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Efficacy of exogenous hormone (GnRHa) for induced breeding of climbing perch *Anabas testudineus* (Bloch, 1792) and influence of operational sex ratio on spawning success

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ABSTRACT

The climbing perch, *Anabas testudineus*, is an air-breathing fish having great consumer preference as a food fish and is considered a prime candidate species for aquaculture. Spawning success is an important issue while using hormones for captive induced breeding. In the first experiment, a trial was conducted to assess the efficacy of a synthetic Gonadotropin Releasing Hormone analog (sGnRHa) on the spawning success of climbing perch. Female fish were administered six different doses each with a single intramuscular injection of sGnRHa hormone at 0.002 (T_{OD1}), 0.005 (T_{OD2}), 0.01 (T_{OD3}), 0.015 (T_{OD4}), 0.02 (T_{OD5}), 0.03 (T_{OD6}) μg/g body weight. Similarly, males were administered half of the hormone dose of females in all the respective treatment groups. The greatest ($P < 0.05$) relative fecundity (715.13 ± 15.0 eggs/g female body weight) and fertilization percentage rates (93.1 ± 8.0%) occurred when female fish were treated at the 0.015 μg/g body weight dose. There was a reduction in relative fecundity and hatching rate in female fish injected with the largest dose (1.5 μL/g body weight) of sGnRHa. A second experiment was conducted to assess the effect of a different male-female ratio on optimum spawning success in climbing perch. For this study a different female to male ratio (1:1, 1:2, 1:3 and 1:4) and male to female ratio (1:1, 1:2 and 1:3) were used. There were a greater ($P < 0.05$) relative fecundity (886.62 ± 17.9 eggs/g female body weight), fertilization (98 ± 6.7%) and hatching (99 ± 5.4%) rates with the female to male ratio of 1:2. This indicated that the hormone dose of 0.015 μg/g body weight and a female-male ratio of 1:2 are optimal for enhanced spawning success in the climbing perch.

1. Introduction

Anabas testudineus, also known as the climbing perch, is an air breathing fish which inhabits freshwater and can survive in water with lesser than typical environmental concentrations of saline. Climbing perch is found in swamps, lakes, canal, rice fields and streams and have omnivorous feeding habits (Zalina et al., 2012). This fish can be cultured in poor quality water bodies which are not suitable for culture of carp (Kumar et al., 2013). The climbing perch has great medicinal value as well as a desirable flavor in human diets (Kumar et al., 2012a). In South-East Asia this fish has attracted great attention as a promising candidate species for freshwater aquaculture (Zworykin, 2012). It is a preferred food fish for human diets with a greater market price than many other fish in India (Sarkar et al., 2005;

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Kumar et al., 2012b). Availability of an adequate quantity of seed has been a constraint for farming of the climbing perch. Breeding attempts by a few researchers using different available hormones such as gonadotropin releasing hormone (GnRH) (Morioka et al., 2009) and pituitary gland extracts (Moitra et al., 1987) have been conducted but there is no report on breeding attempts with climbing perch with use of the synthetic gonadotropin releasing hormone agonist (GONOPRO-FH^{TM,} – sGnRHa). Furthermore, an optimal hormone dose is necessary to stimulate release of gonadotropin so as to induce a successful ovulation. Any sub-or supra-optimal dose of GnRH can lead to unsuccessful spawning and sometimes even loss of brood fish.

The operational sex ratio (OSR) is the ratio of sexually receptive males to receptive females (Emlen and Oring, 1977). The OSR can have a great impact on mate competition and sex roles during breeding (Kavernemo and Ahnesjo, 1996). When the OSR becomes biased towards one of the sexes, competition for mates increase for the most limiting sex and mating opportunities for the gender that is not limited decreases (Emlen and Oring, 1977; Kavernemo and Ahnesjo, 1996). Many studies have shown that presence of a specific sex ratio for a particular animal can increase its breeding/spawning efficiency (Jirotkul, 1999; Barbaro et al., 1997; Meiri et al., 2002; Black and Black, 2013). The present study was, therefore, conducted to ascertain the optimum sGnRHa doses and OSR for greatest spawning success in the climbing perch, *A. testudineus*.

