

Article

Effect of feeding management of broodstock on breeding performance of bata (*Labeo bata*)

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Abstract: *Labeo bata* is one of the important minor carps in Bangladesh with great demand as good table fish. The study was conducted to observe the breeding performance at different management practices in Mafatema, Rupali, Modhumoti and Anan fish hatchery and disinfection treatments of water, eggs and fry in Jessore, Bangladesh during 10 March 2014 to 15 May 2015. An improvement in broodstock nutrition and feeding has been shown to greatly improve seed production. Protein and lipid percentage of broodstock diet have been identified as major dietary factors. Protein level was 24.77%, 23.47%, 18.08%, 17.78% and lipid level was 11.07%, 9.50%, 7.74%, 8.14% in Mafatema, Rupali, Modhumoti and Anan fish hatchery respectively. Three concentrations of four chemical-formalin (10, 20, 30 mg/L), malachite green (1, 3, 5 mg/L), NaCl (1, 2, 3 g/L) and methylene blue (1, 3, 5 mg/L) treatment regimes and a control were compared for efficacy in treating *L. bata* eggs to prevent fungus and bacterial infection and improve hatch and survival rate of fry. Highest correlation value between absolute fecundity and body weight ($r=0.938$, $p<0.05$) and total length ($r=0.891$, $p<0.05$) and gonadosomatic index (26.2%) were found in Mafatema fish hatchery among four experimental hatcheries at 24.77% protein and 11.07% lipid level. Better fertilization rate ($84.2\pm 5.17\%$) and hatching rate ($82.0\pm 4.30\%$) were found in Mafatema and Rupali fish hatchery respectively that has significant difference ($P<0.05$) from that of Modhumoti and Anan fish hatchery at higher protein and lipid level. Lowest deformity rate (6.05 ± 2.65) was observed in Mafatema fish hatchery that was significantly different ($P<0.05$) from that of Modhumoti fish hatchery. In case of disinfection treatment, methylene blue at 1mg/L bath treatment daily for 4 days showed significantly higher hatching rate ($92.33\pm 3.51\%$) and survival rate ($94.33\pm 4.73\%$).

Keywords: effect; feeding management; broodstock; breeding performance; bata

1. Introduction

Bangladesh inhibits 921 (76 are government hatchery) fish hatchery and 10, 802 fish nursery in its main land (DoF, 2012). Up to now, 14 endemic finfish species are used in hatcheries for seed production. Among them catla, rohu, mrigal, calbasu and Asian catfishes (Deshimagur and shing) are leading (Sarder, 2007). In the recent years the gap between supply and demand of fish is increasing with increased human population without concomitant increase in fish production (Ara, 1998). Supply of fingerlings is a prerequisite for the development of aquaculture (Webber and Riordan, 1976). *L. bata* is one of the important minor carps in Bangladesh with great demand as good table fish. It belongs to the family cyprinidae. Recent estimate suggests that worldwide 20% of all fresh water species are extinct, endangered or vulnerable (Moyle and Leidy, 1992). Among 266

species, 14 are going to be extinct, condition of 12 has been severely deteriorated and 28 of them is critically endangered or somewhat endangered freshwater fish species, *L. bata* is one of them (IUCN, 2000). This species is an important source of proteins, fat, vitamins, minerals, iron, and calcium (Tripathi *et al.*, 1997). In our country this fish grow well in ponds and ditches. It grows about 200-300 mm in length (Rahman, 1989). The success of hatchery mainly depends on improved brood rearing technique entailing pond management, including liming, fertilization and feeding and water quality management. Fertilization and supply of artificial feeds along with natural foods have been a common practice in Bangladesh aquaculture. It was found that Urea, TSP, Murate of Potash (MP) and mustard oil cake were used in different ratios in selected hatcheries in Jessore. There are important references on the use and applicability of inorganic and organic fertilizer in fish ponds by several authors (Haque, 1991; Haque *et al.*, 1996; Islam *et al.*, 2002; Wu, Zhong Wen. 2001). Information on the use of different feed sources, their percentage composition and effects on different cultivable species of fish are available from many workers (Springate *et al.*, 1985; Santiago *et al.*, 1988; Haque, 1991; Islam *et al.*, 2002; Haque *et al.*, 1996; Mollah, 1996). There are several limiting factors affecting the aquaculture industry in Bangladesh, including infectious diseases, nutritional disorders, environmental pollution and some managerial factors. In a recent study, Boglione *et al.* (2001) showed that only 4% of wild caught animals are affected by body deformations. This percentage can reach very high values in hatchery-reared larvae (Divanach *et al.*, 1996). Andrades *et al.*, (1996) showed that only a few percent of larvae affected by skeletal (lordotic) malformation can survive after larval development. Because malformed fish are usually rejected by consumers, they are often culled prior to reaching the market (Boglione *et al.*, 2001; Issa, 2008). Due to poor management developmental abnormalities or external deformities occur in the hatchery. The most common deformities observed in fishes include spinal malformations (scoliosis, lordosis and coiled vertebral column), deformed operculum and fin abnormalities, body shape deformities etc. Vertebral deformities like scoliosis (abnormal lateral curvature) lordosis (excessive inward curvature) and ankylosis (abnormal stiffening and immobility of joint due to fusion of bones) though rare but have been recorded in many species of teleosts. These have been attributed to tetragenic effects of environmental contamination, scarcity of nutrient, oxygen deficiency, sudden changes in temperature, water current, inbreeding etc. The objectives of the research were determine the relationship between body weight, total length and fecundity of *L. bata* at selected hatcheries, determine the gonadosomatic index (GSI) of *L. bata* and the breeding performances of *L. bata* such as breeding success, fertilization, hatching and deformities.

2. Materials and Methods

The major part of the experiment was conducted at the four hatcheries of Jessore and the lab work was practiced in central laboratory of Jessore University of Science and Technology (Figure 1) and in nutrition laboratory of Bangladesh Fisheries Research Institute, Bagerhat. The experiment was done from 10 March 2014 to 25 April 2015.

