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Conservation of Critically Endangered Olive Barb *Puntius sarana* (Hamilton, 1822) through Artificial Propagation

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Abstract

The Olive barb *Puntius sarana* (Hamilton, 1822) is a member of the family Cyprinidae and its conservational status has been referred as critically endangered in Bangladesh and vulnerable in India. An experiment on artificial propagation of the critically endangered fish *P. sarana* was carried out to determine the suitable dose of pituitary gland (PG) hormone as well as to determine the effective breeding season for the conservation of this critically endangered fish species. Three breeding trials (April 2010, June 2010 and July 2010) have been taken into consideration with PG doses in three different treatments (4.5, 5.5, 6.5 mg/kg body weight, respectively) having three replications of each. Brood fishes were collected from the Kangsha River (Netrokona) and reared in the experimental ponds, providing special diet upto their maturation. A total of 18 broods (9 female and 9 male) were selected for induced breeding in each trial. To observe the effective dose for induced breeding, the females were first injected at the rate of 4.5, 5.5 and 6.5 mg PG/kg body weight in T_1, T_2 and T_3, respectively in each trial. On the other hand, the males were administrated at the rate of 2 mg PG/kg body weight. The breeding performance in terms of ovulation, fertilization and hatching rate were studied. Induced breeding, in trial-2 obtained the better result in terms of ovulation, fertilization and hatching rate compared to other two trials. In trial-2 among the treatments, T_2 with doses of 5.5 mg/kg body weight showed better result than other two treatments where 4.5 and 6.5 mg/kg body weight PG doses were used in T_1 and T_3, respectively. The findings of the present study can be used in induced breeding of *P. sarana* for the development of hatchery propagation as well as to conserve this valuable critically endangered species.

Key words: Cyprinidae, artificial propagation, pituitary gland, breeding, fertilization, hatching

Introduction

The conservation of endangered species has gained great ecological significance over recent years (Hossain *et al*., 2008a; 2012a; 2012b; Rahman *et al*., 2013). Knowledge on

the life-history characteristics of threatened species is important for the implementation of sound management strategies for conservation (Hossain *et al*., 2008b; 2013a; 2013b). Bangladesh is home to 260 indigenous freshwater bony fish species apposite for human utilization, belonging to 145 genera and 55 families which represent a very wealthy aquatic biodiversity (Hossain, 2010a; 2010b). Fish and Fishery Products have been playing an important role in the socio-economic condition of Bangladesh. In 2010-2011, the contribution of fisheries sector is 4.25% to the GDP along with the second highest export earning and over 63% to the total export earning comes from this sector (BPC, 2012). In the greater part of the 20th century, Bangladesh had abundant capture fishery stocks and hence little interest was shown in culture techniques. However, over-exploitation and habitat degradation due to massive construction of flood control structures, abstraction of water for irrigation, intensive agriculture and development activities, pollution, destruction of mangrove forests all augmenting the already-poor state of the production from natural waters including rivers, floodplains, lakes and paddies (Hossain *et al*., 2012c). Therefore, aquaculture practices have been rapidly expanded over the recent years, because of increasing demand for fish and fishery products, adoption of improved aquaculture technologies and income generation.

Puntius sarana (Hamilton, 1822) commonly known as "Olive barb" is a small fish belonging to the family of Cyprinidae. The conservational status of the fish has been reported as critically endangered in Bangladesh (IUCN Bangladesh, 1998; Ameen *et al.*, 2000) and vulnerable in India (Mijkherjee *et al*., 2002) or. It has high demand for its excellent taste among barb species with high nutritional and market value in Bangladesh as well as other Asian countries (Chakraborty *et al*., 2006). This fish can be used both as food fish and ornamental fish (Froese and Pauly, 2011).

