Rahman, M.M.; Habib, M.A. and Shah, M.S. 2007. Induced breeding of *Cirrhinus reba* (Ham.) and *Labeo bata* (Ham.). *Khulna University Studies*, 8(2): 286-292.

Khulna University Studies, 8(2): 286-292, 2007

RESEARCH ARTICLE **LIFE SCIENCE**

INDUCED BREEDING OF *Cirrhinus reba* (HAM.) *and Labeo bata* (HAM.)

Md. Moksedur Rahman, Md. Ahsan Habib and Md. Saifuddin Shah*

Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna 9208, Bangladesh

KUS 07/01- 210107

Manuscript received: January 21, 2007; Accepted: December 13, 2007

Abstract: Information on artificial breeding of *Cirrhinus reba* and *Labeo bata* is not common in Bangladesh. There are reports on serious decline of natural abundance of these species and are considered threatened under present environmental situation. The species should be brought under culture systems to protect them from being dwindled. For optimizing the breeding technique, the experiment was conducted. Nine pairs of male and female of each species were used. The females of both the species were divided into three treatment groups (T_1, T_2 and T_3). Weight of *reba* female and male ranged from 110 to 140g and 80 to 105g respectively, the same for *bata* ranged from 310 to 388g and 180 to 260g for female and male respectively. The females of *C. reba* were injected a first dose of 0.5, 1.0 and 1.5 mg PG kg^{-1} body weight followed by a second dose of 4.0, 4.5 and 5.0 mg PG kg^{-1} body weight respectively after 6 hours of the first dose. The doses for *L. bata* were 0.5, 1.0 and 1.5 mg PG kg^{-1} body weight as first, followed by a second dose of 4.5, 5.0 and 5.5 mg PG kg^{-1} body weight. A single dose for males of *C. reba* was 1.5 mg PG kg^{-1} body weight and for *L. bata* 2.0 mg PG kg^{-1} body weight. Single pair mating was performed under semi-natural conditions in nine separate hapas with continuous showering at a temperature range of 27-28 ^{0}C. The highest average fertilization and hatching of *C. reba* were obtained at 87.88% and 81.47% respectively and that of *L. bata* were 86.19% and 75.00% respectively from T_2. The percentage of fertilization and hatching obtained against the comparatively low doses of hormone in this experiment was considered noteworthy.

Key words: Induced breeding, *Labeo bata*, *Cirrhinus reba*, fertilization, hatching

Introduction

The inland fisheries of Bangladesh mainly consist of carps, minnows and catfishes. Rahman (1989) recorded 260 species of indigenous fin fish species in fresh water of Bangladesh. There are also about 40-50 indigenous fish species. These species are defined as small species which grow to a maximum length of about 25 cm. (Felt *et al*., 1996). Prior to 1970, many different small indigenous fishes like *koi, bheda, taki, kaski, rani, bata, pabda gulsha, mola, baim, puti, shing, magur* etc. were abundant in almost all the fresh waters of Bangladesh. They were commonly caught by the large number of subsistent fishermen that comprised of a major portion of their intake (Wahab, 2003). Small fishes contain large amount of calcium and most likely also iron and zinc (Thilsted *et al*., 1997). Small indigenous fish contain more calcium and phosphorus than big fish (Carps) (Hossain *et*

* Corresponding author. Mobile: 01711-466866; e-mail: <drmsshahbd@yahoo.com>

DOI: https://doi.org/10.53808/KUS.2007.8.2.0701-L

al., 1999). They also provide subsistence and supplementary income to the poor and disadvantaged fishermen. Moreover, these fishes are most favored for their taste.

Information on different aspects of biology of *C. reba* are available from studies of many authors (Alikunhi and Rao, 1951; Hickling, 1944; Verghese, 1967, 1968, 1969; Bhuiyan, 1989; Tripathi *et al*., 1997; and Hossain, 2001). Information on detail aspects of biology of *L. bata* is scarce. Rahman (1989) provided some information on its biology. Azadi and Naser (1996a and 1996b) worked on its length-weight relationship and morphology, respectively. Chatterjee (1979) provided information on its feeding habit. According to the IUCN (Anon, 2001), natural and anthropogenic activities have adversely affected populations of different indigenous fish species in Bangladesh.

