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Comparative efficacy of ovaprim and ovatide for induced breeding of freshwater dwarf gourami (*Trichogaster lalius*)

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Abstract
An experiment on induced breeding of dwarf gourami *Trichogaster lalius* (Hamilton, 1822) using ovaprim and ovatide hormone was carried out. This study consists of four treatments each with three replications. The objective of the experiment was to find out the effective dose of ovaprim and ovatide for induced breeding. A total of 80 fish individuals were selected for induced breeding, out of which 48 were males and 32 were females. Brood fish were kept in the ratio of 1:1, 2:1 and 3:1 for breeding purpose. Female brood fish were injected at the rate of 0.4, 0.6, 0.8 and 1 ml ovaprim and ovatide/kg body weight while the males were injected with 0.2, .4, 0.6 and 0.8 ml ovaprim and ovatide/kg body weight respectively in T_1, T_2, T_3 and T_4 at the same time. The results indicated variation in fertilization rate, latency period, egg output and hatching rate in response to different treatments. Spawning was occurred between 20-24 hrs of injection in all the experiments at 26.33±0.88 °C water temperature. Among all the experimental trials, the highest fertilization rate was observed in T_3 (96.15±0.60) of E2 and the highest hatching rate was observed in T_3 (92.49±1.00) of E2.

Keywords: Dwarf gourami, induced breeding, ovaprim, ovatide

Introduction
The dwarf gourami, *Trichogaster lalius* (Hamilton, 1822) [8], is well-known in southern Asia, including Bangladesh, Nepal, Vietnam, India, Sri Lanka, Thailand, Malaysia, Pakistan, and a few other continents such as the United States, Australia, and Columbia (Zuanon *et al.*, 2013; Darshan *et al.*, 2019) [7, 9]. It was previously categorized as *Colisa lalia*, and in Bangladesh it is known as Lal Khailsa, Boicha, and Ranga Khailsa. It is an air-breathing fish that lives in both well-oxygenated and hypoxic water bodies, including ponds, rivers, swamps, marshes, pools, beels, flood plains, canals, haors and rice fields (Rahman, 2005; IUCN, 2015) [2, 4]. It is an omnivore, feeding on both zooplankton and vegetable particles from aquatic vegetation (Goodwin, 2003) [10]. *T. lalius* is regarded as a small indigenous target fish by artisanal fishermen who use various traditional fishing equipment such as drag nets, traps, push nets, hook and line, and lift nets (Goodwin, 2003; Roy *et al.*, 2020) [5, 10]. It is in high demand among low-income individuals as a source of animal protein. Furthermore, it is well known and used as a peaceful ornamental fish globally (Talwar & Jhingran, 2001; Froese & Pauly, 2018) [1, 6]. As a result, aquaculturists have devised captive breeding programs. On the other side, wild *T. lalius* is facing numerous environmental stresses like as habitat loss and drying out of shallow water bodies. Despite being a healthy fish, it is classified as a 'least concern' species in India and around the world in terms of conservation.

Implementing induced breeding techniques could potentially be used to examine the conservation of this fish species in both natural and cultural contexts, lowering the risk of extinction. Before beginning induced breeding, it is vital to evaluate the breeding biology and physicochemical water factors, breeding season, breeding behavior, and sexual characteristics (Paul *et al.*, 2021) [3]. The current study attempted to assess the efficacy of Ovaprim and Ovatide in inducing spawning of *Trichogaster lalius* in order to create an appropriate breeding regimen for this species in Madhya Pradesh and aid in its dissemination.

Materials and Methods

Collection and rearing of broodstock

The samples of *Trichogaster lalius* (n=80) were collected from different sampling stations from Narmada River at Narmadapuram (Hoshangabad), Madhya Pradesh and the specimens were kept in aquaria (120 cm × 40 cm × 40 cm) tanks for acclimatization. The aquarium was provided with a constant aerator. Fine sand with small-sized stone covers the bottoms with a thickness of 3 cm and thereby filling it with water to a depth of 18 cm. The provisions of some hideouts are also deliberately constructed in the aquaria for providing the fish enough space for hiding and avoid possible fight among them. The different aquatic plants such as Eichhornia crassipes, Ceratophyllum demersum, Sagitlaria guayayensis and Ludwigia repens were also grown to imitate the natural environment. The fishes were fed with live and formulated feed containing all necessary ingredients at 5% of total body weight at regular basis (in the morning and evening). The water parameters such as pH, water temperature, dissolved oxygen (DO), free carbon dioxide and hardness were regularly monitored (APHA, 2012) [11].

