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Induced Spawning of Native Threatened Spotted Snakehead Fish *Channa punctatus* with Ovaprim

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Abstract

The spotted snakehead, *Channa punctatus*, is one of the native threatened fish species in India. In the present study the efficacy of Ovaprim in stimulating ovulation and spawning performance of this candidate fish was examined under captive condition. A total of 9 matured female and 18 male fishes with weights ranging from 63 to 80g, were randomly selected for three hormone dosages. Both male and female fishes in each hormone dosage were administered a dose of 0.2, 0.4, and 0.6 mL of Ovaprim/kg body weight (BW) respectively. Each breeding set consisted of two males and one female. The hormone-administered fishes were then placed into the concrete cement tanks for spawning. The breeding performance was assessed based on the number of eggs spawned, spawning response, percentage fertilization and percentage hatching. Partial spawning was observed with a dose of 0.2 ml Ovaprim/kg BW, while complete spawning was noticed in the medium dose (0.4 ml of Ovaprim/kg BW) and higher dose (0.6 ml of Ovaprim /kg BW) administered fishes. The highest number of eggs spawned ($p < 0.05$) was recorded when the females were injected with 0.4 ml of Ovaprim/kg body weight than those injected with

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other doses. The latency period and number of spawned eggs ranged from 24 -31 hrs and 2,164-6,538 respectively. The highest fertilization (97.6%) and hatching rates (96.3%) were also observed at the medium dose ($P < 0.05$). With regard to hatching rates, no significant difference was noticed between the medium and low doses of Ovaprim administered groups. From the present study, it is evidenced that the synthetic gonadotropin-releasing hormone with a dopamine antagonist at a dose of 0.4 ml /kg BW could be used as an appropriate spawning agent for successful breeding and seed production of *C. punctatus* under captive conditions.

Introduction

In aquaculture the most important constraint for large scale cultivation of several fish species is the non-availability of quality seed of uniform size, as well as free from diseases, parasites and pests at the time of stocking in culture ponds. These strict requirements are seldom fulfilled when the fingerlings are collected from the wild conditions for culture (Horvath 1997). Further, the wild collected broodstock are reared in captive conditions and may not receive appropriate environmental cues for gonad maturation and spawning hence these can cause reproductive development to be arrested in late vitellogenesis. For this reason, hormonal treatment has been attempted for stimulating gamete maturation and has been successfully used to spawn many commercially important fish species that exhibit arrested reproductive development (Zohar and Mylonas 2001).

It is well known that reproductive processes in fishes are controlled by endogenous biological rhythms as well as by environmental cues (Munro 1990). Endogenous control is mediated through actions of various hormones along the brain-hypothalamus- pituitary-gonadal axis. Under natural conditions environmental stimuli are detected and relayed to the brain, resulting in a release of hormones and neurotransmitters that regulate ovulation (Yaron 1995; Peter and Yu 1997). The most important reproductive hormone is gonadotropin-releasing hormone (GnRH) that regulates gonadotropic hormone, GtH (Peter and Yu 1997). Gonadotropin release in teleost fishes is also influenced by a gonadotropin-inhibiting factor (GnIF) from the hypothalamus. This factor has been identified as dopamine and demonstrated to have inhibitory activity on the release of GtH (Peter et al. 1988). Combinations of GnRH and dopamine antagonists have been used to induce ovulation in several cyprinids including *Carassius auratus* (Sokolowska et al. 1984), *Labeo rohita*, *Cirrhinus mrigala* (Halder et al. 1991), and *Cyprinus carpio* (Driori et al. 1994). The good results in induced ovulation in cyprinid fish species were also obtained after hormonal stimulation from synthetic analogue of gonadotropin releasing hormone (GnRH), frequently with strong dopamine antagonists (Yaron 1995 and Barth et al. 1997). The GnRH, and its super-active analogues have been reported in snakeheads (Haniffa et al. 2001, 2004; Marimuthu et al. 2001a, b). Such a treatment results in the release of the fish's own gonadotropin. Thus, it avoids the administration of exogenous gonadotropin, which might be immunogenic and is biologically species-specific (Bye et al. 1982). The use of exogenous hormones in the induction of spawning of fishes is well documented (Lam 1982), and different doses of hormones and sex steroids gave different results (Ayinla and Nwadukwe 1988;