The Olive barb is widely distributed through the Indian sub-continent including Bangladesh, India, Pakistan, Nepal, Bhutan, Sri- Lanka, Afghanistan and Thailand (Talwar and Jhingran, 1991; Bhat, 2004; Chakraborty *et al*., 2006; Froese and Pauly, 2011; Jena *et al*., 2007). It has also been reported from Vietnam (Yen and Trong, 1988). This small indigenous species inhabits in rivers, streams, ponds, beels, ditches, and floodplains in the South Asian countries (Bhat, 2004; Chakraborty *et al*., 2006; Jena *et al.*, 2007). However, the population has declined drastically or on the verge of extinction due to over exploitation and various ecological changes in its natural habitats (Mijkherjee *et al*., 2002; Chakraborty *et al.*, 2006; Hossain *et al*., 2010).

In Bangladesh, this fish has enormous aquaculture potential and it could be easily grown in fish ponds along with other polyculture species (Jena *et al.*, 2008). In order to do so, a huge quantity of fingerlings will be required which can be met through artificial breeding and successful rearing of fry and fingerlings. Several studies (Akhteruzzaman *et al*., 1992; Chakraborty *et al*., 2002; Chakraborty *et al*., 2003; Parvez and Khan, 2005) have been conducted on artificial breeding of *P. sarana* but most of the fry produced faced the problems of lower growth and survival, susceptibility to disease and deformities thus they could not meet the demand of

farmers. Therefore, the present study aimed to determine the suitable dose of pituitary gland (PG) hormone and to determine the effective breeding season of *P. sarana*.

Materials and methods

The present study was conducted at the Fisheries Field Laboratory Complex, Faculty of Fisheries, Bangladesh Agricultural University, Bangladesh during November, 2010 to December, 2011.

Brood collection

The wild brood fish including male and female *P. sarana* were collected from Kangsha River, Netrokona district, Bangladesh. The collected broods were checked for diseases and acclimatized and were stocked separately in ponds of 18×14×1.3 m^3, situated in the field laboratory complex.

Brood rearing

The brood fish were fed on a special feed enriched with protein and vitamin E. The ingredients and the proximate composition of the feed are given in tables 1 and 2, respectively. This feed was given at the rate of 4-5% of total body weight of fish per day. Additionally, the ponds were treated with manure of cow dung at 15 days interval at the rate of 5 kg/decimal. Similarly, fertilizer application was done using Urea and TSP (Triple Super Phosphate) at the rate of 200g/decimal and 100 g/decimal, respectively.

Brood selection and conditioning

Broods of both male and female were collected from the rearing pond using seine net at 1:1 ratio on the day of breeding trials. Only healthy mature broods of both sexes were selected following the characteristics mentioned in table 3. The selected broods were immediately transferred to the hatchery of the field laboratory complex and kept into *hapa* in rectangular tank for conditioning for about 24 hrs. The males and females were kept in separate tanks and continuous water flow was maintained to ensure sufficient aeration. However, no feed was provided during the conditioning period.

Table 1. Feed ingredients used for rearing of *Puntius sarana* brood for artificial propagation.

Ingredient	Percentage
Fish meal	18.43
Rice bran	18.43
Wheat bran	18.43
Soya bean meal	18.43
Mustard oil cake	11.06
Sesame oil cake	11.06
Wheat flour	03.69
Vitamin-minerals premix	00.46
Vitamin E	00.01

Brood injection and breeding induced

In this experiment three breeding trials (April 2011, June 2011 and August 2011) and three doses of PG hormone (4.5, 5.5 and 6.5 mg/kg for female and 2.0 mg/kg for male fish, respectively) in each trial were performed (Tab. 4). Each trial contained three treatments having three replications of each. So, a total of 18 broods were injected in each trial.

Locally available dehydrated carp PG were collected from market. An electronic balance (College B204-S, Switzerland) was used to weight the required amount of PG. The amount of PG was weighed by using the following formula:

Weight of PG (mg) = $(W_t \times P_t) / 1000$

where, W_t represents total body weight (g) of all the fishes to be injected and P_t

represent the rate in mg PG to be injected/kg body weight under a particular treatment. The weighed PG was then transferred to a tissue homogenizer for thoroughly crushing. The crushed PG was diluted by distilled water to dissolve it and was centrifuged with a centrifuge machine for precipitation. The supernatant solution

Table 2. Proximate feed composition used for rearing *Puntius sarana* brood for artificial propagation.

