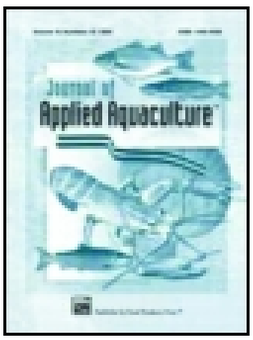

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Captive Breeding of a Gangetic Leaffish *Nandus nandus* (Hamilton-Buchanan) with Three Commercial GnRH Preparations

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Nandus nandus *is a threatened fish species that plays a significant role in the nutrition of India, especially in the Northeastern states. In the present study, induced spawning of a threatened freshwater fish* Nandus nandus *(Hamilton-Buchanan) was conducted using three commercially available synthetic GnRH preparations viz., wova- FH, ovaprim, and ovatide in different intensities. The brooder females were injected one time and left to spawn in the spawning hapa. It was found that at different dosages (0.1 ml, 0.2, and 0.3 ml/kg of body weight) hormone wova-FH and ovaprim could induce the fishes to spawn. No spawning was observed by females treated with ovatide and in control set. The spawning time, fertilization rate, hatching rate, and survival rate were quantified in each set of experiment. The egg output/gm female was higher with the dosage of 0.3 ml in comparison to 0.1 ml/kg and 0.2 ml/kg of body weight of ovaprim and wova-FH. The statistical analysis showed significant effect ($P < 0.05$) between hormonal doses with latency period, fertilization rate, incubation period, hatching percentage, and egg output. The present study suggests that wova –FH and ovaprim at 0.3 ml/kg body weight of fish are more effective in induction of spawning of N.* nandus.

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KEYWORDS Nandus nandus, *captive breeding, commercial GnRH, incubation, fertilization, hatching*

INTRODUCTION

Captive breeding and release of captive bred individuals into the wild are among the techniques used for conservation of threatened fishes. Other than carps, a large number of small indigenous fish species in India is distributed in the water bodies in the floodplains and used as food fish. We selected *Nandus nandus* (Hamilton-Buchanan), a Gangetic leaf fish under family Nandidae; it is one of the species of conservation and management interest in India. The cause of its decline in the wild is due to reduced abundance, habitat degradation, and overexploitation from natural waters. It has been listed as a threatened fish in India (Anonymous, 1993–1994). Locally called *bheda,* it falls under the category of food fish and also has potential aquarium value. They commonly occur in ditches and inundated fields and attain a length of 20 cm. They are carnivorous, preying upon small carps in paddy fields and ditches, and are very tenacious. The market value of the fish in India ranges from Rs. 100–150/kg. The species is an annual breeder and matures within a year and nowadays is considered to be one of the potential candidate species for freshwater aquaculture and captive breeding (Ponniah & Sarkar 2000; Ayyappan, Raizada, & Reddy 2001). However, research effort on this important species has been little. Review of literature reveals that no research has been devoted to the breeding and larval rearing of this species except Pal and colleagues (2003) from Bangladesh. However, some information is available on other biological and ecological aspects (John & Nair 1989; Saxena, Sahai, & Jain 1984; Golder & Chandra 1987; Sarkar & Bain 2007). Keeping those in mind, it is now most important to initiate research for conservation and aquaculture enhancement.

In recent years, several hormones—ovaprim (a combination of dopamine antagonist and GnRH), Ovopel (mammalian GnRH_a and metoclopramidee, the water soluble blocker of dopamine receptors), ovatide (a synthetic preparation of sGnRH and a dopamine antagonist) and wova-FH (synthetic gonadotropin releasing hormone)—have been found promising and used successfully as an ovulation-inducing agent in fishes (Nandeesha, Ramacharya, & Vergese 1991; Sharma & Singh 2002; Brzuska 2001; Pandey, Koteeswaran, & Singh 2002; Sarkar et al. 2005, 2006). However, no trials were undertaken with any of the hormones on this prioritized species, so trials with the different hormones seemed justified. The aim of the present study was to determine the efficiency of three synthetic hormones, ovaprim, wova- FH, and ovatide, in the stimulation of ovulation and to standardize the doses for conservation and aquaculture enhancement.

