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CAPTIVE BREEDING OF AN ENDEMIC MEDIUM CARP PENGBA, *OSTEOBRAMA BELANGERI* (VAL.) WITH WOVA-FH IN MANIPUR

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Induced breeding of the endemic medium carp, *Osteobrama belangeri* (Val.) was conducted using synthetic hormone Wova-FH in three doses @ 0.3, 0.5, 0.7 ml/kg to female and 0.1, 0.2 and 0.3 ml/kg to male body weight in groups A, B and C, respectively. The male and female brooders were injected and left to spawn in the breeding hapas at 2:1 ratio. It was found that Wova-FH could induce the fishes to breed, whereas no breeding was observed in control set. The latency period was found to be 7-8 hours. The egg output/female with Wova-FH at a dose of 0.5 ml/kg of female and 0.2 ml/kg of male body weight in group B was significantly higher ($P<0.05$) than groups A and C. A female of 250 g could produce 55,000-65,000 eggs by induced breeding. Fertilization and hatching rates of 91.7 and 85% respectively, with Wova-FH dose of 0.5 ml/kg of female and 0.2 ml/kg of male body weight was found to be significantly higher ($P<0.05$) than the other doses.

INTRODUCTION

Osteobrama belangeri (Val.) locally known as Pengba is an endemic medium carp available in the State of Manipur in India. Pengba used to migrate from Chindwin river of Myanmar to the upstream of Imphal river of Manipur for breeding in flood plains in early monsoon. It is a major seasonal fishery of Manipur contributing about 40% of the natural fishery in the state (Behera *et al*., 2009). However, over the last few decades its population has significantly declined in Manipur. It is perhaps due to loss of habitat after the construction of Ithai barrage on Imphal river. The barrage possibly prevented breeding migration of the fish from the southern parts of Manipur river, which ultimately flows into Chindwin in Myanmar. This fish species has been listed among the endangered fish species of India (CAMP Report, 1998).

O. belangeri is one of the most important fish of Manipur having high consumer preference. The effect of Wova-FH as inducing agent has been attempted in Indian major carps and other carps (Rath *et al*., 2007). The dosages of other synthetic hormones like

Ovaprim and Ovatide in tropical fishes have also been studied by several authors. However, no experiments have so far been conducted on artificial breeding of *O. belangeri* with synthetic hormone Wova-FH. Although culture, breeding and larval rearing technology of the Indian major carps has been developed, other species of commercial importance have been ignored. Hence, the present study was conducted to determine the dose of synthetic hormone Wova-FH on induce breeding of *O. belangeri* and to study the breeding efficiency of the fish.

MATERIAL AND METHODS

Broodstock collection and maintenance

The captive breeding experiment was conducted at ICAR Manipur Centre Fish Farm located at Imphal, Manipur (24.44 N; 93.58 E and altitude 746 m). *O. belangeri* broods (n=30) were collected from Loktak lake during December-February, 2005 and transported in oxygenated polythene bags. The fishes were maintained in an earthen pond (0.02 ha, average depth 120-130 cm). Pengba is an herbivorous fish and were fed with *Azolla, Lemna,* locally available grass and also with rice polish and mustard oil cake (1:1) at 5% of body weight per day. Adult fish show distinct sexual dimorphism. Male has a slender body, its pectoral fin has rough dorsal surface and the same is longer than that of female; its abdomen does not bulge out as in female. Further, on applying gentle pressure, milt oozes out through the genital aperture. In females, the pectoral fin is smooth, has a reddish, soft swollen and bulging belly. On applying gentle pressure on the belly, eggs ooze out.