Brood fish selection

The healthy and mature male and female brood fish were chosen based on secondary sexual traits such as size, color, and movement of the fish, as well as swelling abdomen and genital openings. Only healthy fish were chosen for induced breeding.

Aquarium set up

Breeding was place in rectangular (120cm × 40 cm × 40 cm) aquaria and round fiber tanks. The aquaria and tanks were supported by sand, gravel, and hideouts. A lid was utilized to keep the fish from diving out of the tank. Filters were installed at the inlet and outflow, as well as fine-meshed nets. Aside from them, water hyacinths were planted to create a natural environment and to hold the sticky eggs.

Experimental design

For induced breeding of *Trichogaster lalius*, firstly broods were collected. The males and females were separated and placed in the tanks and aquaria in three sets (a) 1:1 ratio of males and females; (b) 2:1 ratio of males and females and (c) 3:1 ratio of males and females. Four doses of ovaprim and ovatide hormone treatment (T_1, T_2, T_3, and T_4) with three replications of each were used (Table 1).

Hormone administration

The fishes were caught very carefully and the hormones were administered near the base of the dorsal and pectoral fin at an angle of 450 with the body. The synthetic hormone *viz*., Ovaprim (Glaxo India Ltd.) and Ovatide (Hemmo Pharma, India) were used as inducing agents separately. The amount of hormones was calculated beforehand according to the body weight of the broods. After administration of hormones, the fishes were kept at the sex ratio of 1:1, 1:2 and 1:3 in a separate aquarium, which was equipped with a continuous air and water flow.

Table 1: Doses of hormones applied to both the sexes of *Trichogaster lalius* brood

Treatments	Ovaprim Hormonal doses (ml/kg)		Ovatide Hormonal doses (ml/kg)	
	Male	Female	Male	Female
(Control)	No inducement	No inducement	No inducement	No inducement
Tank T1	0.2	0.4	0.2	0.4
Tank T2	0.4	0.6	0.4	0.6
Tank T3	0.6	0.8	0.6	0.8
Tank T4	0.8	1.0	0.8	1.0

Breeding performance

Trichogaster lalius eggs were found connected to the roots of water hyacinths. The fertilized eggs were translucent with an undamaged nucleus, whereas the unfertilized ones turned dark brownish. After being examined under a magnifying glass, the eggs were separated and numbered using a soft thin brush. The effective fecundity of each female after spawning was assessed using a random sampling of released eggs. The total number of eggs from selected samples was counted and multiplied by the total number of eggs released. The fertilization rate was also measured according to Behera *et al.* (2010) [12].

% Fertilization: No. of fertilized eggs/Total no. of fertilized eggs x 100

The fertilized eggs were then housed separately under close and constant supervision. The varied developmental diameters and stages of eggs were studied using a Leica DM 750 microscope and documented with images at various stages. The hatchlings were kept in circular fiberglass reinforced plastic (FRP) containers. The hatching rate was measured visually and computed using the following formula:

Hatching rate

No. of hatching / Total no. of fertilized eggs x 100

Results

The results were varied significantly with the different hormonal treatments and different sex ratio of male and female in the experiment. Besides this, the variation also depends on the brood size, maturation, season of inducing and aquarium condition. Total 80 fish species were selected for induced breeding out of which 48 were males and 32 were females. Fertilization rate, latency period, egg production and hatching rate in various treatments and sex ratios have been summarized in (Table 2, 3 and 4). No spawning took place in control groups.

Breeding behavior: After hormonal administration, the brooders showed varied mating behaviour in all the treatments (except control). Each female was paired with male, and the mating was preceded by courtship behaviour. Brooders showed breeding behaviour after 10- 14 of injection in all the doses. Latency period varied significantly in different hormonal doses and in different experimental setup. Spawning took place between 20h to 24h of injection in different experiments as shown in the table.