MATERIALS AND METHODS

The brood fishes of both male and female *N.nandus* (n = 30) were collected from the river Punarbhava, located in Maldah district, West Bengal, India, during 2003-2004. The fishes were collected using cast nets and dragnets with the help of local fishermen. Fishes were carried in aluminum *bundi* (60-l capacity) from the river site and kept in a plastic pool installed in the vehicle and transported to experimental farm. The brood fishes were maintained in earthen pond (area 0.05 ha, average depth 70-80 cm). The fishes were fed zooplankton and small-sized live prawns provided at 3% of body weight/day. No mortality was noticed during the stocking period. The length of the fish ranged from 40.6 m to 46.2 mm, and weight of the fish ranged from 17.1 g to 18.6 gm. Captive breeding experiments were conducted at Roy Fish Farm, located at Beldanga (24:57.1390N; 88:20.4770E and altitude 152 m), West Bengal.

In May 2003, both male and female were found to be gravid and easily distinguishable. Adult fish showed sexual dimorphism. The genital papilla was rather pointed and narrow with free oozing milt when slight pressure was applied on the abdomen. In the case of the female pectoral fin, it was smooth and genital papilla was found swollen and slight pink in color; the abdomen was bulging and soft in appearance. Males were identified by their reddish genital openings and the oozing milt that occurred when slight pressure was applied on the abdomen. Females are identified by their bulging abdomen, soft and distended belly with swollen pinkish vent. Among other features of sexual dimorphism, the pectoral fin of male becomes rough during breeding season. Females were larger in size comparison to males**.** The values of physicochemical parameters of spawning hapa were: air temperature 30°C ± 1.0; water temperature 28.5.0°C ± 2.2, pH 7.5 ± 0.2; dissolved oxygen 8.0 ± 1.3 ppm; free CO_2 2.3 ± 0.5 ppm; turbidity 3.5.0 ± 0.5cm; alkalinity 35.8.0 ± 5.0 ppm; and water hardness 60.6 ± 4.6.0 ppm. The methods followed for water quality analysis were as per the American Public Health Association (APHA), American Water Works Association (AWWA), and Water Pollution Control Federation (WPCF) (1998).

The brood stocks were collected from the earthen pond by repeated drag netting followed by dewatering, segregation and transferral into nylon hapa (1.5 × 2.5 × 3.0 m). The spawners were selected for hypophysation in the month of June–July 2004. Healthy and sexually mature brooders were selected. Free oozing males and ripe female were taken in the ratio of 2:1 respectively for breeding. Breeding experiments were commenced during 17.00–18.00 h. Immediately after administering the hormone, the breeding sets were released into the spawning hapa, provided with *Eichhornia crasipes* for hiding purposes. The experiments were conducted in separate nylon hapa. Injections were administered intramuscularly in the dorso-lateral region

of the body. A pair of brooders consisting of one female and two males was kept in each spawning hapa. A control set was maintained in a separate hapa where no hormonal injections were done. Synthetic gonadotropin releasing hormone analogue (SGnRH) 'wova-FH' (a preparation containing synthetic gonadotropin releasing hormone analogue, it is a product of Biostadt Agrisciences, Wockhardt Life Science Ltd., Mumbai, India, and is water-soluble), ovaprim (1 ml of ovaprim contains 20 μg Gn RH a(D-Arg6, Trp 7, Leu 8, Pro9, Net and 10 mg domperidone, Syndel, Lab. Ltd. Vancouver, Canada), and ovatide (Gonadrelin, Gn RH A, 20 mcg and domperidone 10 mg; a product of M/S Hemmo Pharma, Mumbai, India) was used as inducing agent. All females were injected intramuscularly while partial injection was given to male depending upon their maturity status. Three different doses of hormone (Table 1) were given to the females. In the evening the injection was administered and the fishes were transferred into the breeding hapa (11 × 3.5 × 2.7 feet). Water hyacinth collected from uncontaminated water bodies were cleaned in $KMnO_4$ and kept in breeding hapa for providing facilities so that eggs attached. The complete spawned females were released back into the brood stock pond after dip in potassium permanganate ($KMno_4$) solution to avoid infection. After spawning, effective fecundity of each female was determined by randomly taking a representative sample 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 petri dish and then fertilized eggs having intact nucleus were used for calculating fertilization percentage. The ova diameter was measured by keeping approximately 20 eggs in a row along the measuring scale under a dissecting microscope. The total length of the eggs was divided by the number of eggs to obtain the mean diameter of each egg. Different developmental stages were observed under a binocular microscope (Leica MJ 800). The one-day-old hatchlings were maintained in plastic troughs. Aeration was provided in plastic troughs and water was exchanged daily. We analyzed data using a computerized statistical software package, SPSS version 11.5. The traits taken in to consideration were the latency period, egg output per female, fertilization, and hatching rate. The significance of the effects on the investigating traits was checked by *F*-test. A probability level of 0.05 was utilized to account for the statistical difference between the means.