Captive breeding

Spawners were selected for induced spawning experiment in the month of July, 2006. The broods were collected from the earthen pond by repeated netting followed by dewatering, segregated and transferred into nylon hapas (1.5 × 2.5 × 3.0 m) for acclimatization. The experiment was conducted with the female broodstocks of body weight varying from 250 to 260 g and male broodstocks of body weight varying from 170 to 180 g. Three sets of experiments were conducted for three different doses. Group A was induced with 0.3 ml/kg of female and 0.1 ml/kg of male body weight, group B with 0.5 ml/kg of female and 0.2 ml/kg of male body weight and group C with 0.7 ml/kg of female and 0.3 ml/kg of male body weight (Table 1) in separate nylon hapas. The present experiment was designed based on the earlier reports on different related species. It has been proved by many researchers that male requires less amount of hormonal injection than the female (Routray *et al.*, 2007). A control was also maintained where no hormonal administration was done. Free oozing males and ripe females were used for spawning in the ratio of 2:1 respectively. All females and males were injected with Wova-FH (Biostadt Agrisciences, Wockhardt Life Science, Mumbai, India) intramuscularly. Soon after administering the hormone, the brooders were released into the breeding hapas.

Table 1. Results of the captive breeding experiments of *Osteobrama belangiri* by Wova-FH

Groups	Size of female (g)	Wova-FH dosage to female (ml/kg body weight)	Size of male (g)	Wova-FH dosage to male (ml/kg body weight)	Latency period (h)	Egg output/ female	Fertilization (%)	Hatching (%)	Remark
A	252±2.32	0.3	173±3.21	0.1	10	22,000[c]	45.10±3.17[c]	52.30±3.14[c]	Partial spawning
B	257±3.26	0.5	178±2.25	0.2	7	65,000[a]	91.70±2.50[a]	85.40±2.30[a]	Complete spawning
C	254±3.21	0.7	176±3.42	0.3	8	43,000[b]	75.30±3.21[b]	71.90±2.23[b]	Complete spawning
Control	258±3.21	-	175±3.75	-	-	No breeding	Nil	Nil	No spawning

[abc] Means with different superscripts in each column differs significantly ($P<0.05$)

After spawning, effective fecundity of each female was determined by random sampling of eggs in a 10 ml graduated measuring tube from the total eggs released by the female. The total number of eggs in 1 ml were counted and multiplied with total volume of eggs released. The fertilization rate of eggs was determined by randomly taking a sample of approximately 100 eggs from the total eggs in a dish. Fertilized eggs having intact nucleus were only considered for calculating percentage of fertilization. The ova diameter was measured by keeping approximately 20 eggs in a row along the measuring scale under dissecting microscope. The total lengths of eggs were divided by numbers of eggs to obtained mean diameter of each egg. The 1-day old hatchlings were maintained in circular FRP tanks. Aeration was provided in the FRP tanks and water was exchanged daily.

The water quality parameters of broodstock and breeding ponds were analyzed as per APHA (1998). The physico-chemical parameters of broodstock pond were; water temperature (28.2±2.3 ^{o}C), pH (7.8±0.21), dissolved oxygen (6.2±1.8 ppm) and free CO_2 (2.5±0.7 ppm). The values of physico-chemical parameters of breeding pond were; water temperature (28.5±2.5 ^{0}C), pH (7.9±0.27), dissolved oxygen (6.7±1.3 ppm) and free CO_2 (2.4±0.5 ppm).

All the statistical analysis of the experimental data was performed by using SPSS version 16.0 for windows. One-way ANOVA was used to analyse the variance. Post-hoc test was carried out using Duncan's multiple comparison procedures to find out the significant differences (Duncan, 1955).

RESULTS AND DISCUSSION

O. belangeri attained maturity at the age of 2^{+} years and were found to be fully mature during the month of July. A varied degree of response of inducing agent was observed in different doses. Fertilization rate, latency period, egg output and hatching rate were observed and recorded (Table 1). In the present study, out of twelve females selected for induced breeding in three experiment sets, nine responded positively and produced viable eggs. In control set, no breeding behaviour was observed.

