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OBSERVATIONS ON THE INDUCED BREEDING OF *LABEO BATA* (HAMILTON) WITH OVAPRIM AND OVATIDE AS INDUCING AGENTS WITH A NOTE TO ITS DEVELOPMENT

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Induced breeding of *Labeo bata* was conducted using synthetic hormones, Ovaprim in three dose combinations, *viz.*, 0.6 & 0.3 ml/kg, 0.5 & 0.2 ml/kg and 0.4 & 0.1 ml/kg body weight and Ovatide at 0.5 & 0.3 ml/kg, 0.4 & 0.2 ml/kg and 0.3 & 0.1 ml/kg for female and male, respectively. The latency period was found to be 7-8 hours. The egg output per female was highest at a dose combination of 0.5 ml/kg to female and 0.2 ml/kg to male with Ovaprim and at 0.4 ml/kg female and 0.2 ml/kg male with Ovatide. The number of eggs released by the females (40-45 g body weight) ranged 3500-4000. The highest fertilization and hatching rate recorded were 55.7±1.50% and 53.42±2.31%, respectively with Ovaprim at dose of 0.5 ml/kg to female and 0.2ml/kg males.

INTRODUCTION

Labeo bata (Hamilton), a minor carp, is distributed throughout the Eastern and North-eastern States of India except Nagaland and Mizoram. The species in recent years has been categorized as near threatened (Sarkar *et al.*, 2000). In North-eastern parts of the country, the species was available in large numbers in the rivers, canals and Loktak Lake of Manipur. While Loktak Lake was the natural breeding ground of the species, its distribution has been observed to be declining rapidly in Manipur. Due to its market preference, even at smaller size, the species offers a good scope for aquaculture. It is also a suitable species for culture in shallow water bodies and paddy-cum-fish culture. Availability of sufficient quantity of seed of the species, however, has been the major bottleneck for aquaculture development in Manipur. While the seed production of cultivable carps is mainly based on the induced breeding, in recent years synthetic hormones like Ovaprim and Ovatide are used extensively for induce spawning of different species (Borah *et al.*, 1999; Haniffa *et al.*, 2000; Pandey *et al.*, 2002). An attempt has been made in the present study to evaluate the efficacy of Ovaprim and Ovatide in induced spawning of *L. bata* in order to develop a suitable breeding protocol for this species in Manipur and help in the propagation of this species.

MATERIALS AND METHODS

The study on breeding and seed production of *L. bata* was carried out at the ICAR Manipur Centre Fish Farm located at Imphal (Lat. 24.44 N; Long. 93.58 E and Altitude 790 m), Manipur during the month of June, 2007. Brood-stocks were raised in small earthen ponds of 0.02 ha with average depth of 120-130 cm and stocked at the rate of 1500 kg/ha and fed with balanced feed, composed of fish meal 10%, groundnut oil cake 35%, soybean oilcake 20%, rice bran 24.8%, wheat flour 10%, trace mineral mixture 0.1% and vitamin mixture 0.1% at a rate of 5% body weight per day (Singh *et al.*, 2000) in two installments. At monthly intervals the brooders were sampled to assess their growth, health conditions as well as maturity status. The male and female brooders attained their maturity after 1 year of age at a weight range of 35 to 45 g.

The sexually matured brooders were segregated sex-wise during the month of March, 2007 depending upon their secondary sexual characteristics and stocked separately in the ponds. The gravid females could be distinguished by their bulging abdomen, soft ventral abdominal region, comparatively larger size and smooth pectoral fins.

In the month of June, 2007 the brood stocks were collected and the experiment was conducted with the female weighing 40-45 g and male 35-40 g. Ovaprim (Glaxo India Ltd.) and Ovatide (Hemmo Pharma, India) were used as inducing agents separately. Three sets of experiments were conducted at three different doses for both the inducing agents. While doses for Ovaprim were 0.6 & 0.3 ml/kg, 0.5 & 0.2 ml/kg and 0.4 & 0.1 ml/kg body weight for female and male respectively, the corresponding doses of Ovatide injected were 0.5 & 0.3 ml/kg, 0.4 & 0.2 ml/kg and 0.3 & 0.1 ml/kg (Table 1). A control was also maintained in both the cases without any hormone injection. Free oozing males and ripe female were selected in the ratio of 2:1 and were given intramuscular injection. Soon after administration of the hormone, the brooders were released into the portable FRP circular breeding pool for spawning. After spawning, fertilized eggs were transferred to circular hatching pool for hatching.