Zonneveld et al. 1988; Salami et al. 1992). Among the several inducing agents used in fish breeding, salmon gonadotropin releasing hormone (sGnRH) or luteinising hormone releasing hormone (LHRH) analogues in combination with dopamine antagonists have been found to be an effective agent in fish breeding and seed production (Lin and Peter 1996). Some associated problems have been reported using exogenous hormones and endogenous hormones viz, weighing of such low quantity, preparation of these analogues and storage of this hormone in prepared solutions. Due to these difficulties, fish breeders and farmers are reluctant to use them in farm conditions. However, the commercially available synthetic ovulating agents in ready made form containing GnRH_a and dopamine antagonist such as Ovatide, Ovopel, Dagin and Aquaspawn are becoming very popular nowadays and found to be efficient and successful spawning agents in different fish species (Peter et al. 1988, Nandeeshha et al. 1990, Cheah and Lee 2000, Das 2004, Brzuska 1998).

The spotted snakehead, *Channa punctatus* (Bloch, 1793), locally known as spotted murrel, is one of the highly priced freshwater food fish species in India. It is distributed throughout the South East Asian countries. Over the last 10 years, its wild population has undergone a steady decline due to fishing, loss of habitat, introduction of alien species, disease, pollution, siltation, poisoning, dynamite and destructive fishing. These factors not only destroyed the breeding and feeding grounds but also caused havoc to the biodiversity of this important fishery. As a result, according to IUCN status (CAMP 1998), it has been listed among the 66 low-risk near-threatened fish species in India. Due to its hardiness and air breathing nature it has been identified as one of the potential cultivar species for aquaculture in derelict, swampy and oxygen depleted waterbodies. The fish is well known for its good taste, high protein content and fewer intramuscular spines, high nutritive value, recuperative and medicinal qualities, and is recommended as a diet during convalescence (Haniffa et al. 2004). However in India, the culture of snakeheads is still not common due to the lack of quality seed supply and knowledge on their breeding and feeding techniques. Snakeheads breed naturally during the southwest monsoon (June - September) and northeast monsoon (October-December) in flooded rivers, paddy fields, ponds and ditches in southern parts of India (Haniffa et al. 1996). The lack of monsoons also often limits the breeding and seed production. The inadequate supply of quality seed in appropriate time, dependency on collection of seeds from the wild while being unreliable, time consuming and uneconomical are the major constraints for large scale culture of this fish species. Hence to overcome these problems, induced spawning is thought to be the only alternative method for quality seed production/supply. Initially in 1976, Parameshwaran and Murugesan used carp pituitary glands for induced breeding in this species but due to inadequate supply and the cumbersome methods of collection and preservation of pituitary glands, their attempts did not meet the required outcomes. Recently successful spawning through a synthetic analogue of GnRH has been reported in several species of air breathing fishes such as *Clarias batrachus* (Basu et al. 2000), *H. fossilis* (Alok et al. 1993, 1994; Marimuthu et al. 2000), *C. striatus* (Haniffa et al. 1996, 2001; Marimuthu et al. 2001a; 2001b, 2007). Paucity of information on the induction of spawning and artificial propagation in *C. punctatus*, is due to difficulties in preparing synthetic hormone (Parameshwaran and Murugesan 1976; Marimuthu et al. 2001b). Therefore, the present study was conducted to investigate the efficacy of a synthetic GnRH, with a dopamine antagonist for the induction of ovulation and the initiation of spawning in

C. punctatus, and to determine the minimum effective dose of Ovaprim that could be used to spawn and produce seed of the candidate fish species under a controlled captive condition.