Brooders showed chasing behaviour after 3-4 hours of injection of Wova-FH in groups B and C. However, in group A, breeding behaviour was seen after 7 hours of injection. Mating was preceded by elaborate courtship. It was observed that male rubbed its body with female and released its milt and eggs were fertilized externally. Spawning occurred after 10, 7 and 8 hours of Wova-FH injection in groups A, B and C, respectively. In our present study, difference in latency period was noticed. Higher latency period in Wova-FH at the dose of 0.3 ml/kg body weight of female indicates difference in the mode of action of the hormone. Similar observation was reported by Habibi *et al*. (1989) in

Carassius auratus. Longer latency period in low dose of synthetic hormone Ovatide was reported by Pandey *et al.* (2002b). Behera *et al.* (2007) also reported the same during their study on induced breeding of *Labeo bata* in Manipur. According to Billiard *et al.* (1984) and Peter *et al.* (1986), differences in dose requirement may be attributed to varied level of dopamine activity in different species of fish.

The fertilization rate in the present experiment was estimated at 45.2±3.17, 91.7±2.50 and 75.3±3.21 % in groups A, B and C, respectively. Fertilization percentage of group B was significantly higher ($P<0.05$) than the groups A and C. The dose of hormone possibly affected the percentage of fertilization. Administration of over doses of the inducing agents causes early milting; resulting in poor fertilization and under-dosing may cause late inducement in males. Similar finding was also reported by Routray *et al*. (2007) in his review paper. Rath *et al*. (2007) reported the breeding of Indian major carps with different synthetic hormone. Pandey *et al*. (2002a) successfully conducted breeding of Indian major carps using Ovatide at the dose of 0.4 ml/kg body weight for female and 0.1 ml/kg body weight of male. Behera *et al.* (2007) reported the egg output/female, fertilization and hatching rates to be the highest with Ovaprim at a dose of 0.5 ml/kg of female and 0.2 ml/kg of male body weight and with Ovatide at a dose of 0.4 ml/kg of female and 0.2 ml/kg of male body weight in *L. bata*.

The fertilized eggs were spherical, translucent and demersal measuring 2.4±0.03 mm in diameter and non-adhesive. Unfertilized eggs were pale and opaque. In the present study, number of eggs released by the female (250 g body weight) was ranged from 55,000 to 65,000 numbers indicating high fecundity. The number of eggs released by the female in our present study seems to be high fecundity as compared to many fishes reported in India.

Within 8-9 hours twitching movement of the embryo was observed. The fertilized eggs underwent development and the young hatched out within 14-16 h at 28 ^{o}C similar to other Indian major carps. The hatching rate in the present experiment was estimated at 52.30±3.14, 85.4±2.30 and 71.90±2.23 % in groups A, B and C, respectively. Hatching percentage of group B is significantly higher ($P<0.05$) than the groups A and C. Similar to the fertilization rate, the dose of hormone possibly affected the percentage of hatching. Optimal range of physico-chemical parameters of water in the breeding pond (viz., pH, temperature, and dissolved oxygen) also attributed to the high rate of hatching. Behera *et al.* (2007) reported the hatching rate of *L. bata* was highest, while inducing with Ovaprim and Ovatide along with the optimum range of water quality in experimental pond. Rath *et al.* (2007) also reported the higher hatching rate in Indian major carps while administering Wova-FH @ 0.4-0.5 ml/kg of female body weight.

The freshly hatched larvae measured 4.3±0.2 mm in length and 1.6±0.3 mg in weight. The 1-day old hatchlings were maintained in nylon hapas and plastic troughs simultaneously. Movement of hatchlings was very fast, air bladder was prominently visible with regular fanning of pectoral fins. After 2-3 days, mouth was slightly developed and they started feeding external feed after 72 hours. The yolk sac was fully absorbed on 4th day and hatchlings grew up to 6.4±0.3 mm in length and 2.7±0.2 mg in weight. After 4 days of hatching, the spawns were released into well prepared nursery tanks.

