Biodiversity and Conservation (2006) 15:3579–3589
DOI 10.1007/s10531-004-2935-6

Captive breeding of endangered fish *Chitala chitala* (Hamilton-Buchanan) for species conservation and sustainable utilization

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Received 22 January 2004; accepted in revised form 19 August 2004

Key words: Captive breeding, *Chitala chitala*, Conservation, Hatching, Ovaprim, Seed production, Spawning

Abstract. Over the last few decades wild population of *Chitala chitala* (Hamilton–Buchanan) has been declined more than 50% due to various reasons and is presently listed under endangered (EN) category due to reduced abundance. In the present communication wild *C. chitala* were collected from natural habitats and induced to spawn under captivity during July 2002 by injecting three different doses of synthetic hormone Ovaprim. Intramuscular injections were administered to fishes using three different doses (1.5, 1.0 and 0.5 ml/kg body weight). Artificial breeding pool was prepared for each set by encircling area (20 × 5 m) with mosquito net, where wooden country boat (8 × 4 × 2.5 feet with surface area 48.5 sq. feet) was placed inside the breeding pool. Distinct spawning behavior was noticed in the experimental sets with different hormonal dose whereas no spawning activity was noticed in control set. The fertilization rate varied from 48.86–80.2% and total numbers of spawned eggs in two sets of experiments were estimated to be 81,034. The average number of eggs deposited 15 ± 2.1/square inches. The fertilized eggs were large in size (4.5 ± 0.05 mm), adhesive and attached to the hard substratum. The eggs hatch out between 168–192 h after fertilization and about 33,639 hatchlings were produced. Newly hatched larvae measured 10.23 ± 0.03 mm and 0.031± 0.008 gm in weight and the mean diameter of yolk sac was 4.1 ± 0.08 mm. The yolk sac remains attached up to a week. The percentage survival of hatchlings varied from 42.2 to 65.60. Statistical analysis was worked out to determine the relation between the hormone dosage with different breeding parameters like latency period, fertilization rate, egg output, hatching rate and hatchling production.

Introduction

Captive breeding and the release of captive bred individuals into the wild are among the techniques used for conservation of rare and endangered fish species. We selected feather back *Chitala chitala* which constitutes an important component of riverine fisheries in the Indian subcontinent and considered as one of the most commercially important food fish. It belongs to order Osteoglossiformes under family Notopteridae and is distributed in all African and Asian countries like India, Pakistan, Myanmar, Bangladesh, Sri Lanka, Nepal,

Thailand and Indonesia. They are generally carnivorous and insectivorous in nature but some times they feed on crustaceans and plankton also and occasionally they are also cannibalistic in nature. Fish attains up to 122 cm (Chonder 1999) with maximum weight 14 kg (present survey). Featherbacks are known specially for its delicious meat quality and nutritive value. Featherback *C. chitala* is considered as a potential freshwater food as well as ornamental fish, command high market demand and has been prioritized recently as new candidate species for fresh water aquaculture system (Ponniah and Sarkar 2000; Ayyappan et al. 2001). Recently there has been a steady decline in the wild stocks in India and according to Conservation Assessment and Management Plan (1998) workshop the species is categorized endangered (EN). The recent survey made by NBFGR team in the stretches of river Bhagirathi, Farakka, West Bengal, India indicated that the landings of *C. chitala* has been declined rapidly (70%) over the last 10 years. While, few attempts have been made to develop chitala culture along with Indian major carps but no scientific attempts were made to develop breeding protocols under captivity except a few attempts by fish farmers. Due to non-availability of seeds in natural waters and difficulty in artificial breeding of this fish, not much could be achieved towards commercialization of the species or conservation. Keeping this in view, it is now most important to conserve this endangered species in a sustainable manner. In the present study fish was successfully bred for the first time under captivity and captive bred populations are being maintained.