2.1. Experimental fish

The species that was subjected for this experiment was Bata (*L. bata*) (Figure 2).

Kingdom: Animalia
 Phylum: Chordata
 Class: Actinopterygii
 Order: Cypriniformes
 Family: Cyprinidae
 Genus: *Labeo*
 Species: *L. bata*



Figure 1. Map of Jessore sadar upazila, showing the study location, Chanchra.



Figure 2. *Labeo bata*.

2.2. Pond management of brood fish

Pond management plans of the hatchery were done dividing into pre-stocking management, management during culture, post-stocking management.

2.2.1. Pond drying

Most of the ponds retained water round the year but some ponds were completely dewatered by pumping and allowed to dry by sunlight. Mafatema and Rupali fish hatchery practice pond drying every year at the time of October to November but Modhumoti and Anan fish hatchery practice pond drying 3-4 years at the same month.

2.2.2. Water source and color

In the present study underground water was used as the main source of water in the brood pond. As underground water contained significant level of iron, there was likely a reproductive problem where iron creating a layer on eggs of fish. The sub lethal toxicity of iron may be in the form of change in morphology, histology, growth, development, swimming performance, respiration, blood chemistry, enzyme activity, reproduction, endocrinology, reproduction and behavioral changes.

2.2.3. Liming

Though the rate of liming was depended upon pH of the pond water, most of the hatchery owners are not aware of it. They usually spread lime over the pond bottom 5-6 times in a year. The doses were found to be ranged from 1000- 1200 gm/dec in the present study (Table 1).

2.2.4. Pond fertilization

The differences in the rate of fertilizer application in this region was ascertained to be based on the species combination, soil fertility, water quality and on the farmers own experience on culture practices. Most of the farmers were seen using fertilizers at near optimum rates, only a few used at very low levels. Every time TSP and oil cake were soaked in water a night before application (Table 1).

2.3. Stoking density

Stocking density of fish in brood pond was varies from 8-16 kg/decimal in Mafatema and Rupali fish hatchery was 10-12 kg/decimal but 14-15 kg/decimal was kept in Modhumoti and Anan fish hatchery (Table 1).

Table 1. Comparative study on pond management.

Name of hatchery	Drying (month)	liming	fertilization	Stocking density
Mafatema	October-November (every year)	1000-1200 g/decimal	TSP-170g/dec Urea-100g/dec MoP-75g/dec	12 kg/dec
Rupali	October-November (every year)	1000-1200 g/decimal	TSP-150g/dec Urea-80g/dec MoP-80g/dec	10 kg/dec
Modhumoti	October-November (3-4 years interval)	1000-1200 g/decimal	TSP-220g/dec Urea-150g/dec MoP-100/dec	15 kg/dec
Anan	October-November (3-4 years interval)	1000-1200 g/decimal	TSP-200g/dec Urea-120g/dec Mop-100/dec	14 kg/dec

2.4. Feeding

In selected fish hatcheries homemade feed was used 3-4 days/week. Homemade feed ingredients are shown in Table 2 and Table 3.

Table 2. Gross ingredients of prepared experimental diets.

Name of hatchery	Mafatema (T1)	Rupali (T2)	Modhumoti (T3)	Anan (T4)				
Feed ingredients	Rice bran	40%	Rice bran	55%	Wheat bran	30%	Rice bran	30%
	Oil cake	15%	Soybean cake	10%	Cow dung	20%	Oil cake	15%
	Corn	10%	Flour	8%	Rice polish	16%	Corn	17%
	Fishmeal	15%	Fishmeal	12%	Oil cake	15%	Flour	10%
	Soybean cake	12%	Dial calcium	4%	Fishmeal	14%	Fishmeal	15%
	Flour	3%	Oil cake	8%	Molasses	3%	Soybean cake	10%
	Dicalcium phosphate	2%	Vitamin premix	1%	Salt	2%	Molasses	2%
	Molasses	1.5%	Molasses	1%			Salt	1%
	Salt	1.5%	Salt	1%				

Table 3. Feeding management of Bata (*L. Bata*) in different hatchery.

Name of hatchery	Rate of feeding/ body wt.		Supply of feed a week	of feed in		Crude protein level (%)		Average crude protein level (%)	Crude lipid level (%)		Average crude lipid level (%)
	F. F	C. F		F. F	C. F	F. F	C. F		F. F	C. F	
Mafatema	3%	2.5%	Mega feed	4 day	2days	22.5	29.3	24.77	14.5	4.2	11.06
Rupali	3%	2%	Quality carp feed	4 days	2days	21	28.4	23.47	11	6.6	9.50
Modhumoti	2%	2%	Teer fish feed	3 days	2days	13.8	24.5	18.08	9.3	5.4	7.74
Anan	2-3%	2%	Aftab fish feed	3 days	2days	12.9	25.1	17.78	10.1	5.2	8.14

2.4.1. Determination of proximate composition

After formulating of feeds proximate composition of the formulated feeds were determined following the standard methods given by Association of Official Analytical Chemists, in the Laboratory of Fisheries and Marine Bioscience Department and Bangladesh Fisheries Research institute, Bagerhat. The detailed procedure for the determination of protein, lipid, moisture and ash were given below. Each experiment was conducted with the replication of three.

2.4.2. Determination of protein

The protein content of the sample was determined by micro-kjeldahl method. Protein content of feed is shown in Table 4.