Materials and Methods

Brood fishes weighing from 63 to 80 g, were maintained in earthen ponds (3 X 3 X 1m) at the Centre for Aquaculture Research and Extension (CARE), Palayamkottai, Tamilnadu, India. The fishes were fed cleaned chicken viscera *ad libitum* daily for their normal growth and gonadal development. Mature male fish was identified by a slightly pointed genital papilla, and mature females by a swollen abdomen and a reddish, swollen vent (Haniffa et al. 1996). In addition, maturity of the female was confirmed by slightly pressing along the ventral side of the fish for the release of eggs. The eggs were collected by hand-stripping and immersed in a solution containing 70% acetic acid and 30% ethanol for clarification of the cytoplasm. About three minutes subsequent to immersion, the position of the oocyte nuclei was determined. Migration of the nucleus from the center of eggs to its periphery indicates the readiness of fish for breeding by hormonal stimulation. Only those females containing the highest percentage of mature oocytes having germinal vesicle in the center or initial stage of migration were selected for the hormonal treatment (Billard et al. 1984).

One day before the experiment, the required fishes were selected and transferred to cement tanks (3x1x1 m) of 1500 L capacity filled to a water level of 50 cm of de-chlorinated water. Each breeding set consisted of two males and one female (Haniffa et al. 1996). The selected fishes were randomly assigned to three treatment groups and were injected intramuscularly either with 0.2, 0.4, or 0.6 mL of Ovaprim/kg body weight (BW). For each dose, three breeding trials were made to find out the differences in breeding response by the fish and to observe the variation in latency period, the rate of fertilization, and percentage of hatching in each treatment groups.

The hormone-treated brood fishes were introduced into the cemented breeding tank (3 X 1 X 1 m). Aquatic macrophytes such as *Eichhornia crassipes* and *Hydrilla verticillata*, were introduced into the breeding tank to allow breeding in a protective condition. After spawning, eggs were collected from the breeding tank, and the number of eggs spawned (spawning fecundity) and rate of fertilization was calculated. Dead eggs were removed from the egg batches by siphoning. The water quality parameters recorded during the study were as follows, temperature, $29 \pm 1^\circ\text{C}$; dissolved oxygen, 5.8- 6.5mg/L; and pH 7.5 – 8.1. Two hours post-spawning, a total of 500 fertilized eggs from each breeding set were collected and incubated in glass aquaria to determine the incubation period and hatching rate. The fertilization and hatching rate were calculated as follows:

Fertilization rate (%) = number of fertilized eggs/total number of eggs counted X 100;

Hatching rate (%) = number of eggs hatched/ total number of eggs in a batch X 100.

Statistical analysis

The data obtained for mean number of eggs spawned, fertilization rate, latency period, and hatching rate from each hormone dose were analyzed using one-way analysis of variance (ANOVA) to find significant difference among the hormone doses and each treatment mean were analysed by Duncan's multiple range tests ($P = 0.05$) using SPSS package Version.11.

Results

The spawning performance of *C. punctatus* induced at different Ovaprim dosages are presented in table 1. The hormone-induced fishes showed breeding behavior after 3-5 hours of injection irrespective of dosages. Each female paired with a single male. At all times, the more active and aggressive male paired with the female while the other one was found to be passive and idle in the corner of the breeding tank. Mating was preceded by elaborate courtship. Spawning rituals commenced after 8 - 12 hours of the hormone injection and continued until the release of gametes. Spawning was noticed from 24.0 to 30.0 hours after the hormone administration. Minimum latency period was observed in the medium dose (0.4 ml Ovaprim/kg BW) but no significant difference ($P > 0.05$) was recognized between the medium and high hormone doses.