The total egg released by the female was determined through representative samples of egg in 10 ml. The fertilization rate of eggs was determined by sampling of approximately 100 eggs in a Petri dish. Fertilized eggs having intact nucleus were only considered for calculating percentage of fertilization. The ova diameter was measured from 20 eggs with the help of microscope. One-day old hatchlings were maintained in circular FRP tanks. Aeration was provided in the FRP tanks with daily water exchange. The water quality parameters of brood stock pond and breeding pond were analyzed as per APHA (1998). The physico-chemical parameters of brood stock pond were: water temperature 27±1.3°C, pH 7.9±0.2, dissolved oxygen 6.2±1.4 ppm and free CO_2 2.4±0.6

ppm, while the values of in breeding pond were: water temperature 27.3±1.5^{0}C, pH 7.6±0.23, dissolved oxygen 6.4±1.6 ppm and free CO_2 2.6±0.2 ppm.

Table 1. Results of the captive breeding experiments of *Labeo bata* by Ovaprim and Ovatide

Size of female (g)	Dosage of hormone to female (ml/kg)	Size of male (g)	Dosage of hormone to male (ml/kg)	Latency period (h)	Egg output/ female (40-45 g)	Fertilization (%)	Hatching (%)
Ovaprim							
43±2.32	0.4	38±2.21	0.1	13	1,200	25.3±1.17	22.10±2.13
42±1.26	0.5	37±1.25	0.2	7	4,000	55.7±1.50	53. 42±2.31
44±1.21	0.6	39±1.00	0.3	8	3,400	45.3±2.21	42.90±2.25
41±1.00	Control	36±1.00	Control	-	No breeding	Nil	Nil
Ovatide							
42±2.52	0.3	36±2.21	0.1	14	1,100	22.5±1.17	20.15±2.14
44±1.28	0.4	38±1.25	0.2	7	3,700	52.3±1.50	51. 48±2.11
41±1.24	0.5	37±1.00	0.3	8	3,200	43.7±2.21	40.70±2.35
43±1.20	Control	36±1.00	Control	-	No breeding	Nil	Nil

RESULTS AND DISCUSSION

Varied degrees of response to the inducing agents were observed at different doses and the differences in fertilization, latency period, egg output and hatching rates observed in the present experiment is presented in Table 1. The female injected with Ovaprim at 0.4 ml/kg and Ovatide at 0.3 ml/kg did not respond, though they showed breeding behaviour after 7-8 hrs of injection. In the control set as expected no breeding behaviour was observed. Brooders showed chasing behaviour after 3-4 hrs of injection of Ovaprim and Ovatide at 0.6 and 0.5 ml/kg body weight and 0.5 and 0.4 ml/kg of body weight respectively. Spawning took place within 7-8 hours after injection at 27-28^{o}C.

The fertilized eggs were spherical, translucent and demersal measuring 3.5±0.5 mm in diameter and non-adhesive. Unfertilized eggs were pale and opaque. The fertilization rates estimated in the present experiment were 25.3, 55.7 and 45.3% at the doses of 0.3, 0.5 and 0.6 ml of Ovaprim per kg body weight of female respectively. In case of Ovatide, the estimated fertilization rates were 22.5, 52.3 and 43.7% at 0.3, 0.4 and 0.5 ml/kg respectively. In the present study, number of eggs released by the female (40-45 g) ranged from 3500 to 4000.