Reagents:

a) 0.2 (N) HCl=>37%.....100

1%.....100/37

36.5%.....100×36.5/37=98.65

So for 1(N) HCl 98.65/1.19 (Leveling 1liter=1.19kg) in abottle

0.2 (N) HCl= 16.58 ml volume into 1 liter distilled water.

b) Digestion mixture => 100 gm K₂SO₄+10 gm C_uSO₄.5H₂O +1 gm selenium powder

c) Mixed indicator =>100 ml ethanol + 0.1 gm methylene blue + 0.2 gm methyl red

d) 33% N₂S₂O₃ => 165 gm N₂S₂O₃ volume into 500 ml distilled water

e) 10 (N) or 40% N_aOH solution => 400 gmN_aOH volume into 1 liter distilled water

f) Methyl orange indicator =>4% = Dissolve 1 gm methyl orange in 100 ml deionized

g) 4% HBO₃ solution => 20 gm HBO₃ volume into 1 liter distilled water

h) 0.1 N Na₂CO₃ standard = Dissolve 5.3 gm Na₂CO₃ in 1L deionized

Procedure:

- 0.5 gm sample in paper to digestion tube. (W)
- 1.1 gm digestion mixture or 1 digestion table in digestion tube
- 10 ml H₂SO₄ (98%) in digestion tube
- Digestion for 1 hour at 420°C temp
- Cooling
- Add 50 ml distilled water = 5 ml 33% N₂S₂O₃ solution

Distillation:

- Distillation for 3:30 min 925 ml 4% boric acid = 2-3 drop mixed indicator in conical flask)
- Allow distillation jar to add 10 (N) NaOH 30 ml = Distilled water 30 ml
- Collect 100 ml distilled sample

Titration with 0.2 (N) HCl (titration value t₁)

Percentage of protein = (t₁×0.2×0.014)/W×6.25×100%

2.4.3. Determination of lipid

Set up the soxhlet apparatus on a hot water bath using iron stand and clamp. Then 5-10 g of prepared sample is taken in a thimble filter and places it inside the soxhlet apparatus. Pour sufficient amount of petroleum ether or acetone (250 ml) in to the round joint flask carefully on water bath at suitable temperature depending on boiling point of the solvent. The solvent evaporates upon heating but allowed drop slowly after condensing on the sample inside the thimble. The solvent gradually accumulates in hollow space up to a level where the solvent containing fat drains out to the round joint flask through siphoning. The process lipid continues for 12 hours until all the fat content in the sample is extracted. Finally the solvent containing lipid transfers to a pre-weighed beaker. The residual lipid content is obtained after removal of solvent by evaporating on heating on water bath. Lipid content of feed is shown in Table 4.

$$\text{Percentage (\%)} \text{ of lipid} = \frac{\text{Weight of lipid} \times 100}{\text{Weight of sample}}$$

2.4.4. Determination of moisture

For determining the moisture, at first crucibles were cleaned, dried and then the weight of the crucible was taken. Then 2-3 g sample was placed in the crucible and weighted again. Difference between the two is the sample weight. Then the crucible with sample weight was put in a controlled oven and was dried at 105°C for about 24 hours until constant weight was reached and colled in desiccators and weighted several times till the constant weight was achieved. Moisture content of feed is shown in Table 4.

The percentage of moisture content will be calculated by the following equation:

$$\text{Percentage (\%)} \text{ of moisture} = \frac{\text{Weight loses} \times 100}{\text{Original weight of sample}}$$

2.4.5. Determination of ash

Heat a crucible in a furnace at 550-600°C for 20 minutes. Remove and cool in a desicator. Weight the crucible accurately. Add about 5 g of the prepared sample. Spread it out evenly then reweight accurately. dry and char the portion by heating carefully. Then heat at 550-600°C in the muffle furnace for four hours. Cool in a desicator and reweight. Ash content of feed is shown in Table 4. The percentage of moisture content will be calculated by the following equation:

$$\text{Percentage (\%)} \text{ of ash} = \frac{\text{Weight of ash} \times 100}{\text{Weight of sample}}$$

Table 4. Proximate composition of formulated feeds (%) on Dry matter content.

Name of hatchery	Crude protein (%)	Crude lipid (%)	Moisture (%)	Ash (%)
Mafatema	22.5	14.5	7.5	5.63
Rupali	21	11	12	7.5
Modhumoti	13.8	9.3	16.5	5.2
Anan	12.9	10.1	14.8	6.7

2.5. Brood collection

The male (20) and female (10) broods were collected from each hatchery of Jessore.

2.6. Conditioning

Collected brood fishes (N=30) from each species groups were kept in the rectangular tank (1200 liter) under showering condition to induce the breeding for 16 hours.

2.7. Observation of sex determination and sex ratio

Male and female fishes could be clearly distinguished only in the breeding season. During the early stages of brood fishes to successfully complete the breeding activity the sexual characteristics of male and female and their sex ratio approximately 4 hatcheries has been observed (Table 5).

Table 5. Observation of sex ratio and sex determined characteristics of selected hatcheries.

Species	Sex determination				Sex ratio	
	External feature or characteristics		Pelvic fin		Male	Female
Bata	Abdomen					
	Male	Female	Male	Female		
	Normal	Swollen	Rough	Soft	2	1

2.8. Measurement of total length and body weight

In vitro, total length (TL) and body weight (BW) were measured. At first the fishes were washed with tap water. Excessive water from the fish body was wiped off by a piece of dry cloth. Total length (cm) of each fish was measured from the tip of the snout to the end of the tail and was recorded in cm Body weight (gm) was taken by an electronic balance of .001 accuracy.

2.9. Preparation of PG

The PG (1mg-3mg/each) was collected from the market in acetone frozen condition. It was then dried with filter paper and weighted with an electric balance. Then it was crushed with tissue homogenizer and diluted with distilled water. The mixture was then centrifuged for precipitation and resultant supernatant solution was then taken slowly in 3ml syringe for injection.

2.10. Hormone administration

For hormone administration the brood fish were collected from conditioning tank very carefully with net and placed on sponge. Then they were wrapped with soft and moist cloth and the hormone was administered pushing the injection syringe near the base of the pectoral fin. Required dose for male and female to selected species are shown in Table 6.

Table 6. Required dose for male and female to selected species.