Fertilization rate: The fertilization rate in Experiment-1 (M: F=1:1) of Ovaprim doses was 72.54±2.54 in T3 and no fertilization took place in all the other doses of Ovaprim in

Experiment-1. In case of Ovatide, the estimated fertilization rates were 76.55±2.47 in T_3 and 72.24±1.57 in T_4 and no fertilization took place in control, T_1 and T^2.

In Experiment-2 (M: F=2:1) of Ovaprim doses significantly higher fertilization rate was observed in T_3 (91.12±1.86) and the lowest was in T_1 (83.21±1.65) and no fertilization took place in control. While as, in case of Ovatide the higher fertilization rate was observed in T3 (95.24±1.33) and the lowest was in T_1 (85.45±1.95) and no fertilization took place in control.

In Experiment-3 (M: F=3:1) of Ovaprim doses fertilization was observed only in T_2, T_3 and T_4 where in T_2 fertilization rate was highest (72.24±2.47). The fertilization rate of Ovatide doses was observed in all Treatments except in control, where in T3 fertilization rate was highest (79.36±2.42).

The highest fertilization rate of Ovaprim doses was found in T_3 (91.12±1.86) of Experiment-2 in all our experimental calculation. In case of Ovatide, the highest fertilization rate was also found in T_3 (95.24±1.33) of Experiment-2 in all our experimental calculation.

Table 2: (Experiment-1): Captive breeding experiment of *Trichogaster lalius* (M: F=1:1)

Size of female		Dosage of hormone to female (ml/kg body weight)	Size of males		Dosage of hormone to male (ml/kg body weight)	Latency	Fertilization rate (%)	Hatching rate (%)
W (gm)	**L (cm)**	**F**	**W (gm)**	**L (cm)**	**M**			
Ovaprim								
14.55±0.41	16.35±0.74	Control	9.54±0.42	13.48±0.62	Control	--	--	--
13.15±0.75	15.25±0.46	0.4	8.47±0.28	13.14±0.61	0.2	--	--	--
12.56±0.86	14.85±0.91	0.6	7.58±0.18	11.29±0.69	0.4	--	--	--
14.33±0.68	15.74±0.98	0.8	7.43±0.35	12.81±0.38	0.6	24	72.54±2.54	76.11±4.21
13.25±0.54	15.19±0.63	1.0	8.68±0.24	13.16±0.52	0.8	--	--	--
Ovatide								
13.15±0.38	14.46±0.71	Control	7.95±0.54	11.21±0.47	Control	--	--	--
14.54±0.45	16.65±0.24	0.4	9.11±0.52	12.15±0.68	0.2	--	--	--
13.47±.85	15.75±0.69	0.6	8.21±0.23	11.22±0.16	0.4	--	--	--
12.16±0.55	13.16±0.48	0.8	7.12±0.41	10.92±0.74	0.6	24	76.55±2.47	78.12±3.84
13.21±0.75	13.89±0.42	1.0	9.21±0.24	12.57±0.52	0.8	24	72.24±1.57	74.16±2.46

Table 3: (Experiment-2): Captive breeding experiment of *Trichogaster lalius* (M: F=2:1)

Size of female		Dosage of hormone to female (ml/kg body weight)	Size of males		Dosage of hormone to male (ml/kg body weight)	Latency	Fertilization rate (%)	Hatching rate (%)
W (gm)	L (cm)	F	W (gm)	L (cm)	M			
Ovaprim								
13.24±0.54	15.5±0.11	Control	9.65±0.54	13.41±0.24	Control	--	--	--
12.85±.84	13.95±0.64	0.4	7.87±0.22	12.55±0.62	0.2	24	83.21±1.65	84.62±2.54
11.57±0.69	14.21±0.55	0.6	7.82±0.49	12.18±0.34	0.4	22	87.54±3.25	85.47±2.58
13.12±0.82	14.32±0.47	0.8	7.15±0.23	12.69±0.74	0.6	22	91.12±1.86	90.10±2.36
13.16±0.45	12.92±0.54	1.0	8.27±0.11	13.25±0.21	0.8	20	90.22±2.54	87.43±4.11
Ovatide								
12.40±0.66	13.91±0.39	Control	8.95±0.29	12.71±0.48	Control	--	--	--
13.14±0.87	15.21±0.22	0.4	7.54±0.34	11.90±0.85	0.2	22	85.45±1.95	88.62±2.54
13.27±0.85	13.12±0.31	0.6	6.12±0.14	11.73±0.75	0.4	22	89.21±2.71	87.22±2.12
13.85±0.57	14.89±0.47	0.8	7.73±0.38	12.43±0.52	0.6	20	95.24±1.33	92.27±2.11
11.29±0.78	13.84±0.26	1.0	7.92±0.54	11.90±0.11	0.8	18	93.42±1.55	89.56±3.24