Materials and methods

Broodstock transportation and maintenance

Brood fishes of 2–3 years old (12 female, 24 male) of *C. chitala* were collected from river Bhagirathi, Punarbhava, Ganga and Mahananda during November 2001–February 2002. They were carried in aluminum hundi (60 l) from the sites and kept in a plastic pool installed in the vehicle and transported. The brood fishes were stocked in the stocking pond (area 2 ha, average depth 1 m) and the size ranged from 81 to 90 cm with a weight range of 1.35–2.91 kg. The brood fishes were maintained in a polyculture system and fed with small live prawns, trash fishes, rice bran and mustered oil cake (2:1) at 4% of the body weight up to 4 months. After 4 months of rearing the fishes were found mature enough for captive breeding. The male and female are not easily distinguishable. We have observed that in maximum cases females are bigger in length than male. After dissection we observed that both male and female gonads are spongy in appearance and situated on the left side of the abdomen. The shape of the gonad is sac like structure in which the eggs are embedded like fimbrae. The sexual dimorphism is distinguishable in male and female. The female brooder shows bulgy abdomen than male and there is no marked coloration at the base of fins. Urinogenital papilla of female is stouter, fleshy, broader, less pointed and not

tipped with red color. Fully mature female shows freely oozing ova. In males, the abdomen is not bulgy and the urino-genital papilla is thin, muscular, hard conical in shape, more pointed and tipped with reddish color. The males shows diffused vent and with red coloration at the base of paired and anal fins.

Induced breeding experiment

We collected brood stocks from the stocking pond by repeated drag netting, segregated and transferred into nylon hapa for acclimatization (7–8 h). Eight female and 16 male with an average weight of 2.44 and 1.64 kg, respectively were selected from available brood stock. We followed 2:1 ratio (male:female) for breeding experiments and conducted experiments on 24.06.2002 and 26.06.2002 in a dry bunds (1 ha.), inundated with floodwater during rainy season. Hypophysation was carried out with synthetic hormone 'Ovaprim' (Syndel, Lab. Ltd. Vancouver, Canada) (Figure 1). A control set was maintained for each experiment. We tested three different doses (1.5,1 and 0.5 ml) in each experiment(Table 1) and each dose administered once to male and female. After Ovaprim injection, each set having two males and one female were released into separate dry bunds (0.2, 0.21 and 0.25 ha) where floodwater was inundated during monsoon. We prepared artificial breeding pool for each set by encircling area (20×50 m) with mosquito net. Wooden country boat ($8 \times 4 \times 2.5$ feet with surface area 48.5 sq. feet) was placed inside each breeding hapa in order to provide artificial substratum required for mating, spawning and parental care. Chasing behavior was observed after 14–18 h of injection and female released eggs and male released milt. Symptom of injury in fish has been occurred due to chasing. After 3–4 days spent fishes were removed from the breeding pool, washed into $KMnO_4$ solution and released back into stocking pond. Since eggs are adhesive in nature this facility provides good protection to the eggs. We fixed boat in water tied with woody logs inside the breeding pool (Figure 2). We estimated fertilization rate by counting eggs randomly deposited on per unit surface area of the boat. The fertilized eggs were clearly distinguished from unfertilized ones. The former was having bright milky white in color, spherical in shape with visible yolk material whereas the unfertilized ones were dull and yolk material was not visible. The size of eggs were measured by digital caliper with accuracy level of 0.01 mm. Hatching rate was estimated on seventh day after spawning by randomly counting eggs in which complete hatching process was seen on the boat surface. Fecundity was calculated prior to spawning (Bagenal and Braum 1968). After 13–14 days hatchlings were transferred to nylon hapa ($7 \times 3 \times 1.5$ feet) for further rearing up to next 15 days. The supplementary feed provided was paste of boiled eggs yolk and small sized prawn (1:1) at 8% of total body weight. Along with that, zooplankton (Rotifers and Brachionous) were also cultured separately and supplied. The survival of hatchling were calculated by randomly taking samples from nylon hapa up to 15 days at 7 days interval. The physicochemical

Table 1. Results of captive breeding experiments using different doses of Ovaprim.