For conservation and management of the small indigenous species, their culture systems should be introduced in the country (Wahab, 2003; Mazid and Kohinoor, 2003). Akhteruzzaman and Kaiya (1998) carried out polyculture of *mola, bata* and *reba* obtaining a production of 3 ton ha^{-1} $month^{-8}$. This suggests for good prospects of inclusion of the small indigenous species in carp polyculture system in ponds of the country but no serious attempt has so far been made in this regard. The study was undertaken for the purpose of optimizing rates of fertilization and hatching by using various doses of PG hormone.

Materials and Methods

Collection of broods: The study was carried out in a private hatchery, Puratan Kasba, Jessore during May–June, 2004. Mature brood fishes were collected from the brood stock pond of the farm.

Selection of broods: Nine pairs of male and female broods were selected for the experiment. Female broods were selected with bulged abdomen, soft post-abdominal region, swollen genital pore and comparatively larger in size. Male broods were comparatively slimmer and smaller in size having rough pectoral fins and with the presence of milt with gentle pressure on the abdomen. Weight of *reba* female and male ranged from 110 to 140 g and 80 to 105 g respectively, the same for *bata* ranged from 310 to 388 g and 180 to 260 g for female and male respectively.

Conditioning of the brood: Prior to hypophysation, the males and females were kept in two separate tanks for 24 hours under water shower. The tanks were (3m×2m×1m) fitted with outlet pipes and filled with deep tube well water up to 30 cm.

Hormone administration: The broods were taken out from the tank, weighed and amount of hormone required was calculated out. The alcohol preserved PGs were first dried with tissue paper weighed and homogenized with half the quantity of the distilled water. After that it was diluted with rest of the distilled water and centrifuged. The supernatant solution was then drawn out in a syringe for injection. The injection was administered in the muscle at the base of pectoral fin. Time interval between the first and second dose was 6 hours and the doses for males of the respective species were same for all the treatments (1.5 mg for *reba* and 2.0 mg for *bata*) The different doses (mg kg^{-1} body weight) applied for the females are shown in Table 1.

Spawning: Single pair semi-natural spawning was performed in nine separate hapas under constant water showering. The male was seen chasing the female vigorously and release of eggs and sperm of *reba* and *bata* took place 6 and 8 hours, respectively, after the second dose of injection to the female. The breeders were removed from the hapa after ovulation had been completed. Four hours after ovulation the fertilization rate was reckoned.

***Fertilization and hatching*:** For determining fertilization rate a sample of eggs were taken in a Petri dish and the total number of eggs and the number of fertilized eggs were carefully counted. The fertilized eggs were clear and transparent and the unfertilized eggs were opaque. Thus the percentages of fertilization of each replicate and the average of the percentages within each treatment were determined using the following formula:

Percentage of fertilization = (Number of fertilized eggs/Total number of eggs in the sample) x 100

For determining the rate of hatching a special contrivance was applied. A sample of known number of water-hardened fertilized eggs was transferred to especially hand-made incubation jar. The jars were prepared by cutting 5 cm dia PVC pipe in to 15 cm length. The bottom of the pipe was closed with fine meshed nylon cloth. The jars were made to float in the hatching funnel with polystyrene foam attached to the rim of the pipe. For circulation of water in the hatching jars, a few holes were made on the upper end of the pipe; they were similarly closed with the nylon cloth. The water temperature of the incubation jar was recorded with the help of a centigrade thermometer. After 18±2 hours of fertilization the newly born hatchlings came out from egg shell.

The percentages of hatching from each replicate and the average percentages within each treatment were calculated by counting the number of hatchlings coming out from the fertilized eggs, according to the following formula:

Percentage of hatching = (Number of hatchlings/Total number of fertilized eggs in the sample) x 100

Data analysis: The significance of the differences obtained between the three treatments was tested with one-way ANOVA by using the Microsoft Excel 2002 program.