RESULTS

Brooders of *N. nandus* were found to be mature enough during early June 2004. Comparative results on the artificial spawning using three hormonal

TABLE 1 Results of the Induced Spawning Experiment (Mean ± SE) of *N. nandus* with Commercial GnRH Preparations and ANOVA's with Dose and Breeding Parameters

Actual Doses (ml/kg)	Size and Number of Females in Each Treatment (mm)	Av. Size of Male (g)	Latency Period (h)	Fertilization (%)	Egg Output/ gm Female	Hatching (%)	Incubation (h)	Remark
1. Ovaprim								
0.1	45.3 (n = 4)	18.6 ± 1.9	9.0 ± 2.3	48 ± 2.8	30.5 ± 10.0	45 ± 3.6	14	Partial spawning
0.2	46.2 (n = 4)	17.8 ± 1.5	8.5 ± 2.9	75 ± 3.0	44.3 ± 10.5	50 ± 4.0	13	Complete spawning
0.3	42.8 (n = 4)	18.2 ± 1.9	7.6 ± 3.4	85 ± 3.2	51.5 ± 12.5	57 ± 3.8	11	Complete spawning
p value	0.001*	0.014*	0.02*	0.004*	0.003*			
Actual Doses (ml/kg)	Size of female (g)	Av. size of male (g)	Latency period (h)	Fertilization (%)	Egg output/ gm	Hatching (%)	Incubation (h)	
2. Wova-FH								
0.1	40.6 (n = 4)	17.4 ± 2.4	8.5 ± 2.8	47 ± 3.2	28.5 ± 10.2	51 ± 4.1	14	Partial spawning
0.2	41.5 (n = 4)	18.0 ± 3.2	7.5 ± 3.0	70 ± 2.5	46.4 ± 11.5	80 ± 3.5	13	Complete spawning
0.3	42.6 (n = 4)	17.3 ± 2.0	6.5 ± 2.5	80 ± 2.8	55.0 ± 12.0	90 ± 4.0	11.5	Complete spawning
p value	0.005*	0.008*	0.03*	0.02*	0.001*			
3. Ovatide			No spawning					
0.1	41.5 (n = 4)	18.4 ± 2.4						
0.2	40.7 (n = 4)	17.0 ± 3.2						
0.3	43.1 (n = 4)	17.1 ± 2.0				No response		
4. Control	42.4 (n = 4)	17.4 ± 2.0				No response		

*Indicates level of significance.

preparations are presented in Table 1. We found response of inducing agents wova-FH and ovaprim in all intensity levels. However, there were differences in fertilization rate, latency period, egg output, and hatching rate in the present experiment. Of the total 12 females selected for induced breeding in three experimental sets with three synthetic hormones, eight responded positively and produced viable eggs. The females maintained immobile in the hapa throughout the inducing process, but shortly before ovulation, started erratic swimming repeatedly. After breeding, eight brooders survived. Females injected with ovatide did not responded to any of the doses administered. In control set, no breeding behavior was observed.