The number of eggs spawned in fishes administered with 0.2, 0.4, and 0.6 ml/kg BW of fishes was 2164 ± 168 , 6538 ± 154 and 4318 ± 214 respectively. Significantly, the highest ($P < 0.05$) spawning fecundity was obtained in fishes administered with a dose of 0.4 mL/kg BW, than those with 0.2 mL and 0.6 mL/kg BW doses of Ovaprim. The highest fertilization rate ($97.6 \pm 1.5\%$) and hatching rate ($96.3 \pm 1.5\%$) were observed in the medium dose of 0.4 mL/kg BW and were significantly higher than the fishes injected with 0.2 mL and 0.6 mL/kg BW (Table 1). Hatching rates were not statistically significant between the doses of 0.2 mL and 0.4 mL/kg BW administered fishes. The incubation period ranged from 24 to 28 hrs at the water temperature of $29 \pm 1.5^{\circ}\text{C}$. In the present study, no size differences of larvae were observed, despite the varied hatching performances among the hormone doses tested.

Table 1. Induced spawning of *Channa punctatus* using a synthetic hormone Ovaprim

Hormone dose mL/kg BW	Fish weight (g)	Latency period (hours)	Total spawning fecundity	Fertilization rate (%)	Hatching rate (%)
0.2	72.41 ± 13.58^a	29.10 ± 1.0^b	2164 ± 168^a	83.3 ± 2.0^a	91.3 ± 2.5^b
0.4	63.36 ± 11.40^a	24.5 ± 0.5^a	6538 ± 154^c	97.6 ± 1.5^b	96.3 ± 1.51^b
0.6	76.82 ± 18.50^a	26.0 ± 2.5^a	4318 ± 214^b	78.30 ± 6.6^a	84.3 ± 5^a

Values in each column followed the same superscript are not statistically different ($P > 0.05$).

Discussion

In the present study, a single intramuscular injection of synthetic hormone, Ovaprim resulted in successful spawning of *C. punctatus*. Table 2 summarizes the results of induced spawning and the success rates of different fish species using Ovaprim. To the best of our knowledge, this is the first successful attempt to use Ovaprim as a stimulating agent for induced spawning of this commercially-important native threatened fish species in a controlled captive condition. Successful spawning using Ovaprim and its analogues has also been reported in several fish species viz., singhi, *H. fossilis* (Vijayakumar et al. 1998); carps (Nandeeshha et al. 1990); striped snakehead, *C. striatus* (Haniffa et al. 1996 and Francis et al. 2000); catfish, *Neosilurus ater* (Cheah and Lee 2000); *Ompok bimaculatus* (Sridhar et al. 1998).

The latency period of *C. punctatus* ranged from 23.5 to 31.0 hrs at $29 \pm 1.5^{\circ}$ C in the three doses tested. The latency period was longer than those reported in *H. fossilis* administered with Ovaprim (Vijayakumar et al. 1998) but it was similar to those in *C. striatus* using Ovaprim (Haniffa et al. 1996) and in *C. striatus* using Ovatide (Marimuthu et al. 2007). In contrast, short latency periods using Ovaprim ranged between 5- 6 hrs in *O. bimaculatus* (Sridhar et al. 1998), 9 – 15 hrs in different carp species have been reported (Table 2). The latency period is related to water temperature and often decreases with an increase in temperature (Clemens and Sneed 1962).