Results

Reba and *bata* are the popular minor carp species in Bangladesh. These species used to be largely available in the freshwaters of rivers, ponds, lakes, *beels* and *baors* but in recent time their abundance has been drastically reduced and concerns have been expressed for their conservation through habitat preservation and bringing them in to culture system. The most important requisite for culture technology development of a species is the optimization of breeding and seed production of the species. With this view in mind the present experiment was undertaken on the optimization of dose of hormone for artificial breeding of the two species.

In *reba*, the average fertilization rates, 44.45%, 87.88% and 72.22% and the average hatching rates, 42.66%, 81.47% and 67.65% (Table 1 and Fig. 1) were obtained against the doses of 0.5, 1.0 and 1.5 mg kg^{-1} body weight as initial doses, followed by the resolving doses of 4.0, 4.5 and 5.0 mg kg^{-1} body weight, respectively, at an interval of 6 hours between the first and the resolving dose to the females; males however, got a single dose of 1.5 mg kg^{-1} body weight at the time of resolving dose to the females.

Table 1. Treatment doses of PG (mg kg^{-1} body weight) to the females of *reba* and *bata* and fertilization and hatching rates obtained with the doses.

Treatments (each with 3 replications)	1st dose to both of the species	2nd dose		Fertilization rate		Hatching rate	
		reba	*bata*	*reba*	*bata*	*reba*	*bata*
Treatment -1	0.5	4.0	4.5	44.45	68.70	42.66	56
Treatment -2	1.0	4.5	5.0	87.88	86.19	81.47	75
Treatment -3	1.5	5.0	5.5	72.22	73.19	67.65	43

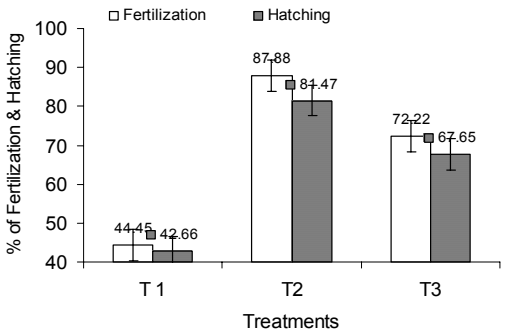

Figure 1. Fertilization and hatching rates of *C. reba* at different doses of PG.

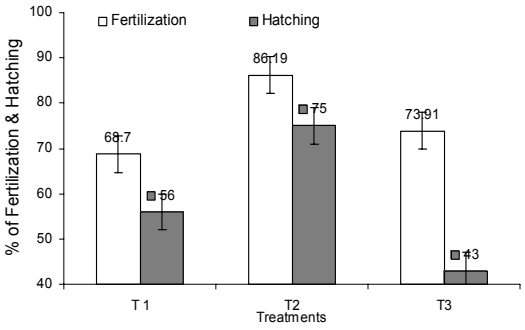

Figure. 2. Fertilization and hatching rates of *L. bata* at different doses of PG.

From Fig. 1 it can be seen that the highest average fertilization and hatching rates were obtained at 87.88% and 81.47% respectively from treatment 2; the second highest rates of fertilization and hatching were obtained at 74.22% and 67.65% from the treatment 3.

The Fig. 2 reveals the average fertilization and hatching rates of *bata* and it shows that the average fertilization rates 68.7%, 86.19% and 73.91% and average hatching rates 56%, 75% and 43%, were obtained against the doses of 0.5, 1.0 and 1.5 mg kg $^{-1}$ body weight as initial doses, followed by the resolving doses of 4.5, 5.0 and 5.5 mg kg $^{-1}$ body weight, respectively with an interval of 6 hours between the initial and the resolving dose of injection to the females; the male fishes got the single dose of 2.0 mg kg $^{-1}$ body weight administered at the time of second dose given to the females. The highest rates of fertilization (86.19%) and hatching (75%) were obtained from the second treatment. The second highest rate of fertilization (73.19%) and hatching (56%) were obtained from the third and the first treatment respectively.