In our study, the brooders showed chasing behavior after 4 h to 5 h of injection of both wova-FH and ovaprim at the dose of 0.2 and 0.3 ml/kg of body weight. However, fishes administered at the dose 0.1 ml/kg body weight showed delayed breeding behavior (Table 1). Ovaprim-induced fish at 0.3 ml/kg of body weight showed higher latency period (7.6 h) as compared to wova-FH (6.5 h) induced spawners of *N. nandus*. The latency period was longer with lower doses (0.1 ml/kg) of ovaprim and wova-FH and ranged from 8.5–9.0 h. Each female was found to pair with a single male. At all times the more active and aggressive male paired with the female, and the other male was found passive and idle in the corner of the breeding hapa. Mating was preceded by short courtship. Male rubbed its body with female and released its milt and the eggs were fertilized externally. In all the spawning attempts, the male was more actively involved in the courtship and spawning. Parental care was not noticed in this species.

The fertilization rate estimated in the present experiment was varied from 55% to 85% and 55% to 80% in the three hormonal doses of 0.1, 0.2, and 0.3 ml/kg of body weight of ovaprim and wova-FH, respectively. The rate of fertilization was varied between hormones and hormonal doses. Maximum fertilization (85%) was recoded from ovaprim-induced experiments at 0.3 ml/kg, while in case of wova-FH it was 80%. Analysis of variance (ANOVA) was done between the hormone dosage with different breeding traits like latency period, incubation, fertilization rate, egg output, and hatching percentage (Table 1). The analysis showed significant variation between doses of both ovaprim and wova-FH with latency period, incubation period, fertilization rate, egg output, and hatching percentage.

Eggs are small in shape, bright pearl white in color, and semi-adhesive in nature. The fertilized eggs were demersal and small in size. Freshly fertilized eggs were 0.59 ± 0.05 mm in diameter, semi-adhesive, and attached to the root of *Eichhornia crasipes*. Fertilized eggs were hatched out between 11–14 h. Twitching movements were noticed after 11–12 h of spawning. After 13–14 h of spawning, fertilized eggs were hatched out. When comparing the hatching data, it appears that when using the same size dose of different hormones, hatching success varies depending on the hormorne used. In wova-FH it was 90 ± 4.0% as compared to 57 ± 3.8% with ovaprim.

The one-day-old larvae were maintained in nylon hapa and plastic troughs simultaneously and behavior was studied. Larvae moved very fast, and air bladder was prominently visible with regular fanning of pectorals fins. The survival of the one-day hatchlings varied from 50%–60% and showed schooling behavior. The two day old larvae of *N. nandus* were brownish in colour with light and dark band alternately. At this stage, larvae attained 2.6–2.9 mm length with a mean length of 2.7 mm and had a small yolk sac attached beneath the head region. At the early life stage of *N. nandus* (2–3 d), mouth clearly developed and started accepting external feed. The survivals of the hatchlings were varied from 60%–75%, up to 7 days. In the later phase, larvae showed sluggish behavior with tendency to aggregate in the bottom. After 2–3 d, mouth was slightly developed and they started feeding external feed. The yolk sac completely absorbed after the third or fourth day and head fully developed. After fifth day, post larvae developed, attaining a length of 7.2 mm.

DISCUSSION

While no scientific reports are available on artificial spawning of *N. nandus* in captivity by any of the inducing agents, there are numerous reports on carps and catfishes. The doses of Ovaprim selected for induced spawning of murrels (*Channa* spp.) ranged from 0.3–0.6 ml/kg of body weight (Haniffa 2000). Singh, Ram, and Singh (2002) reported a significant increase of ovulated eggs/fish in *Heteropneustes fossilis* injected at 0.2 ml/kg of body weight. However, no reports are available in standardizing the doses of wova-FH in most of the potential freshwater food fishes. The results obtained in the present experiment described here show distinctly higher hatching success and short latency period in wova-FH induced treatments. With both wova-FH and Ovaprim, the yield of viable eggs was satisfactory with no significant difference. Higher hatching rate in wova-FH-induced fishes indicate better egg quality. In experiments conducted with European catfish females treated with Ovaprim, 20% of fish yielded eggs of very poor quality [0%-20% live embryos after 24-h incubation of eggs (Brzuska & Adamek 1999)]. Sharma and Singh (2002) failed to breed *Cirrhinus mrigala* by inducing Ovaprim at 0.25–0.5 ml/kg body weight, while other carp *Labeo rohita* responded well with same dose.

