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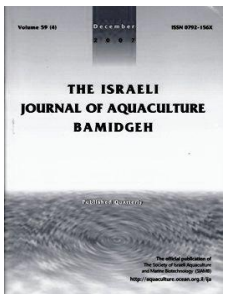

Breeding of Climbing Perch (*Anabas testudineus* Bloch, 1792) Induced with Ovatide

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Key words: climbing perch, *Anabas testudineus*, synthetic hormone, Ovatide, conservation, restoration

Abstract

Climbing perch (*Anabas testudineus*) is a much demanded fish in the northeastern part of India, yet the absence of a standardized method to induce breeding remains a major constraint in the development of aquaculture of this species. In this study, breeding was induced using 0.1, 0.2, or 0.3 ml/kg body weight of the synthetic hormone Ovatide and compared with fish injected with 30 mg carp pituitary extract (CPE) per kg body weight or 0.5 ml saline (control). Male and female brooders were injected once with an identical dose and left to spawn in tubs at a ratio of 2:1. No breeding occurred in the saline-injected control fish. There was partial spawning in the 0.1 and 0.2 Ovatide treatments and complete spawning in fish injected with 0.3 Ovatide. Spawning and number of eggs in fish injected with 0.3 Ovatide did not statistically differ from results in fish injected with CPE ($p \geq 0.05$). The present experiment suggests that Ovatide at 0.3 ml/kg body weight is optimal for seed production of climbing perch held in captivity and can be used for species restoration.

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Introduction

Anabas testudineus, locally called ukabi, is an economically important fish species in India. It inhabits fresh and brackish waters of the Indian subcontinent and southeast Asia. The natural food spectrum of *A. testudineus* juveniles is very wide; larvae and young fry feed on phytoplankton and zooplankton while large fry (and adults) feed on crustaceans, worms, mollusks, algae, soft higher plants, and organic debris (Potongkam, 1972). Adults feed mainly on insects (Ahyaudin, 1992). Reproduction occurs in low lying swamps, paddies, lakes, pools, small pits, ditches, streams, rivers, and irrigation canals. *Anabas testudineus* attains sexual maturity in the first year. It is categorized by the International Union for Conservation of Nature and Natural Resources (ICUN) as a vulnerable species. Artificial hypophysation of the climbing perch was first attempted by Khan and Mukhopadhyay (1972) while the effect of Wova-FH on breeding was observed by Sarkar et al. (2005). The aim of the present study was to determine the efficiency of the synthetic hormone Ovatide (containing sGnRH+dopamin) on the reproduction and breeding behavior of *A. testudineus* and to standardize the dose for seed production.

Materials and Methods

Induced breeding experiments were conducted at the farm of Krishi Vigyan Kendra, Thoubal, located at Wangbal. The farm is situated in the southern part of Manipur (93°45′-94°15′ E, 23°45′-24°45′ N). The experiment was conducted on ≥1-year-old adult *A. testudineus* (40-100 g). Forty gravid males and 20 females were collected from a grow-out pond in May 2008. Males are darker and have a more accentuated knife-edged anal fin than females. The pectoral fin of males becomes rough during the breeding season and the genital papilla is rather pointed and narrow with free-oozing milt when slight pressure is applied on the abdomen. The pectoral fin of females is smooth, the genital papilla are swollen and pinkish, and the abdomen is bulging and soft.

The fish were divided into five treatment groups. Three groups were injected with 0.1, 0.2, or 0.3 ml Ovatide per kg fish. Another group was injected with 30 mg crude pituitary extract (CPE) as a positive control and a fifth with 0.5 ml of 0.7% saline solution as a negative control (Table 1). All free-oozing males and ripe females were injected intramuscularly with the same dose. Immediately after injecting the hormone, the brooders were randomly distributed into five plastic tubs (56 cm diameter, 20 cm depth) at a male:female ratio of 2:1. Room temperature was 28±2°C, water temperature 27±1.5°C, and pH 7.5±0.5.

After spawning, the fecundity of each female was determined by random sampling of eggs in a 10-ml graded tube. The number of eggs in 1 ml were counted and multiplied by the total volume of released eggs. The fertilization rates of eggs were determined by randomly sampling approximately 100 eggs in a petri dish. Fertilized eggs with an intact nucleus were counted to determine the percent fertilization. The significance of the effects of Ovatide on egg output, fertilization, and hatching rates were calculated by analysis of variance (ANOVA) with the statistical software package SPSS version 12.0. The significance of the effects on the investigated traits was checked by F-test with a probability level of 0.05 considered statistically significant.