Moisture	Ash	Protein	Lipid	Fibre	Nitrogen Free Extract (Carbohydrate)
12.12	18.46	29.59	18.27	8.14	13.62

Table 3. Characteristics to select the brood of male and female *Puntius sarana* for artificial propagation.

Characters	Male	Female
1. Scale	Rough and sandy texture.	Scales smooth and silky.
2. Operculum	Rough with sandy tuber.	Operculum smooth.
3. Pectoral fins	Rough on dorsal surface of pectoral fin.	Pectoral fins very smooth and slippery.
4. Abdomen	Round, firm and not very soft to touch.	Soft, round, bulging out belly on both sides.
5. Genital opening	Elongated slightly and whitish colour.	Round, flesh and pinkish genital opening, papillae prominent.

Table 4. Doses of PG hormones for male and female brood of *Puntius sarana* in three breeding trials.

Trials	T	Pbf	Wbf		DPG
Trial-1 (April 2011)	T_1	3	Female	205.5±5.1	4.5
			Male	187.3±3.9	2.0
	T_2	3	Female	201.3±7.3	5.5
			Male	185.4±4.8	2.0
	T_3	3	Female	193.7±3.2	6.5
			Male	176.1±9.2	2.0
Trial-2 (June 2011)	T_1	3	Female	212.5±5.1	4.5
			Male	197.3±3.9	2.0
	T_2	3	Female	205.3±7.3	5.5
			Male	185.4±4.8	2.0
	T_3	3	Female	223.7±3.2	6.5
			Male	203.1±9.2	2.0
Trial-3 (August 2011)	T_1	3	Female	222.5±5.1	4.5
			Male	191.3±3.9	2.0
	T_2	3	Female	215.3±7.3	5.5
			Male	195.4±4.8	2.0
	T_3	3	Female	233.7±3.2	6.5
			Male	211.1±9.2	2.0

T = Treatment, Pbf = Pairs of brood fish, Wbf = Weight of brood fish (g), DPG = Dose of PG (mg/kg body weight of fish)

of hormone was taken in a 1 ml syringe for injection. Then fish was caught carefully by scoop net and kept on sponge. They were wrapped up by wet soft cloth and accurate dose of hormone solution was then administered by intra-muscular injection on muscles beneath the dorsal fin slightly above the lateral line. After injection, the broods were kept in separate breeding tanks for each treatment.

Ovulation and fertilization

The brood was found to ovulate between 5 and 6.5 h after injection. The fishes were removed from hapa when the ovulation was completed. Then the eggs were stripped out from the female and fertilized with stripped milt using a feather. The fertilized eggs were then transferred into mini circular tank. Thereafter, a continuous flow of water was maintained for aeration to ensure the environmental conditions were optimal for the hatching process.

Determination of ovulation, fertilization and hatching rate

For determination of fertilization and hatching rate, approximately 100 eggs were placed in bowls of 1.25 L capacity with three replications each having water flow from porous PVC pipe and outlet facility. At first the number of fertilized and unfertilized eggs of each bowl was counted with naked eyes. After 18-23 h of fertilization, it was observed that hatching almost completed and the number of hatchlings in each bowl was counted. Ovulation rate, fertilization rate and hatching rate were calculated using the following formula:

Ovulation rate (%) = No. of fish ovulated/ Total no. of fish injected × 100

Fertilization rate (%) = No. of fertilized eggs/ Total no. of eggs × 100

Hatching rate (%) = No. of eggs hatched/ Total no. of fertilized eggs × 100

First feeding

Although the hatchlings of *P. sarana* retained yolk sac up to 72 h after hatching, the larvae started first feeding from 36-42 h of post-hatching at ambient temperature of 26.5-29.5^{0}C. Boiled egg-yolk was provided as first food for the hatchlings.

Statistical analysis

Statistical analyses were performed using Graph Pad Prism 5 software. Tests for normality of each group were conducted by visual assessment of histograms and box plots, and confirmed using the Kolmogorov-Smirnov test. Only percent data had to be arcsine transformed before analysis. Where the normality assumption was met, the one-way analysis of variance (ANOVA) was used to compare the variables among treatments, followed by a post hoc Dunn's multiple comparison tests. A Chi-square test was used to check the ovulation, fertilization and hatching rates between treatments. All statistical analyses were considered significant at 5% ($p<0.05$).