Species	Sex	Dose: PG (mg/kg of body weight)		Time interval between the doses
		1 st dose	2 nd dose	
Bata (<i>L. bata</i>)	Male		2	6
	Female	1	5	

2.11. Determination of gonadosomatic index (GSI)

At first the fish were killed by heating on the head. Ovary was removed from each fish by dissecting out the abdomen. Ovaries were washed and cleaned with distilled water. Excess moisture from the surface of the ovaries was removed with blotting paper before weighting. Then the ovaries were weighted in gm on an electronic balance of 0.001 gm accuracy. The gonadosomatic index of the female was calculated using the following formula cited by Parameswarn *et al.* (1974).

$$GSI = \frac{\text{wet weight of gonad in gm} \times 100}{\text{total weight of fish in gm}}$$

2.12. Estimation of fecundity

For the estimation of fecundity direct counting method can be used when the numbers of eggs were not much and the sizes of the eggs were also comparatively larger. The total weight of the ovary was taken by electronic balance. A small portion of the ovary was cut from the total ovary and weighted. Then it was allowed to soak in water contained in petridish and the number of the egg was counted with the help of needle and magnifying glass. The number of egg on an individual was obtained by multiplying the total number of eggs taken from the

cut portion with the total weight of small portion of the ovary of the particular individual. Thus the fecundity was obtained by using the following formula-

$$\text{Fecundity} = \frac{\text{total gonad weight(gm)} \times \text{No. of eggs in the sample}}{\text{weight of small portion total gonad(gm)}}$$

2.13. Determination of fertilization rate

After 6 hrs the eggs were placed on a petridish containing acetone and observed under a magnifying glass. The fertilized eggs were counted with the help of soft thin brush. The fertilized eggs were separated from the unfertilized eggs in terms of color of the egg shell. Transparent eggs were identified as fertilized ones and opaque ones were identified as unfertilized eggs. The fertilization rate was determined by the following formula-

$$\text{Fertilization rate(\%)} = \frac{\text{no. of fertilized egg} \times 100}{\text{total no. of egg}}$$

2.14. Determination of hatching rate

Hatching started after 20±2 hrs of fertilization. The yolk sac absorption was observed by microscopically that took place after 70±2 hrs of hatching. When hatching was completed, the hatchlings were collected in a petridish and counted by visual observation using magnifying glass and recorded. The hatching rate was determined by the following formula-

$$\text{Hatching rate(\%)} = \frac{\text{number of hatchlings} \times 100}{\text{total number of fertilized eggs}}$$

2.15. Collection of water sample

The water samples were collected from three selected hatcheries in Jessore region. For collecting water sample at first three plastic bottles were collected and the capacity of the volume of each sample bottle was 250 ml. Then bottles were washed well with clean water for 3 to 4 times. Finally the outside of each bottle was covered with black plastic tape to avoid the chemical change due to sunlight. Water samples were collected just before the eggs released in to the incubation tank.

2.16. Physicochemical parameters

Physicochemical parameter is an important factor in hatchery operation. Proper maintenance of water quality is prerequisite for any hatchery operation. The water quality parameters are shown in Table 7.

Table 7. Physicochemical parameters of the four hatcheries water.

Parameter	Methods/instruments	Name of hatcheries			
		Mafatema	Rupali	Modhumoti	Anan
pH (Hydrogen Ion Concentration)	Microprocessor pH meter	7.2	7.4	6.3	6.6
Dissolved Oxygen(mg/l)	DO meter	5.8	5.6	4.2	4.3
Temperature(°c)	Thermometer	27±1	28±1	30±1	29±1
Alkalinity(mg/l)	Titrimetric method	153±17	136±15	195±22	170±19
Hardness(mg/l)	Titrimetric method	324±15	307±17	342±13	349±18

2.17. Larvae sample collection

The samples were collected immediately after hatching from four selected hatchery named Mafatema fish hatchery, Rupali fish hatchery, Modhumoti fish hatchery and Anan fish hatchery in Jessore where the fish breed artificially. Initially the larvae were collected just after hatching. Fifty larvae were sampled every day at the same time for observing external deformity.

2.18. Observation of external deformity of larvae

Deformities were recorded at post hatching stage. Deformities were categorized into different forms and were conducted. Deformities were categorized on the basis of presence or absence of eye, slightly curved body shape, completely curved body shape etc.

2.19. Examination of deformities

The preserved larvae were kept in glass slide and examined in the laboratory of Fisheries and Marine Bioscience department. Any deformities in the larvae were identified with the help of electronic microscope.

2.20. Determination of deformity rate

The deformity rate was calculated by visual observation. Then deformity rate was determined by the following formula-

$$\text{Deformity rate (\%)} = \frac{\text{No. of deformed hatchlings} \times 100}{\text{Total no. of hatchlings}}$$

2.21. Data analysis

The results obtained in the study were subjected to statistical analysis. Qualitative and quantitative analysis of all kinds of data were carried out. MS Excel and Graph Pad Prism 6 were used to store all the data. MS Excel was also used for presentation of the tables and graphs obtained from different types of data. ANOVA was done for the test of significance of fecundity, rate of fertilization rate and hatching rate of *L. bata* among different hatcheries using SPSS 16.0.

3. Results

3.1. Body weight – fecundity relationship

Linear and positive regression line was obtained between body weight (kg) and fecundity (number) of *L. bata* in selected hatcheries. The highest correlation coefficient for the relation was 0.938 found in Mafatema fish hatchery and the lowest value was 0.776 found in Rupalii fish hatchery. It indicated that fecundity was highly correlated with body weight in Mafatema fish hatchery than other fish hatcheries. Relationship between body weight (g) and fecundity of *L. bata* in different hatcheries are shown in Figure 3 and Figure 4.

3.2. Total length – fecundity relationship

Linear and positive regression line was obtained between total length (cm) and fecundity (number) of *L. bata* in selected hatcheries. The highest correlation coefficient for the relation was 0.893 found in Mafema fish hatchery and the lowest value was 0.663 found in Rupali fish hatchery. It indicated that fecundity was highly correlated with total length in Mafatema fish hatchery than other fish hatcheries. Relationship between body length (mm) and fecundity of *L. bata* in different hatcheries are shown in Figure 5 and Figure 6.

