.* (1988) conducted an experiment on rohu (*L. rohita*) fry and obtained higher survival with the lower stocking density. They stocked spawn at 6.25-8.75 million^{ha} and found survival rates of 57.41-73.94% and average length of 28.2-30.2 mm in four weeks. Kohinoor *et al.* (1994) also found higher survival in lower stocking density in case of *Puntius gonionotus*. Similar result was found in the present study when the different stocking densities were maintained.

The water quality variables in the present study was almost within the characteristics of suitable water for fish culture mentioned by Mumtazuddin and Khaleque (1987) for mrigal (*C. mrigala*) rearing ponds.

Conclusion

The breeding behaviour and successful induced breeding of the endangered *Labeo bata* have been reported for the first time in Bangladesh. Females move to the water surface during ovulation. The best dose for female was 1.5 mg and 5.0 mg PG^{-K_s} body weight for the first and second injection respectively. The highest (63%) survival rate was achieved with the lowest stocking density (8.65 Kg^{-ha}). Most of the people of Bangladesh do not know the actual breeding season of natural population, so they are engaged in fishing during breeding season, as a result natural population is going to be declined. There is a need to identify the natural breeding season of *L. bata* and to protect indiscriminate fishing during breeding period for conservation of its gene pool and biodiversity. Therefore, in depth long term investigation of breeding behaviour, breeding technique and spawn nursing are necessary.

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