Results

Through the proper management of brood-stock, the fishes were found to be fully gravid and are ready to breed. Although all the fish did not mature at the same time but first growing fish were found to have matured in early breeding season. During the experimentation the range of water temperature, DO and pH was 26.5 to 31.1°C, 5.1 to 6.5 ppm and 6.7 and 7.4, respectively.

The average ovulation, fertilization, hatching, survival rate of three trials is shown in table 5. In trial-1, the result showed marked differences in T_3 than other two treatments. The average ovulation rate (33%) was recorded in T_3, whereas no fish was found to have ovulated in T_1 and T_2. In trial-2, the result expressed that there was no difference in ovulation rate between T_2 and T_3. The highest (100%) ovulation rate was found in T_2 and T_3 whereas T_1 showed the lowest ovulation rate (33%) and the ovulation rate of T_2 and T_3 were

significantly (p<0.05) higher than T_1. Similarly in trial-3, the highest ovulation rate (100%) was found in T_3 followed by T_2 and T_1 (66% and 33%, respectively). The ovulation rate was significantly different from each other.

In trial-1, the average fertilization rate was 25% in T_3. No fertilized eggs were found in T_1 and T_2. In trial-2, the average fertilization rates were recorded as 58%, 81% and 74% in T_1, T_2 and T_3, respectively. The highest fertilization rate (81%) was recorded in T_2 whereas the lowest fertilization rate (58.1%) was found in T_1. The fertilization rate of T_1, T_2 and T_3 were significantly (p<0.05) different from each other. In trial-3, the highest fertilization rate (69%) was recorded in T_2 followed by T_1 and T_3 (64% and 53%, respectively). In this trial T_1 and T_2 were not significantly different from one another but T_3 differ significantly (p<0.05) from T_1 and T_2.

Table 5. Breeding performance of *Puntius sarana* with different doses in different trials.

Trials	Treatments	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)	Survival rate (%)
Trial-1 (April 2011)	T_1	00.0±0.0^b	00.0±0.0^b	00.0±0.0^b	00.0±0.0^b
	T_2	00.0±0.0^b	00.0±0.0^b	00.0±0.0^b	00.0±0.0^b
	T_3	33.3±11.1^a	25.3±2.3^a	32.7±5.1^a	28.5±4.5^a
Trial-2 (June 2011	T_1	33.3±11.1^b	58.1±1.7^c	52.7±2.6^b	78.4±2.2^a
	T_2	100.0±0.0^a	81.3±3.3^a	74.3±7.6^a	70.3±1.3^b
	T_3	100.0±0.0^a	74.7±3.8^b	68.3±5.1^a	67.5±4.5^b
Trial-3 (August 2011)	T_1	33.33±22.2^c	64.6±6.6^a	58.0±3.3^b	50.0±6.0^c
	T_2	66.66±0.0^b	69.0±3.6^a	77.0±4.6^a	77.0±3.0^a
	T_3	100.0±0.0^a	53.3±2.6^b	56.3±4.0^b	60.0±1.6^b

(mean±standard error); Values of the parameter in each column with different superscripts (a, b and c) differs significantly (p<0.05).

During the experimentation with different PG doses of *P. sarana* the average hatching rate was 32% in T_3 found in trial-1. On the other hand the average hatching rates were found to be 52%, 74% and 68% in T_1, T_2 and T_3, respectively found in trial-2. The highest hatching rate was recorded as 74% in T_2 and the lowest hatching rate was recorded as 52% in T_1. The hatching rate in T_1 and T_2 were significantly (p<0.05) higher than that of T_3. Similarly in trial-3, the highest hatching rate (77%) was found in T_2 followed by T_1 and T_3 (58% and 56%, respectively). The hatching rate of T_2 was significantly (p<0.05) higher than T_1 and T_3 and there was no significance difference between T_1 and T_3.