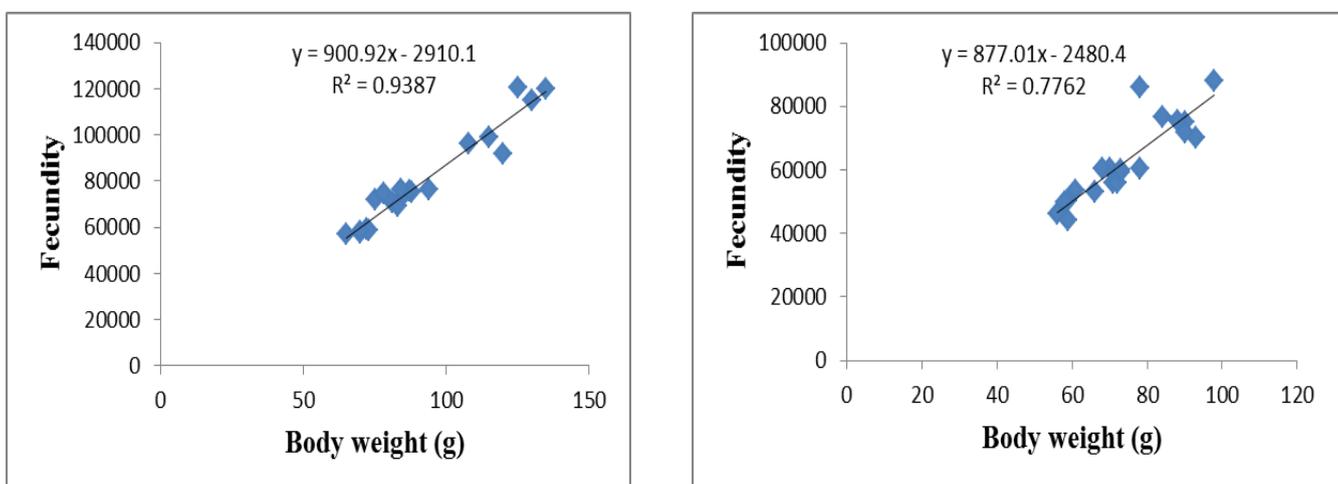


Figure 3. Relationship between body weight (g) and fecundity of *L. bata* in Mafatema fish hatchery (Left) and Rupali fish hatchery(Right).

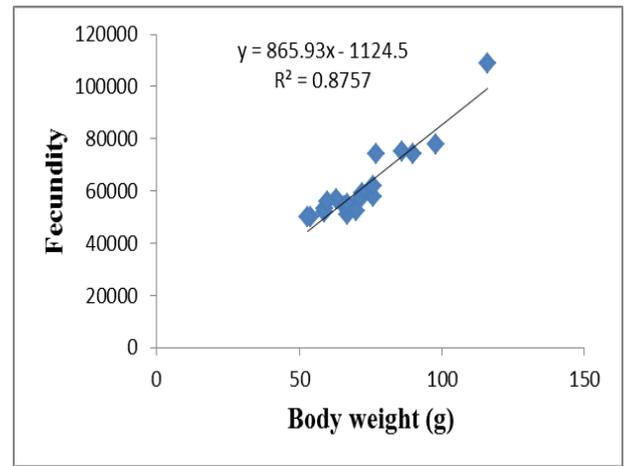
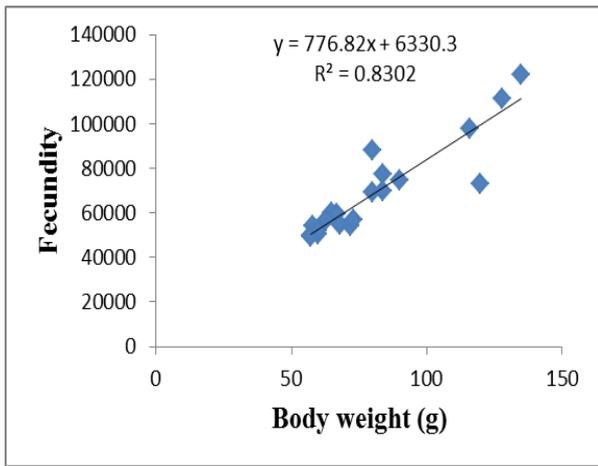


Figure 4. Relationship between body weight (g) and fecundity of *L. bata* in Modhumoti fish hatchery (Left) and Anan fish hatchery (Right).

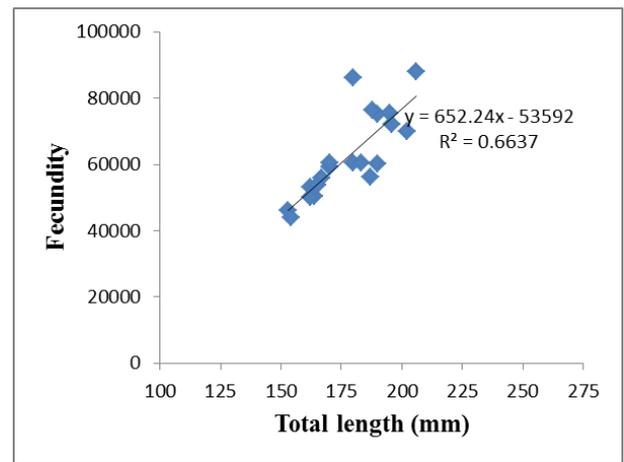
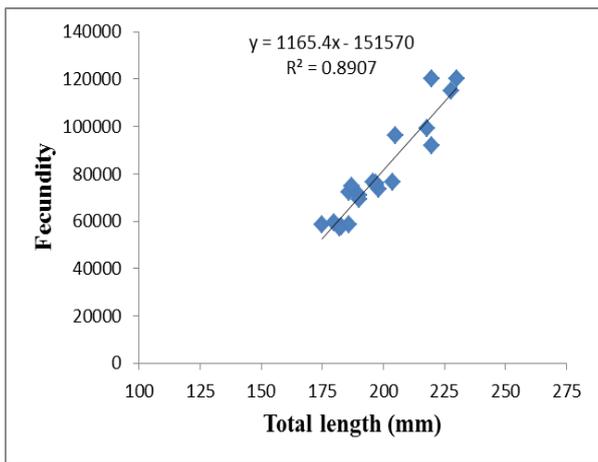


Figure 5. Relationship between total length and fecundity of *L. bata* in Mafatema fish hatchery (Left) and Rupali fish hatchery (Right).