Results

Male brooders exhibited chasing behavior 8-10 h post injection of Ovatide. None of the negative control fish spawned, however, all groups injected with Ovatide spawned. All females paired with the more aggressive male. Active males released milt in concurrence with the release of eggs. Parental care was not observed.

Analysis of variance showed a significant effect of Ovatide dose on egg output and hatching rate but the fertilization rate did not significantly differ between treatments (Table 1). The spawning rate and number of eggs in fish treated with 30 mg/kg CPE or 0.3 ml/kg Ovatide did not statistically differ.

One-day-old hatchlings were held in hapas. Three days after hatching, their mouths were slightly developed and feeding on external feeds started. Yolk sacs were completely absorbed between days 3 and 4. Mixed plankton collected from natural water bodies was

fed to larvae from day 3, onwards. Fish were released into small earthen ponds on day 7. Fish reached an average of 21.5 mm after one month of rearing.

Table 1. Induced breeding (means±SEM; n = 4 females) in climbing perch (*Anabas testudineus*) injected with Ovatide, carp pituitary extract (CPE) as a positive control, or saline as a negative control. Doses were administered in equal amounts to males and females (40-100 g).

Treatment	*Spawning (%)*	*Egg production (no./g)*	*Fertilization (%)*	*Hatching (%)*	*Remark*
0.1 ml/kg Ovatide	43.2±14.5[a]	151.62±4.1[a]	90.3±6.5	48.7±3.9[a]	Partial spawning
0.2 ml/kg Ovatide	89.5±9.9[b]	404.41±10.3[b]	89.6±4.6	69.2±4.9[b]	Partial spawning
0.3 ml/kg Ovatide	100[c]	505.84±7.6[c]	90.2±7.1	92.3±6.1[c]	Complete spawning
30 mg CPE	100[c]	495.87±6.7[c]	91.3±5.1	83.5±3.8[d]	Complete spawning
0.5 ml saline (0.7%)	0	0	0	0	No spawning

Different subscripts indicate significant differences (p<0.05).
Spawning rate = 100(no. fish spawned/no. fish injected)
Egg production = no. eggs released/g body wt of female
Fertilization rate = 100(no. eggs with faint streak/no. eggs in sample)
Hatching rate = 100(no. hatched eggs/no. tail bud embryos)

Discussion

GnRH and sGnRHa successfully induce breeding in teleosts (Levavi-Sivan et al., 2004). Our observations indicate that climbing perch spawn normally with the commercially available hormone, Ovatide, that contains sGnRHa and dopamin. The dose of 0.3 ml/kg induced 100% ovulation and spawning and affected egg production and the hatching rate. Similar results were obtained in climbing perch using the synthetic hormone Wova-FH (Sarkar et al., 2005) and a single dose of 8-12 and 4.0 mg pituitary gland extract per kg body weight for females and males, respectively (Mazid and Kohinoor, 2003).

In the present observation, egg production at the dose of 0.3 ml/kg was significantly similar to that of the positive control group and significantly higher than in fish given a lower dose. This shows that 0.3 ml/kg Ovatide is sufficient to achieve ovulation with similar results as those obtained with CPE. Similar results were obtained in *Channa striatus* treated with 0.4 ml/kg Ovatide (Marimuthu et al., 2007), however, a higher dose of 1 ml/kg Ovatide was required to obtain complete spawning in *Clarias batrachus* (Sharma et al., 2010). When Ovaprim was used, a higher dose of 0.5 ml/kg was ineffective in inducing ovulation in *A. testudineus* (Pius, 2010).

The optimum water temperature for breeding *A. testudineus* under laboratory condition is 28±1ºC (Moitra et al., 1979). In the present study, the water temperature was 27±1.5ºC, quite favorable for breeding. The high hatching rate may be attributed to the optimum water temperature.

The objective of the present study was fulfilled. Ovatide administered at 0.3 ml/kg body weights produces the highest spawning rate, egg production, and hatching rate in *A. testudineus*. The positive response of both males and females to a single dose of Ovatide is significant for commercial seed production. This breeding protocol does not require a high investment, so it can be adopted by small farmers for seed production as well as be used for species restoration and conservation.

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