2. Materials and methods

2.1. Experimental animals

The present study was conducted in the Air Breathing Fish Breeding and Culture Unit of the ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA), Bhubaneswar, India. Brood fish for the study were taken from brood stock housed in outdoor concrete tanks based on the secondary sexual characteristics (i.e., round and bulged belly and reddish swollen genital opening in females and slender body with oozing milt in male fish). Female fish had an average body weight of 36.83 g, whereas male fish weighed 23.33 g. Fish were kept in 2000 L concrete tanks overnight for acclimatization with appropriate aeration and water recirculation. Breeding trials were conducted the following morning.

2.2. Experiment I: optimization of inducing hormone dose

One male and one female fish were randomly assigned to six experimental groups that were maintained in concrete tanks with a 1000 L capacity in duplicate, using a completely randomized design. Fish were maintained in the Air Breathing Fish Breeding and Culture Unit, Aquaculture Production and Environment Division of ICAR-CIFA, Bhubaneswar. Female fish were administered a single injection of sGnRHa (Trade name – GONOPRO-FH^{TM} manufactured by APC NUTRIENT PVT. LTD, Secunderabad, India and each ml of GONOPRO-FH^{TM} contains 20 μg sGnRHa) with doses of 0.002 (T_{OD1}), 0.005 (T_{OD2}), 0.01 (T_{OD3}), 0.015 (T_{OD4}), 0.02 (T_{OD5}) and 0.03 (T_{OD6}) μg/g body weight with a BD Ultra-Fine^{TM} 29 gauge 1 mL insulin syringe. Similarly, male fish were administered GONOPRO-FH^{TM} in half the dose amount of females in the respective treatment groups. All the hormone doses were diluted to 50 μL in saline solution (0.9%). Fish were injected intramuscularly at the base of pectoral fin and kept in the respective experimental breeding tanks which were covered with net to prevent the escape of the fish from the tanks and to also to provide dark environment during spawning.

With this study design, the Optimal Dose (OD) of sGnRHa (GONOPRO-FH^{TM}) for induction of spawning was determined.

2.3. Experiment 2: effect of different operational sex ratios (OSR) on spawning success

Experiment 2 was conducted to examine the effect of operational sex ratio (OSR) on spawning success. The average body weight of female fish was 41.01 g, whereas male fish was 24.33 g. The different treatment groups in duplicate were as follows, T_{OSR1} (one male and one female), T_{OSR2} (one male and two females), T_{OSR3} (one male and three females), T_{OSR4} (one male and four females), T_{OSR5} (two males and one female) and T_{OSR6} (three males and one female). All the female fish were injected intramuscularly with sGnRHa at 0.015 μg/g body weight, whereas male fish were injected with half the size of dose as female fish. After hormone injection, fish were maintained in net-covered breeding tanks for undisturbed spawning. With this study design, the Optimal Sex Ratio (OSR) was determined.

2.4. Latency period and spawning performance

The duration between hormone injection and first appearance of spawned eggs is termed as latency period. After complete spawning, all eggs were collected and transferred to separate incubation tanks for different treatment groups for hatching. Total fecundity was determined by counting the number of eggs in 1 mL and multiplied by the total volume of released eggs. Relative fecundity was calculated as total number of eggs/weight of female fish. Egg size was also measured under the microscope.

Fertilization rate (%) was determined by calculating the number of fertilized eggs/total number of eggs counted X 100 and hatching rate (%) was determined by the number of eggs that hatched/total number of fertilized eggs X 100.

2.5. Nursery rearing of larvae and survival

Twelve glass aquaria (25 L) were arranged in a wet laboratory in the Air Breathing Fish Breeding and Culture Unit, APED, ICAR-CIFA, Bhubaneswar, India. The larvae obtained from the six treatment groups of Experiment 1 were reared in glass aquaria in duplicate. Glass aquaria were filled with 10 L of water and each aquarium was stocked with 25 larvae that were 3 days post hatching (dph). Larvae were fed ad libitum with mixed zooplankton. Every day, before feeding fecal matter was siphoned. About 15% to 20% of water was replaced to keep optimum water quality content. Mortality