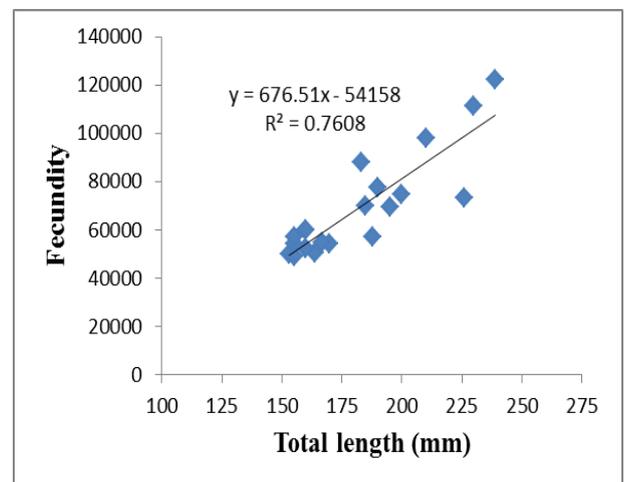
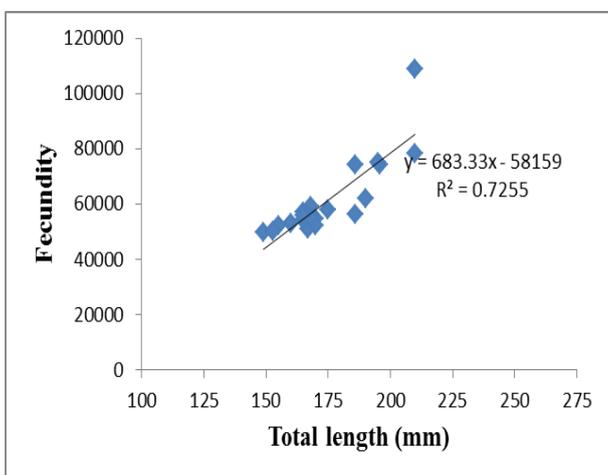


Figure 6. Relationship between total length and fecundity of *L. bata* in Modhumoti fish hatchery (Left) and Anan fish hatchery (Right).

3.3. Observation of breeding success

Breeding success of *L. bata* was observed. Highest rate of breeding success of *L. bata* was found in Mafatema fish hatchery and the rate was 96 ± 3.3 (%) (Figure 7).

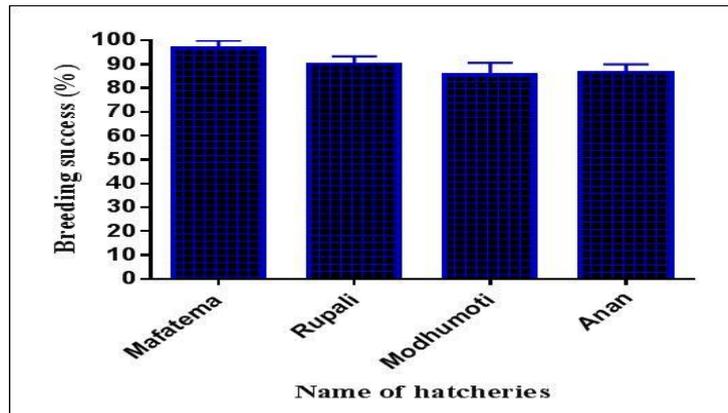


Figure 7. Breeding success of *L. bata* in selected hatcheries.

3.4. Gonadosomatic index

The GSI values of *L. bata* obtained in the present study varied between 26.2 to 20. In selected hatcheries highest value was found in Mafatema fish hatchery (26.2) that was significantly different from that of Modhumoti fish hatchery (Figure 8).

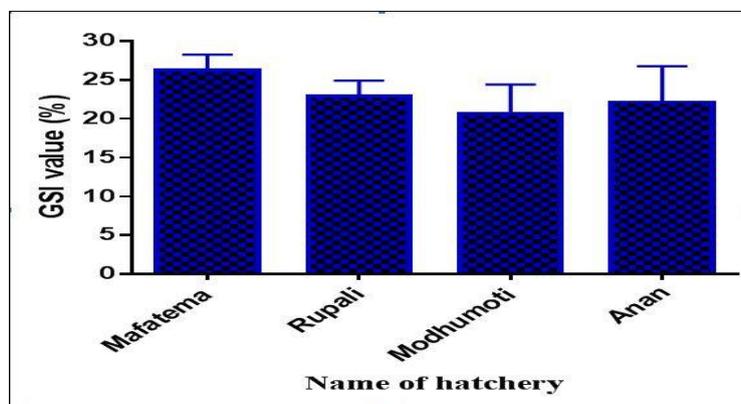


Figure 8. GSI value of fish in selected hatcheries.

3.5. Fertilization rate

Fertilization rate of *L. bata* was obtained 84.2 ± 5.17 , 80.6 ± 6.02 , 69.4 ± 6.31 and 65.2 ± 4.32 (%) in Mafatema hatchery, Rupali hatchery, Modhumoti and Anan fish hatchery respectively (Figure 9).