In trial-1, the survival rate of *P. sarana* larvae up to first feeding was 28% found in T_3. The average survival rates were recorded as 78, 70 and 67% in T_1, T_2 and T_3, respectively found in trial-2. The highest survival rate (78%) was recorded in T_1 whereas the lowest survival rate (67%) was found in T_3. The survival rate of T_1 and T_2 were significantly (p<0.05) higher than T_3. In trial-3, the highest survival rate (77%) was recorded in T_2 followed by T_3 and T_1 (60% and 50%, respectively). The survival rate of T_1, T_2 and T_3 was significantly (p<0.05) different from each other.

Discussion

Brood stock management is one of the major aspects of successful induced breeding of any fish species. In hatchery

management, maintenance of brood fish for the development of modern aquaculture activities has become one of the most important concepts. Brood fish should be maintained under optimum condition for their proper growth and maturation. Proper care of brood stock is very important for assuring the production of eggs, fry and fingerlings (Robert *et al.,* 1982). The daily and seasonal rates of feeding of brood stock diets have direct effects on fecundity and egg size (Jones and Bromage, 1987, Bromage and Cumaranatunga, 1988). After successful completion of the brood stock management with balanced feed that comprising of adequate amount of protein, lipid, and carbohydrate, especially enriched with vitamin-E, the fish *P. sarana* attained gonadal maturity in late April. In the present study, it was found that *P. sarana* successfully bred at June and August. The peak-breeding season was in June-July and it continued till August. Sobhan and Nair (1974) reported that *P. sarana* has prolonged spawning season extending from May to November. On the other hand Sinha (1975) reported the spawning period of *P. sarana* from July to August. Several studies (Talwar and Jhingran, 1991; Khanam *et al*., 2008) concluded that mature *P. sarana* bred in running, shallow waters during the monsoon season (April-Sepetmber). In the present study, breeding of *P. sarana* was performed at an ambient temperature of 26.5 to 31.1^{0}C. This range of temperature is suitable for breeding of most indigenous small fishes (Islam and Chowdhury, 1976; Akteruzzaman *et al*., 1992). Temperature ranging from 26.5 to 35.0^{0}C is reported to be appropriate for spawning of major carps (Ibrahim *et al.,* 1968). *P. sarana* being a minor carp seemed to have similar environmental requirement with other Indian major carps. Use of vitamin mineral premix might have some positive effect for the maturation of fishes. Hoque (1990) reported that diets containing 1% vitamin premix showed better result in all aspects viz., selectivity, spawning success, fertilization and hatching rate. Spawning performance of the reared broods indicated that the spawner might have been at their optimal breeding condition. This might be due to good management practices of brood stock, which elongated their breeding season too in artificial condition.

In this experiment the breeding trials of *P. sarana* were treated with PG extract. The PG was used as an inducing agent for *P. sarana* observed by Akhteruzzaman *et al.* (1992) and Chakraborty *et al*. (2002). Chakrabortey *et al*. (2002) also reported the successful use of ovaprim but the use of human chorionic gonadotropin (HCG) and Profasi did not respond to ovulation. *P. sarana* was treated with the single PG dose to avoid more chances for getting injured during handling of the fish. In the present study, injection of PG extract at the rate of 5.5 mg/kg body weight in the trial-2 gave the best result in consideration of ovulation, fertilization and hatching rates (100%, 81% and 74%, respectively). Chakraborty *et al.* (2002) found the fertilization and hatching rate 90% and 80%, respectively by using a single dose of PG at the rate of 6.5-mg/kg body weight. In this study further increase or decrease in the amount of hormone doses from 5.5 mg/kg body weight resulted in lower reproductive performances. Dose specificity of PG extract as observed in the present study was in conformity with the findings of Akhteruzzaman *et al*. (1992) who found better spawning performances of

P. sarana at 6.0 mg PG/kg body weight. Kohinoor *et al*. (1995) found no ovulatory response of *P. sarana* to PG extract below 4.0 mg/kg body weight of the female breeders. They recommended that the spawning of *P sarana*, single doses of 5 to 6 mg PG/kg body weight would be required. The result of the present study also confirmed the above findings.

In conclusion, this study would be beneficial for apposite management of induced breeding programs of *P. sarana* and other minnow carps as well as to conserve this valuable critically endangered species in Bangladesh and neighboring countries.

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