One-way ANOVA tests were done on the differences between the rates of fertilization and hatching obtained in different treatments in both the species. The tests showed that in *reba,* the differences in rates of fertilization between treatment 1 and 2 and between 1 and 3 were significant at 5% level; the difference between 2 and 3 was not significant at 5% level. The differences of the rates of hatching obtained in the three treatments were similarly tested and were found not to be significant at 5% level.

Incase of *bata* also, the difference between treatment 1 and 2 was found significant at 5% level; the differences between treatment 2 and 3 and between treatment 1 and 3 were not significant. With regard to the rates of hatching the differences between treatment 1 and 2 and between treatment 2 and 3 were found to be significant at 5% level; the difference between treatment 1 and 3 was not significant.

Discussion

Published information on the biology, pertinently reproduction and breeding of *reba* and *bata* are very meager in Bangladesh. The situation in case of *reba* is comparatively slightly better; there are a few published references available on induced breeding of this species

in Bangladesh and India. In *bata*, however, there has not been any attempt known on induced breeding of the species.

At the backdrop of rapidly decline in abundance of these species from the natural waters of Bangladesh, necessitating technologies for bringing the species under culture system, the present attempt on the artificial breeding of the species can be demanding. There are three important factors that need optimization for successful induced breeding of a species. These are type of hormone used, dose used, and the interval time between the first and the second injection given to the female. These factors were seen to vary in the available references on the induced breeding of *reba.*

The success of induced breeding mostly depends on the administration of appropriate dose of hormone (Pillay, 1993). Hossain (2001) reported using PG doses at 2 mg kg^{-1} and 6 mg kg^{-1} body weight respectively in the first and second injection to the female of *reba*; with the male getting dose at 2 mg kg^{-1} body weight. The time interval between the first and second injection to the female was maintained at same as the present case at 6 hours. The rates of fertilization and hatching were obtained at 92% and 86% respectively. Verghese (1969) obtained fertilization and hatching rates both at 70% in *reba* where the females were injected with PG doses of 4 mg and 2 mg kg^{-1} body weight in the first and second injection respectively at an interval of 5 hours; the males were injected with the dose 5 mg kg^{-1} body weight at the time of second injection. The doses of PG hormone used by Tripathi *et al*. (1997) were also higher; 4 and 8 mg kg^{-1} body weight to the females and 4 mg kg^{-1} to the males. Mazid and Kohinoor (2003) reported obtaining fertilization and hatching rate of *reba* at the range of 51-88% and 55-86% respectively with a single dose of PG at 8 mg kg^{-1} body weight where the male got the dose at 2 mg kg^{-1} body weight.

The rates of fertilization and hatching obtained by Hossain (2001) were just marginally higher than the rates obtained in the present study. The water exchange in the hatching jar is an important determinant of rates of fertilization and hatching (Webber and Riordan, 1976). In the present study the hatching was performed in hand made especial hatching funnel. The reduced rate of water exchange in the funnel most plausibly could undermine hatching and explicate the comparative lower rates of hatching in the present study. Nevertheless, the rates obtained by either Verghese (1969), Mazid and Kohinoor (2003) or Tripathi *et al.* (1997) are lower than the rates obtained in the present study. The doses of hormone producing the highest average percentage of fertilization and hatching in the present study were 1.0 mg and 4.5 mg kg^{-1} body weight in the first and second injection respectively to the females. The dose of injection given to the male was 1.5 mg kg^{-1} body weight. The doses of hormone used in the present study are evidently lower than the doses used by authors of the foregoing references which is definitely a positive advantage considering the cost of hormone required in induced breeding.

Response to hormone injection for breeding is always optimum at the peak of the breeding season. In Bangladesh the breeding season of *reba* and *bata* ranges from April to August (Hossain, 2001). Verghese (1967) reported breeding season of *reba* and *bata* to be from June to October. Fish may not respond optimally to breeding if it does not coincide with its breeding readiness. Immature or over matured fish generally require higher dose of hormone than the matured ones. The present study was conducted at the peak of the

breeding season in May–June, thus needing comparatively lower doses of hormone to breed them. Sex ratio of the breeders in the breeding hapa is also an important consideration. The sex ratio was maintained at 1:1 in the present study which is same as that of Hossain (2001), however, Verghese (1969) reported using sex ratio at 1:2 male to female.