In the present study significantly, the highest number of eggs spawned was observed in the medium dose (0.4 mL mL/kg BW of Ovaprim). Further, complete spawning was observed at the doses of 0.4 mL and 0.6 mL Ovaprim/kg BW whereas 0.2 mL/kg BW induced partial spawning. Complete spawning has been reported using Ovaprim in carps (Nandeeshha et al. 1990); in *Neosilurus ater* (Cheah and Lee 2000); in *Puntinus japonicus* (Azad and Shimray, 1991); in *Clarias batrachus* (Basu et al. 2000) and using Ovatide in papda catfish, (Mukherjee and Das 2001); and singhi (Marimuthu et al. 2000). No significant differences were noticed in the hatching rates between the doses tested (0.2 and 0.4 mL/kg BW). However the overall hatching rates of Ovaprim treated fish were high compared to those reported in the candidate fish using pituitary extract (Parameshwaran and Murugesan 1976). Similar results were also observed in other air breathing fishes, *C. striatus* and *H. fossilis* (Haniffa et al. 1996; Francis et al. 2000; Vijayakumar et al. 1998). In general, the response of fish to Ovaprim was found to be better, considering the spawning success, number of spawned eggs, and, percentages of fertilization and hatching. Further, the synthetic hormones like Ovatide, and Ovaapel, Ovaprim are known to act at the pituitary level leading to the secretion of the fish's own endogenous gonadotropins, while in the case of hypophysation technique and administration of HCG, exogenous gonadotropins are directly delivered into the body (Habibi et al. 1989; Goswami and Sharma 1997; Zairin et al. 1992). Endogenous gonadotropins appear to significantly enhance the secretion of the right type of steroids in appropriate quantities enabling complete maturity of ova for spawning. The results of the present investigation demonstrate the possibility of using the synthetic hormone, Ovaprim at a dose of 0.4 mL/kg BW for induced spawning and will be appropriate for mass seed production of *C. punctatus*. Low and higher doses are reported to affect the egg quality, lead to

partial spawning or reduced fertilization and hatching rate. Therefore, the findings that emerged from the present study would immensely be helpful for quality seed production in snakehead and other threatened freshwater fishes as well as for their conservation and rehabilitation. Further studies are required to examine the development and growth performances of larvae and fry produced by propagation with Ovaprim.

Table 2. Summary of induced breeding in different fish species administered with Ovaprim

Species	Ovaprim (ml/kg.bw)	Latency period (hr)	Spawning success	Fertilization rate (%)	Hatching rate (%)	References
<i>Acanthopagrus latus</i>	0.5	59	-	82.5	88.3	Leu and Chou (1996)
<i>Aristichthys nobilis</i>	0.6	8	Complete	98	100	Nandeesh et al. (1990)
<i>Catla catla</i>	0.4	9-10	Complete	80	90	
<i>Channa striatus</i>	0.4	21	-	93	-	Francis et al. (2000)
<i>Channa striatus</i>	0.5	24	-	-	-	Haniffa et al. (1996)
<i>Cirrhinus mrigala</i>	0.35	15	Complete	86	90	Nandeesh et al. (1990)
<i>Clarias batrachus</i>	2 - 2.5	16 - 17	Complete	60-65	-	Mohapatra et al. (2000)
<i>Clarias batrachus</i>	2.0	16 - 18	Complete	80	60	Basu et al. (2000)
<i>Ctenopharyngodon idella</i>	07	8	Complete	95	100	Nandeesh et al. (1990)
<i>Heteropneustes fossilis</i>	0.6	14 - 12	Complete	-	-	Alok et al. (1993)
<i>Heteropneustes fossilis</i>	0.4	11 - 12	Complete	80-84	-	Francis (1996)
<i>Heteropneustes fossilis</i>	0.5	10 - 24	-	-	80	Vijayakumar et al. (1998)
<i>Hypophthalmichthys molitrix</i>	0.5	12	-	-	-	Peter et al. (1988)
<i>Hypophthalmichthys molitrix</i>	0.7	15	Complete	53.3	-	Nandeesh et al. (1990)
<i>Labeo rohita</i>	0.35	10 - 13	Complete	76-98	90-95	Nandeesh et al. (1990)
<i>Neosilurus ater</i>	0.5	17 - 23	Complete	89.9	78.7	Cheah and Lee (2000)
<i>Ompok bimaculatus</i>	0.5	5-6	-	75	60	Sridhar et al. (1998)
<i>Puntius japonicus</i>	0.4	8	Complete	90	58.5	Azad and Shimray (1991)
<i>Tor putitora</i>	0.2	24	-	70-80	60-65	Pandey et al. (1998)

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