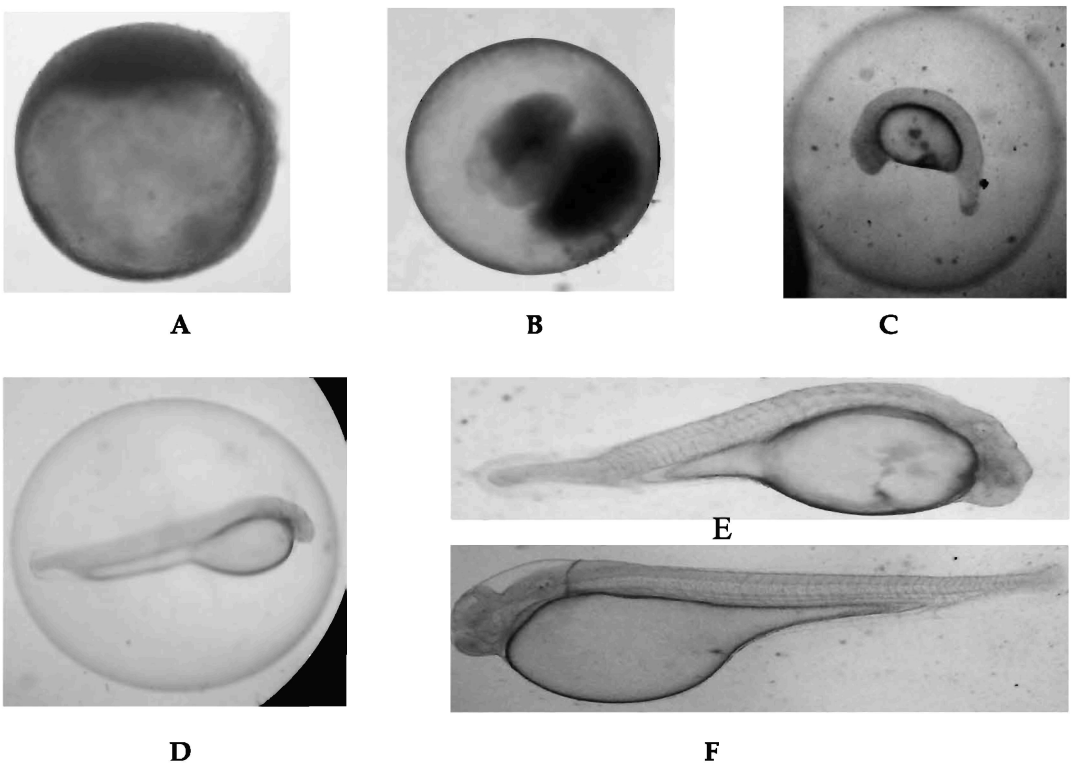

Fig. 1. *Labeo bata* embryo development at 27 ^{0}C : (A) 2h post fertilization (pf); (B) 4h pf; (C) 10h pf embryo prior to hatching; (D) 13h pf embryo prior to hatching; (E) Just hatched out larva (3.8 ± 0.2 mm); (F) larva of *Bata* 4h post-hatch (4.2 ± 0.3 mm)

The fertilized eggs underwent development and the hatching of larvae took place between 14-18 hours after ovulation at 27-28^{o}C. The freshly hatched larvae measured 3.8±0.2 mm in length and 1.7±0.2 mg in weight. After 2-3 days, mouth was slightly developed and they started feeding external feed after 72 hours. The yolk sac was found to be fully absorbed on 3 rd day and hatchlings grew to 7.6±0.4 mm in length and 3.7±0.3 mg in weight.

The dosage of synthetic hormones Ovaprim and Ovatide in tropical fishes has been experimented by several workers. Inducing agent Ovaprim at a dose of 0.3-0.5 ml/kg body weight of fish was found to be effective in bringing out ovulation in *Puntius sarana* (Borah *et al.*, 1999). Pandey *et al.* (2002) was successful in breeding of Indian major carps using Ovatide at 0.4 ml/kg to female and 0.1 ml/kg to male. Higher latency period in Ovaprim and Ovatide at the dose of 0.4 and 0.3 ml/kg female indicates difference in the mode of action of the hormone. Longer latency period in low dose of Ovatide was

reported by Pandey *et al.* (2002). According to Billiard *et al.* (1984) and Peter *et al.* (1986), the differences in dose requirement may be attributed to varied level of dopamine activity in different fish species.

The type of hormones and the doses found to affect the level of fertilization, egg output, hatching rate and spawn production. The present observations indicated that optimum doses for normal spawning of *L. bata* with Ovaprim to be 0.5 and 0.2 ml/kg of body weight to female and male respectively and with Ovatide 0.4 and 0.2 ml/kg. While present study was limited to only a few trials, extensive studies may be necessary to optimize the dose and to assess the efficiency of two different hormones. However, considering the present seed demand of *L. bata* in Manipur, both for aquaculture and also conservation, the present information may be effectively utilized for mass-scale seed production.

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