The time required for ovulation varies from species to species. In the present study *reba* and *bata* females ovulated 6 and 8 hours respectively after the second injection. Similar observations were also made by Tripathi (1997) and Hossain (2001). Rahman *et al*. (1985) reported ovulation time of mrigal to be 4-5 hours after the second injection. Temperature is an important determinant of incubation period. The period is generally shorter at elevated temperature and vice-versa. In the present study the incubation period was recorded to be 18±2 hours at 27-28 ^{o}C. Hossain (2001) had the same observation. Alikunhi and Rao (1951) recorded incubation of *reba* to be about 15 hours at temperature 28 ^{o}C. Rahman *et al*. (1985) recorded incubation period in major carps ranging from 16-18 hours at temperature 28-31 ^{o}C, while Chaudhuri (1960) reported the period to be 15-18 hours at temperature 24-31 ^{o}C.

The rates of fertilization and hatching obtained for both *reba* and *bata* in the present study by using comparatively lower doses of PG hormone than the available references on the species can be considered satisfactory. However, there is scope for further improvement in the process.

Acknowledgement: The experiment was conducted at the premise of the hatchery of Arabpur Fish Farm, Jessore. The owner of the hatchery Mr. Abdus Salam Sarder is very much thanked for his help and cooperation in conducting the experiment.

References

Akhteruzzaman, M and Kaiya, K.U. 2003. Production performance of small indigenous fish species under different management systems for sustainable rural livelihood. pp.37-46. In: Wahab, M.A.; Thilsted, S.H. and Hoq, M.E. (eds.), *Small Indigenous Fish Species in Bangladesh*. Technical Proc. of BAU-ENRECA/ DANIDA Workshop on *Potentials of Small Indigenous Species of Fish in Aquaculture and Rice-field Stocking for Improved Food and Nutrition Security in Bangladesh*. 30-31 October, 2002, BAU, Mymensingh 2202, Bangladesh, 166 pp.

Alikunhi, K.H. and Rao, S.N. 1951. Notes on the early development, growth and maturity of *C. reba*. *Journal of Zoological Society of India*, 3 (1): 85-98.

Anon. 2001. *Red Book of Threatened Fishes of Bangladesh*. IUCN-The World Conservation Union, Dhaka, Bangladesh, 116 pp.

Azadi, M.A. and Naser, A. 1996a. Length-weight relationship and relative condition factor of a carp, *Labeo bata* (Ham.) from Kaptai reservoir, Bangladesh. *Chittagong University Studies*, Part 2, 20(2): 19-25.

Azadi, M.A. and Naser, A. 1996b. Morphology of *Labeo bata* (Hamilton) from Kaptai reservoir, Bangladesh. *Chittagong University Studies*, Part 2, 20(2): 133-136.

Bhuiyan, A.S. 1989. On the food and feeding habit of the fry of *C. reba*. (Ham). *The Rajshahi University Studies*, 17: 245-255.

Chatterjee, A. and Siddiqui, A.Q. 1979. Feeding habits of *Labeo bata* (Ham.) in the river Kali. *Journal of the Asiatic Society of Bangladesh (Science)*, 5 (2): 33-41.

Chaudhuri, H. 1960. Experiments on induced spawning of Indian carps with pituitary injection. *Indian Journal of Fisheries*, 7(2): 20-48.

Felts, R.A.; Rajts, F. and Akhteruzzama, M. 1996. Small indigenous fish species culture in Bangladesh. IFADEP Sub-Project-2. Development of Inland Fisheries, Department of Fisheries, Dhaka, 41 pp.

Hickling, C.F. 1944. *Tropical Indian Fisheries*. 2nd edn., The Camelot Press Ltd., London, 295 pp.