CONCLUSION

The observation in the present study indicates that optimal spawning of *O. belangeri* occurred with hormone (Wova-FH) at a dose of 0.5 ml/kg body weight and 0.2 ml/kg body weight of females and males respectively. The dose of hormone administration possibly affected the percentage of fertilization, egg output, hatching rate and spawn production. So, it can be summarized that, the commercial scale seed production of *O. belangeri* could be achieved in captivity through induced breeding technique using Wova-FH. Since the breeding protocol is simple and cost effective, this can be taken up by small farmers for seed production. This technique would be useful in conservation and artificial propagation of endemic medium carp, Pengba (*O. belangeri*) in Manipur and other parts of India.

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REFERENCES

APHA, AWWA, WPCF (American Public Health Association, American Water Works Association and Water Pollution Control Fedearation), 1998. Standard Methods for the Examination of Water and Waste Water. 15thedn. American Public Health Association, Washington, DC, USA, 1134 pp.

Behera, B. K., P. Das, N. S. Singh and A. K. Sahu, 2007. Observation on the induced breeding of *Labeo bata* (Hamilton) with Ovaprim and Ovatide as inducing agents with a note to its development. *J. Aqua.*, **15**: 11-17.

Behera, B. K., P. Das and S. V. Ngachan, 2009. Strategies for improving fish production in Loktak Lake. *In*: *Ecology, Aquatic Bio-resources and conservation of wetlands of North East India* (ed. Kosygin, L.), Akansha Publishing House, New Delhi, pp. 29-40.

Billiard, R., K. Bieniarz, R. E. Peter, M. Sokolowska, C. Weil, 1984. Effect of LHRH and LHRH-a on plasma GtH levels and maturation/ovulation in common carp, *Cyprinus carpio* kept under various environmental conditions. *Aquaculture*, **41**: 245-254.

CAMP Report, 1998. Report of the workshop on "Conservation assessment and management plan (CAMP) for freshwater fishes of India". Zoo Outreach Organization and NBFGR, Luckhnow, 22-26 September, 1997, p.156.

Duncan D. B., 1955. Multiple range and multiple F tests. *Biometrics* **11**: 1-42.

Habibi, H. R., T. A. Marchant, C. S. Nathorniak, H. Van der Loo, R. E. Peter, J. E. Rivier and W. W. Vale, 1989. Functional relationship between receptor binding and biological activity for analogs of mammalian and salmon gonadotropin-releasing hormones in the pituitary of goldfish (*Carassius auratus*). *Biol. Reprod*., 40: 1152-1161.

Pandey, A. K., C. T. Mohapatra, G. Kanungo, M. Sarkar, G. C. Sahoo and B. N. Singh, 2002a. Ovatide induced spawning in the Indian major carps, *Labeo rohita* (Hamilton-Buchanan). *Aquacult*, 3: 1-4.

Pandey, A. K., R. Koteeswaran and B. N. Singh, 2002b. Breeding of fishes with synthetic hormone drug Ovatide for mass scale seed production. *Aquacult*, **3**: 137-142.

Peter, R. E., R. Sokolowska and C. S. Nahorniak, 1986. Comparison of (D-Arg6, Trap7, Leu8, Pro9 Net) luteinising hormone (LHRA) in combination with pimozide, in stimulating gonadotropin release and ovulation in the goldfish *Carassius auratus*. *Can. J. Zool*., 65: 987-991.

Rath, S. C., S. K. Sarkar, S. D. Gupta and N. Sarangi, 2007. Comparative account of induced breeding of Indian major carps with ovaprim, ovatide, wova- FH and carp pituitary extract. *Ind. J. Anim. Sci*., **77**: 1057-1060.

Routray, P., D. K. Verma, S. K. Sarkar and N. Sarangi, 2007. Recent advances in carp seed production and milt cryopreservation. *Fish Physiol. Biochem*., **33**: 413-427.