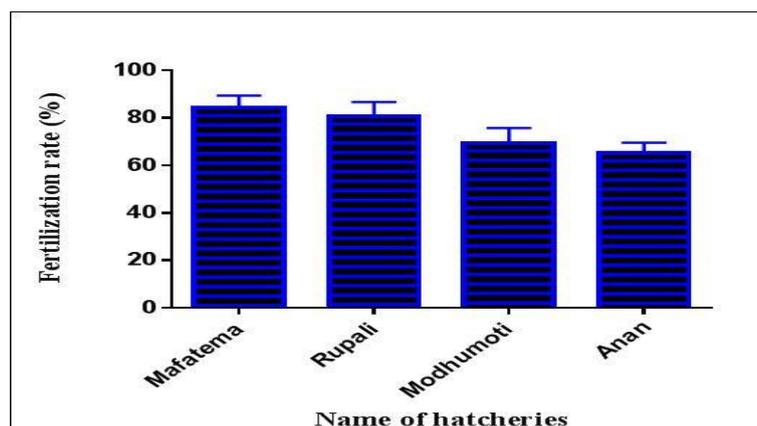


Figure 9. Fertilization rate of *L. bata* in selected hatcheries.

3.6. Hatching rate

Hatching started after 20 ± 2 hrs of fertilization. Hatching rate of *L. bata* was obtained 80.2 ± 6.50 , 82.0 ± 4.30 , 69.2 ± 5.72 and 71.6 ± 3.21 (%) in Mafatema hatchery, Rupali hatchery, Modhumoti and Anan fish hatchery respectively (Figure 10).

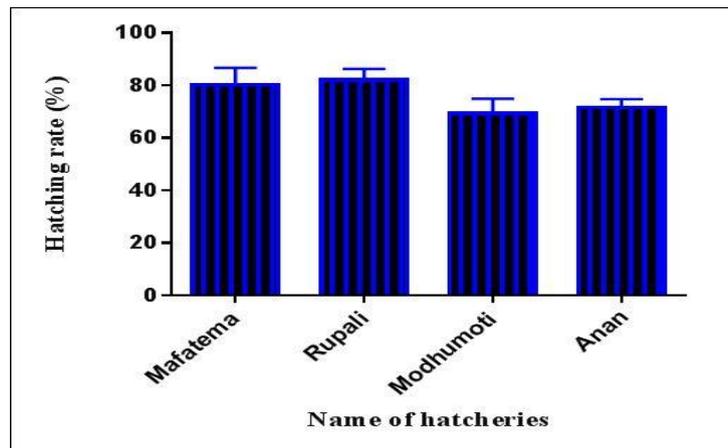


Figure 10. Hatching rate of *L. bata* in selected hatcheries.

3.7. Deformity rate of *Labeobata* larvae

The deformity rate of *Labeobata* in different hatcheries was ranged from $6.50 \pm 2.65\%$ to 11.75 ± 3.59 . The highest rate of deformity was observed in Modhumoti Fish Hatchery and the lowest rate of deformity was found in Mafatema Fish Hatchery. No significant difference was found among four hatcheries (0.05 level of significance) (Figure 11).



Figure 11. Deformity rate of *Labeo bata* larvae in four selected hatcheries after four days.

3.8. Type of deformities of *Labeo bata* larvae

Deformities were recorded at three different categories those are absences of eye, slightly curved body shape and completely curved body shape. Among three different hatcheries the highest rate of absent eye deformity was showed by the Modhumoti Fish Hatchery. Highest rate of slightly curved body shape deformity was showed by the Mafatema Fish Hatchery and the highest rate of completely curved body shape deformity was showed by the Anan Fish Hatchery. Types of deformities of *Labeo bata* larvae are shown in Table 8.

Table 8. Types of deformities of *Labeo bata* larvae

Name of hatchery	Type of deformities		
	Mean rate of absence of eye (%)	Mean rate of slightly curved body shape (%)	Mean rate of completely curved body shape (%)
Mafatema	15.38	61.53	23.08
Rupali	13.33	53.33	33.33
Modhumoti	25.53	40.43	34.04
Anan	10.25	38.47	51.28

4. Discussion

In view of economic importance and food value of *L. bata*, it is very important to have data and information on the reproductive biology particularly in relation to fecundity, fertilization and hatching, for better scientific management and conservation of *L. bata*, study of breeding biology is a must. In the present study the fecundity varied from environment to environment due to different factors such as age, size, nourishment, ecological condition of the water body etc.

The number of eggs produced by a female depends on many factors like size, age, condition and types of samples (Lagler, 1956). Generally, it has been found that the number of eggs increased with the size of fish. Sato *et al.*, (1995) suggested that variation in fecundity was primarily a reflection of variation in the size of fish at maturity. Greater the fecundity, smaller will be the size of egg. Large size fish will have more fecundity than small sized fish. It is also believed that larger fish lay larger egg (Bagenal, 1966). Survey of available literature revealed that works on the fecundity of different fishes have been done in this country by many researchers like Miah and Dewan (1984) and Kabir *et al.*, (1998). Breeding success of *L. bata* in Mafatema hatchery was highest and the rate was 96.66 ± 3.33 due to proper brood stock management and poor physical stress.

It is widely known that a complete broodstock diet is necessary to improve spawning quality and consistency. A high seed production demands particular nutrition of broodstock which significantly affect fecundity and survival (Bromage *et al.*, 1992). The importance of broodstock nutrition has been stressed earlier by Cerda *et al.*, (1994) while Muchlisinet *et al.*, (2006) reported that dietary protein levels influenced parameters such as weight gain and proximate composition of brood fish, quantity and quality of eggs and larval viability.