Hossain, M.A.; Afsana, K. and Azad Shah, A.K.M. 1999. Nutritional value of some small indigenous fish species (SIS) of Bangladesh. *Bangladesh Journal of Fisheries Research*, 3(1): 77-85.

Hossain, Q.Z. 2001. Induced breeding of the fish *C. reba* by pituitary gland extract and survival of spawn in nursery pond. *Journal of Asiatic Society of Bangladesh*, 27(2): 205-213.

Mazid, M.A. and Kohinoor, A.H.M. 2003. Research and conservation of small indigenous fish species. pp. 79-86. In: Wahab, M.A.; Thilsted, S.H. and Hoq, M.E. (eds.), *Small Indigenous Fish Species in Bangladesh*. Technical Proc. of BAU-ENRECA/ DANIDA Workshop on *Potentials of Small Indigenous Species of Fish in Aquaculture and Rice-field stocking for Improved Food and Nutrition Security in Bangladesh*. 30-31 October, 2002, BAU, Mymensingh 2202, Bangladesh, 166 pp.

Pillay, T.V.R. 1993. *Aquaculture: Principles and Practices*. 3rd edn., Blackwell Science Ltd., London, pp. 156-175.

Rahman, A.K.A. 1989. *Freshwater Fishes of Bangladesh*. Zoological Society of Bangladesh, Dhaka, 352 pp.

Rahman, M.A.; Dewan, S.; Rahmatullah, S.M. and Das, M. 1985. Experiment of induced breeding of indigenous major carps by pituitary hormone injection. *Bangladesh Journal of Aquaculture*, 6-7 (1): 51-57.

Thilsted, S.H.; Roos, N. and Hassan, N. 1997. The importance of small indigenous fish species for improved human nutrition in rural Bangladesh. pp 65-69 In: Wahab, M.A.; Thilsted, S.H. and Hoq, M.E. (eds.), *Small Indigenous Fish Species in Bangladesh*. Technical Proc. of BAU-ENRECA/ DANIDA Workshop on *Potentials of Small Indigenous Species of Fish in Aquaculture and Rice-field stocking for Improved Food and Nutrition Security in Bangladesh*. 30-31 October, 2002, BAU, Mymensingh 2202, Bangladesh, 166 pp.

Tripathi, S.D.; Mazid M.A. and Mazumder, D. 1997. On farm seed production of a small indigenous species, *Cirrhinus reba* (Ham.) for Aquaculture and Biodiversity Conservation. *Proceedings of National Workshop on Small Indigenous Fish Culture in Bangladesh*, Rajshahi, 79-84 pp.

Verghese, P.U. 1967. Prolongation of spawning season in the carp *Cirrhina reba* (Ham.) by artificial light treatment. *Current Science*, 36(17): 465-467.

Verghese, P.U. 1968. Preliminary experiments on the modification of the reproductive cycle of an Indian carp *Cirrhina reba* (Ham.) by control of light and temperature. *Proceedings of Indo-Pacific Fisheries Council*, 13(2): 171-184.

Verghese, P.U. 1969. Effect of pituitary hormone injection on the carp, *C. reba* (Ham.) in relation to day length and temperature. *Indian Journal of Zoology*, 10: 155-160.

Wahab, M.A. 2003. Small indigenous fish species of Bangladesh: Potential for culture and conservation. pp 1-12. In: Wahab, M.A.; Thilsted, S.H. and Hoq, M.E. (eds.), *Small Indigenous Fish Species in Bangladesh*. Technical Proc. of BAU-ENRECA/ DANIDA Workshop on *Potentials of Small Indigenous Species of Fish in Aquaculture and Rice-field stocking for Improved Food and Nutrition Security in Bangladesh*. 30-31 October, 2002, BAU, Mymensingh 2202, Bangladesh, 166 pp.

Webber, H. and Riordan, P.F. 1976. Criteria for candidate species for aquaculture. *Aquaculture*, 7: 107-114.

Rahman, M.M.; Habib, M.A. and Shah, M.S. 2007. Induced breeding of *Cirrhinus reba* (Ham.) and *Labeo bata* (Ham.). *Khulna University Studies*, 8(2): 286-292.