Date of experiment	Average weight of female (kg)	Ovaprim dosage (ml/kg body weight)	Average weight of male (kg)	Ovaprim dosage (ml/kg body weight)	Latency period (h)	Number of eggs spawned	Fertilization (%)	Hatching (%)	No. of hatchlings produced	Remarks
24.06.2002	2.41	1.5	1.35	0.5	18–20	13,200	75.23	60.2	5958	Complete spawning
24.06.2002	2.62	1.0	1.62	0.5	17–18	14,014	76.54	65.6	6972	Complete spawning
24.06.2002	2.52	0.5	1.43	0.5	25–27	12,500	50.12	42.3	2631	Partial spawning
24.06.2002	2.20	Control	1.45	Control	–	No breeding	Nil	Nil	Nil	No spawning
26.06.2002	2.83	1.5	1.55	0.5	17–19	15,400	75.54	62.5	7266	Complete spawning
26.06.2002	2.91	1.0	2.21	0.5	18–20	16,800	80.26	65.3	8798	Complete spawning
26.06.2002	1.94	0.5	1.45	0.5	27–29	91,20	48.86	60	2014	Partial spawning
26.06.2002	2.12	Control	2.07	Control	–	No breeding	Nil	Nil	–	No spawning

Figure 1. Hormonal injection to *Chitala chitala*.

Figure 2. Breeding pool encircled with mosquito net.

parameters of broodstock pond were; air temperature (30 ± 1.1 °C), water temperature (31 ± 2.2 °C), pH 7.5 ± 0.23, dissolved oxygen (8.0 ± 2.3 ppm), free CO_2 (2.3 ± 0.5 ppm) and turbidity (2.5 ± 11 cm). The values of physicochemical parameters of breeding pool were; air temperature 30 ± 1.0 °C, water temperature 29.0 ± 2.2 °C, pH 7.5 ± 0.2, dissolved oxygen 8.0 ± 1.3 ppm, free CO_2 2.3 ± 0.5 ppm, turbidity 5.0 ± 1.1 cm, alkalinity 60.0 ± 5.0 ppm and water hardness 200 ± 10.0 ppm.

Data analysis

We analyzed data using with a statistical software package SPSS version 11.5. A probability level of 0.05 was utilized to account for the statistical difference between the means.

Results

The results of breeding trials of *Chitala chitala* are summarized in Table 1. It has been observed that early spawning (17–20 h) occurred in the fishes injected with the doses of 1.0 and 1.5-ml/kg body weight as compared to lower dose (0.5 ml/kg) and it took 25–29 h for spawning. The first two doses resulted complete spawning whereas partial spawning occurred in lower dose. No spawning activity was noticed in control set. One male was found with a female. The spawning pairs were seen moving together on the boat in search of suitable breeding place. The pairs moved erratically for certain time, become aggressive within a limited area, closed to each other, nudge themselves and found to be settled on the boat. This process repeated several times until spawning. The fertilization rate in experimental sets varied from 49–80.26%. Low rate of fertilization was recorded in case of lower dose (0.5 ml/kg) whereas very little difference was observed in first two doses of ovaprim. No marked differences in breeding and spawning behavior were observed in case of males injected at 0.5 ml/kg. The fertilized eggs were large in size (4.5 ± 0.05 mm) and adhesive and were found to be stuck on the inner surface of boat (Figure 3). The eggs hatch out between 168–192 h after fertilization. Total 33,639 hatchlings were produced as output of two sets of experiments. The average numbers of eggs per square inches deposited in both the experiments were 15 ± 2.1. The changes in color of eggs and other characteristics were noticed during embryonic development. The eggs were milky white in color, spherical in shape at the beginning (0–2 days) then become light yellowish (2–4 day) and little collapsed, turned yellow orange (4–6 days), elongated shape and finally became bright orange red in color (6–7 days) with distinctly visible form of larvae. The average hatching percentage ranged from 56.03–62.6%. Hatching rate in both the experiments was comparatively higher for the dose of 1.0 ml/kg. The total numbers of eggs spawned in two separate trials were 39,714 and 41,320, respectively (Table 1). The post spawners were shifted to

Figure 3. Fertilized eggs attached inside the boat surface.

stocking pond. The average fecundity estimated was 5286 eggs/kg body weight. Fish exhibited parental care up to 10–15 days. Throughout the hatching period male attended assiduously for fanning over the eggs, keeping the eggs aerated and guarding eggs and hatchlings.