It was found in the experiment that fecundity of *L. bata* ranged from 79884.40 ± 20520.90 to 61525.70 ± 14320.84 in selected hatcheries and highest fecundity was found in Mafatema fish hatchery at 24.77% protein level. The lowest (17.78%) dietary protein showed lowest relative fecundity and hatching rate of *L. bata* in Anan fish hatchery. De Silva and Radampola (1990), Chong *et al.*, (2004) and Khan *et al.*, (2004, 2005) reported that dietary protein level influence relative fecundity. Similar observations were made by Ling *et al.*, (2006) for *Xiphophorus helleri* where fry production was maximal at 30% protein. Dahlgren (1980) reported that *Poecilia reticulata* did not show significant difference in mean fecundity at varying protein levels. On contrary, Shim *et al.*, (1989) and Santiago *et al.*, (1991) published that varying dietary protein level can influence fecundity in dwarf gourami (*Colisalalia*) and bighead carp (*Arisfichthys nobilis*) respectively. Similar observations were recorded in the present study. Besides pond management mainly pond preparation in Modhumoti and Anan fish hatchery was poor. They dry pond 3-4 years interval and they use more fertilizers than other two hatcheries resulting algal bloom every year may cause impact on brood fish. Though stocking density is kept 14-15 kg/decimal higher than Mafatema and Rupali fish hatchery. The fishes were fed at 2.5-3 % of body weight, 5-6 days in a week. The feeding rate was followed in the initial stage of brood preparation, however, as the breeding season approached, the rate was decreased to 1-1.5% of body weight. 2.5-3 % body weight of feed was used in Mafatema and Rupali fish hatchery while Modhumoti and Anan always use 2-2.5 % body weight of feed. The feeding rates were found to depend on species combination. Brood pond management relating to fertilization and feeding was the prime consideration for producing quality broods and that in turn allowed the availability and quality seed for successful aquaculture in the country.

Linear positive relationship between fecundity and total length was found in this experiment. The increased fecundity with the increase in length was also found by Das (1989) for hilsha, Shafi and Mustafa (1976) for *Anabustudineus*, Mustafa *et al.*, (1980) for *Nandus nandus*. It was found that in this experiment very few fish of selected species in different hatcheries did not provide proportional fecundity due to storing fat in gonad for extra feeding. This problem was highly found in heavy weighted fishes.

In the present study a linear and positive relationship was found between fecundity and body weight (kg). Linear positive relationship between fecundity and body weight was reported by many scientists like Shafi and

Mustafa (1976) in different fish species. A linear relationship between fecundity and total length was observed. Weight of the fish and weight of ovary has been established by several authors (Khan, 1993; Pillay, 1954; Das, 1964; Pathani, 1981). The fecundity was strongly correlated with gonad weight than other factors with fecundity. This type of strongest relationship of fecundity with gonad weight was reported by Shafi and Quddus (1974), Das *et al.*, (1989) for other fishes.

Fertilization and hatching rates of fish are determined for various breeding purposes. Because it can determine how many young can be hatched from a certain number of fecund female and how many are lost and why. It also indicates the status of well being of the broods used in breeding and also the quality of hatchery management. It helps to improve the hatchery product and their by the production. The highest fertilization rate of *L. bata* was 84.2 ± 5.17 % in Mafatema fish hatchery. After 20 ± 2 hrs of fertilization the hatching was started. Hatching was carried out in specially made hatching jar and highest hatching rate of *L. bata* was 82.0 ± 4.30 % in Rupali fish hatchery and Mafatema was also good. Protein level was 24.77 % and 23.47% in Mafatema and Rupali fish hatchery respectively while protein level 17.78 % and 18.08 % causing lowest fertilization and hatching in Anan and Modhumoti fish hatchery respectively.

Other responsible factor that might affect the fertilization and hatching percentage are temperature, photoperiod, dissolved oxygen, salinity and water flow. Temperature plays a vital role in gonadal reappearance and timing of ovulation. The embryonic development is closely dependent upon oxygen supply and water flow. The hatching time, size, and growth rate of developing embryos is proportional to the dissolved oxygen concentrations up to 8 mg/L or greater. The dissolved oxygen content of the water should not be below 4-5 mg/L, at below 2 mg/L the embryo will develop abnormally. In the present study the dissolve oxygen level was 5.8 mg/L and 5.6 mg/L due to presence of aerator on overhead tank in Mafatema and Rupali fish hatchery respectively resulting better fertilization (84.2 ± 5.17 %) and hatching rate (82.0 ± 4.30 %) while dissolved oxygen was 4.7 mg/L and 4.3 mg/L in Modhumoti and Anan fish hatchery . McMahan (1983) recommends dissolved oxygen levels be 8 mg/L for good fertilization rate, hatching rate and high survival of fry. USEPA (1986) concluded that the embryonic and larval stages of salmonid development will experience no impairment when water column dissolved oxygen concentrations are 11 mg/L.

In the present study deformity rate was ranged from 6.50 ± 2.65 % to 11.75 ± 3.59 %. Highest deformities rate was found in Modhumoti fish hatchery. In addition to these, poor brood stock management, nutritional problem and inbreeding problem in hatcheries added a series of new parameters that may exert considerable impact on developing fish (Von Westernhagen *et al.*, 1988). A study involving striped bass (*Moronesaxatilis*) and blueback herring (*Alosaestivalis*) eggs exposed to heat shock conditions demonstrated increased incidence of deformed larvae at hatching (Koo and Johnston, 1978). In this experiment, highest deformity was found in modhumoti fish hatchery where protein level of feed was lower (18.45%) than other fish hatcheries. It is also observed deformity rate decrease with the increase of protein level in feed in selected hatcheries. Diet components of broodstock diet are prerequisite for normal embryonic and larval development (Izquierdo *et al.*, 2001).

5. Conclusions

Carp brood management is the prime consideration for producing quality broods as well as quality seed for successful aquaculture in the country. Jessore is the most important region which has got recognition as the most promising areas for hatchery technology of Indian major and minor carps. Thus it is important to get an idea about the practices of brood rearing relating to pond fertilization and feeding of broods. As Bangladesh is highly dense populated country in the third world, they can only be met their protein requirement through aquaculture. To fulfill this requirement, fish farmers have to be ensured with good quality seed through production of quality broods. This research provides ample evidence on the effect of broodstock management specially diet (protein and lipid) on the breeding performances of *Labeobata*. In Mafatema fish hatchery 24.77% protein and 11.06% lipid in feed show better result in case of breeding success, GSI, fertilization, hatching, deformity and survival rate.

Conflict of interest

None to declare.

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