The observations indicate that normal spawning of *N. nandus* occurred at the Ovaprim and wova-FH doses at 0.2 and 0.3 ml/kg of body weight, and the dose of the hormones affected the percentage of fertilization, egg output, and hatching rate, respectively, as shown in Table 1. No breeding activity was observed with any of the doses of Ovatide-induced experiments and in control set. Table 1 shows that latency period was considerably higher in Ovaprim-induced experiments as compared to wova-FH-induced

experiments. However, latency period was longer in lower doses (0.1 ml/kg) for both Ovaprim and wova-FH. Similar observation was reported by Habibi and colleagues (1989) in *Carassius auratus*. Longer latency period in low dose of synthetic hormone Ovatide was reported by Pandey, Koteeswaran, and Singh (2002). Nandeesha, Ramacharya, and Vergese (1991) reported latency period of 8-15 hr in Ovaprim-induced Indian major carps(*Catla catla, Labeo rohita, Cirrhinus mrigala*). The latency period of Ovaprim-induced air-breathing fishes is 18 h for *Channa punctatus* and *Heteropneustes fossilis* (Haniffa 2000). Pandey, Koteeswaran, and Singh (2002) reported varied interspawning period between 8-15 h in *H. fossilis* injected in the doses of 0.3 to 1.0 ml/kg of body weight of synthetic hormone Ovatide. According to Billard and colleagues (1984) and Peter, Sokolowska, and Nahorniak (1986), differences in dose requirement may be attributed to varied levels of dopamine activity in different species of fish. Haniffa (2000) observed differences in survival rate of the larvae within Human Chorionic Gonadotrophin-induced parent and Ovaprim-induced larvae. Undetermined environmental factors such as one that affected that ovulatory response of carps to LH-RH (Foster et al. 1984) could be regarded as a reason.

In the present observation, the average number of eggs/ml was 2,200 and 2,245 in Ovaprim and wova-FH, respectively. The mean egg diameter after hydration was 0.61 ± 0.05 and 0.60 ± 0.04, respectively for Ovaprim and wova-FH treated experiments, indicating not much variation in egg dimensions after hydration. Singh, Ram, and Singh (2002) reported significantly higher hydration of eggs of *H. fossilis* administered at the dose of 0.2 ml/kg than lower dose of 0.1 ml/kg body weight.

An important observation was made in the present experiment concerning the incubation period of fertilized eggs. It took almost same time duration for both wova-FH and Ovaprim. It is worth noting that statistical analysis between the two hormone dosages showed significant effect with latency period, fertilization rate, egg output, and hatching rate for all sets of experiments except control. The egg output/gm female was ranged from 30.5–51.5 and 28.5–55 for Ovaprim- and wova-FH-treated fishes, respectively. Interestingly, egg output/gm female induced at 0.3 ml/kg Ovaprim and wova-FH was more than other doses. However, egg output was less in both the hormones at 0.1 ml/kg.

In the present study, four females out of six injected with wova-FH responded, while no breeding activity was observed in control sets, indicating effect of inducing agent under captivity. The high rate of hatching in the present experiment may be attributed to the optimum range of physiochemical parameters of water (viz., pH, temperature, and dissolved oxygen). Ovulation response greatly depends upon weather conditions; cool rainy days were conductive for successful spawning in carps (Bhowmick et al. 1977). In our study, temperature of breeding hapa recorded in the experiments was 28.5°C ± 2.2 for water and air temperature was 30°C ± 1.0, indicating it

was quite favorable for breeding. There are reports of better spawning of carps between 29°C–31°C (Sharma & Singh 2002). They reported a high rate of hatching in Indian major carps with Ovaprim at lower dose (0.3–0.4 ml/kg).

The present study has potential implications in the context of biodiversity conservation and aquaculture enhancements. The objective of the present study was fulfilled, and maximum output (egg output, hatching rate) was observed in the dose of 0.3 ml/kg of body weight of both Ovaprim and wova-FH. Our results clearly demonstrate the possibility of using both the synthetic fish hormone Ovaprim and wova-FH for effective induced spawning and seed production of *N. nandus*. Since the breeding protocol does not require higher investment, it could be taken up by small and marginal farmers for seed production. However, more research is required in larval rearing of fish.

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