In the present study one way ANOVA and *F*-tests were done between the hormone dosage with different breeding parameters like latency period, fertilization rate, egg output, hatching rate and hatchling production for two sets of experiments. The analysis showed that relationship between hormone dose versus fertilization rate and hatching rate was significant ($p \leq 0.05$) whereas the dosage of Ovaprim versus latency period was significant but negatively correlated (Table 2). However, the analysis between dose with egg output and hatchling production indicated non significant results.

After 4 days of embryonic development, notochord flexations was clearly visible on the periphery of the egg shell. Rapid twisting movement was noticed after 6 days of fertilization while after 7–8 days the eggs were completely hatched out. One to two days old hatched larvae were considered as newly hatched larvae (Figure 4) measured 10.23 ± 0.02 mm and 0.031 ± 0.01 gm in weight and the diameter of yolk sac measured 4.1 mm. Yolk sac was attached to the ventral side where blood capillaries and eyes were clearly visible. A long

Table 2. Correlation between the hormone dosage and other breeding parameters.

Variables	24.06.2002	26.06.2002
Latency period	−0.771*	−0.9449*
Fertilization rate	0.843*	0.7881*
Egg output	0.461	0.7677
Hatching rate	0.733*	0.4714*
Hatchlings production	0.732*	0.7381*

*Correlation significant at $p < 0.05$.

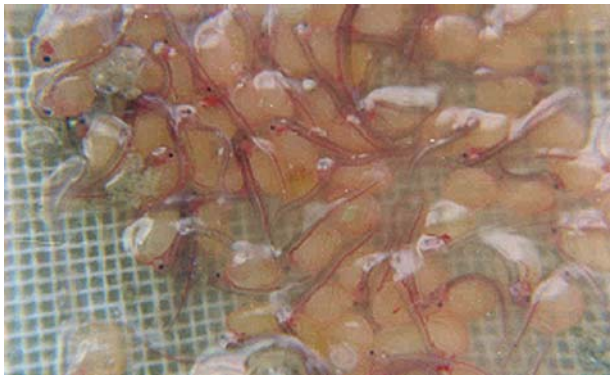

Figure 4. Newly hatched larvae (1–2 days old).

Figure 5. 15–20 days old hatchlings of *Chitala chitala*.

blood capillary line was also seen lying below the notochord flexation. The early hatchlings were still adhesive in nature and light pink in color. The yolk sac remained attached up to a week. After complete yolk sac absorption the larvae were considered as hatchlings. Five to six days old larvae were considered as hatchlings. At this stage hatchlings started free swimming and accepted natural food. The average percentage survivals of these hatchlings were 58.68%. The mean length of the hatchlings after 5–6 days were 12.51 ± 1.5 mm and mean weight was 0.033 ± 0.009 gm. After 8–9 days the hatchlings (13.8 ± 0.031 mm, 0.031 gm) were able to float in water. A characteristic pattern of congregation with their heads converging together with their heads converging together and the tail radiating outwards like petals in a flower. Hatchlings of 10–12 days was also consisted with little yolk sac and was completely absorbed after 13–15 days (Figure 5). By the time newly formed larvae were started to take external food. Hatchlings were transferred to nylon hapa (7 × 3 × 1.5 feet) for further rearing up to next 15 days. The hatchlings attained an average length of 20 ± 0.092 mm and weight of 0.062 ± 0.01 gm after 21 days in nylon hapa. After 31 days they attained up to 30 ± 0.10 mm in length. Numerous light and dark bands were appeared on the body surface. Gradually, the number of bands reduced, turned in to silvery color and scales appeared on the body after 28–35 days. Active feeding on zooplanktons and spawns of Indian major carps was observed at this stage. Now, this stage of fish was ready to stock into well maintained ponds for rearing.

Discussion

The results showed that complete spawning of *Chitala chitala* occurred at the doses of 1.0 and 1.5 ml/kg body weight of female and the dose of the hormone significantly affected the percentage of fertilization, egg output, hatching rate and hatchling production respectively (Tables 1 and 2). However no

remarkable effect of hormonal dose was recorded in breeding and spawning process for males. Higher latency period in Ovaprim at the dose of 0.5 ml/kg of body weight indicates difference in the mode of action of the hormone. Similar observation was reported by Habibi et al. (1989) in *Carassius auratus*. In the present study statistical analysis (ANOVA, *F*-test) of both the experiments (24.06.2002 and 26.06.2002) between hormone dose versus fertilization rate and hatching rate was significant ($p \leq 0.05$) whereas the dosage of Ovaprim versus latency period was significant but negatively correlated (Table 2). However, Ovaprim dose with egg output and hatchling production indicated non significant results. Singh et al. (2002) showed that number of ovulated eggs/fish was significantly higher in higher dose of Ovaprim tested in catfish *Heteropneustes fossilis*.

In our study water temperature of breeding pool recorded in two experiments were 29 ± 2.2 °C indicating quite favorable for breeding. In the present study all the females injected with Ovaprim responded while no breeding activity was observed in control sets indicating effect of inducing agent under captivity. Review of literature shows that some aspects of natural breeding behavior of *C. chitala* in the pond environment (Singh et al. 1980). They observed natural spawning in fry carrier (75 × 45 × 50 cm) after rains where eggs were found sticking to stones, brick walls, tin carriers and wooden box submerged at the corners of the pond. The average numbers of eggs per square inches deposited in our experiments were 15 ± 2.1 whereas 100 eggs/25 cm^2 surface of the substratum was reported by Singh et al. (1980). The percentage of fertilized eggs obtained in the present study was ranged from 76.54 to 80.26 in the experiments where Ovaprim used at 1 ml/kg of body weight. The percentage of fertilization was 60% in natural breeding as reported by earlier studies (Singh et al. 1980) and the fecundity was estimated to be around 4000 eggs/kg body weight. Natural breeding of *Chitala chitala* was also reported during May–June by Hossain (1999). The average fecundity in the present study (5286 per kg body) was higher in comparison to earlier reports of Singh et al. (1980). The breeding habit of *Chitala chitala* in the river Ganges was reported by Southwell and Prasad (1918). Mobarek (1980) also attempted hypophysation technique for another featherback *Notopterus notopterus*. Later, induced spawning and hatching of *N. notopterus* by injecting Ovaprim have been reported but the details are not available (Anonymous 2002). In the present study parental care of male fish was observed throughout the hatching period by fanning over the eggs, keeping the eggs aerated and guarding eggs and hatchlings. Similar parental behavior of male was reported by Singh (1996). However, Chonder (1999) reported that both male and female participate actively in parental care of *C. chitala*.

Based on the present experiments the ovaprim dose of 1 ml/kg body weight for female and 0.5 ml/kg for male can be recommended. Nandeesha et al. (1990) and Haniffa et al. (1996) have applied different dosages (0.3–0.6 ml/kg body weight) of ovaprim selected for induced spawning in carps and murrels. In *Heteropneustus fossilis* the dosage of ovaprim was given 0.3–0.7 ml/kg body

weight and the number of eggs spawned increased with increasing dosage upto 0.7 ml/kg (Haniffa et al. 2000).

Our results clearly demonstrate the the possibility of using synthetic fish hormone Ovaprim for effective induced spawning and seed production of *Chitala chitala* at experimental level. In conclusion it is recommended that the seed of *Chitala chitala* could be produced in captivity through scientific management of eggs, larvae and hatchlings. Evidently, ranching programme could be undertaken for species restoration and conservation. The successful development of protocols for captive breeding is likely to pave way towards commercialization of the technology, which may introduce an exciting entrepreneurial area. The present dosage of 1.0 ml/kg body weight for female and 0.5 ml/kg body weight for male of Ovaprim exhibited encouraging results for induced spawning and hatching and may be used as a standard doses in future breeding of *C. chitala*.

Acknowledgements

We express our sincere thanks to Project Implementation Unit, National Agricultural Technology Project (NATP), ICAR, New Delhi for financial assistance. The authors would like to thank Mr Dipak Roy, Progressive Fish Farmer for providing farm and hatchery facilities for carrying out the experiment.

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