Table 4: (Experiment-3): Captive breeding experiment of *Trichogaster lalius* (M: F=3:1)

Size of female		Dosage of hormone to female (ml/kg body weight)	Size of males		Dosage of hormone to male (ml/kg body weight)	Latency	Fertilization rate (%)	Hatching rate (%)
W (gm)	**L (cm)**	**F**	**W (gm)**	**L (cm)**	**M**			
Ovaprim								
16.45±0.65	17.25±0.35	Control	9.64±0.24	12.55±0.41	Control	--	--	--
14.51±0.21	15.69±0.28	0.4	9.54±0.85	11.95±0.21	0.2	--	--	--
14.19±0.38	15.26±0.37	0.6	8.26±0.57	11.76±0.38	0.4	24	72.24±2.47	75.28±2.63
13.41±0.58	14.91±0.24	0.8	8.23±0.32	12.14±0.24	0.6	22	71.57±2.81	81.34±1.76
13.97±0.12	14.94±0.68	1.0	8.75±0.42	12.46±0.25	0.8	22	70.27±1.56	84.24±2.79
Ovatide								
15.16±0.24	16.94±0.35	Control	8.47±0.32	12.42±0.24	Control	--	--	--
13.54±0.67	15.17±0.65	0.4	9.14±0.22	12.47±0.37	0.2	--	71.52±1.25	75.14±1.69
13.75±0.17	14.71±0.68	0.6	7.97±0.24	11.24±0.64	0.4	22	78.21±2.17	79.37±2.81
13.52±0.11	14.87±0.27	0.8	8.16±0.37	12.13±0.49	0.6	22	79.36±2.42	85.45±1.95
13.16±0.28	14.82±0.68	1.0	7.95±0.21	11.92±0.34	0.8	20	78.91±1.94	88.28±2.73

Hatching rate

In case of Ovaprim doses, a contorted movement of the embryos was distinguished within 12-16 h of spawning and hatching was observed within 20-22h of fertilization. Calculated hatching rate was significantly higher in Experiment-2 compared to Experiment-1 and Experiment-3 and the maximum hatching rate was found in T3 (90.10±2.36). The observation suggested that the highest fertilization and hatching rate was observed in the experiment where the male and female ratio is M: F= 2:1. Similarly in Ovatide doses, a contorted movement of the embryos was distinguished within 12-14 h of spawning and hatching was observed within 18-20h of fertilization. Calculated hatching rate was significantly higher in Experiment-2 compared to Experiment-1 and Experiment-3 and the maximum hatching rate was found in T_3 (95.24±1.33).

Water parameters: Different water parameters such as pH, water temperature, Free CO2, DO, total alkalinity etc. in experimental tanks and aquarium under different treatments of *Trichogaster lalius* were monitored and are presented in Table-5.

Table 5: Range of certain water parameters in the experimental tanks and aquariums

Parameters	Breeding and hatching tanks
pH	7.26±0.8
Water Temperature (ºC)	25.40±0.57
Free CO2 (mg/l)	4.87±0.14
DO (mg/l)	8.13±0.28
Total alkalinity (mg/l)	57.64±0.39
Total Hardness (mg/l)	56.49±0.64
Chloride (mg/l)	9.21±0.37

Conclusion

Captive breeding is an effective approach for saving many species from extinction while also increasing seed output through induced breeding. It was expected that providing natural environmental conditions in a restricted aquarium would encourage spawning. Drawf Gourami. *Trichogaster lalius* is a potential ornamental fish merchant species in eastern India. Breeding of this species in the wild is gradually declining due to habitat loss, environmental degradation, and other factors that limit natural seed collection. Management and breeding in controlled environments are becoming increasingly vital for the species' conservation and culture. The present observation largely corroborated the notion, as the fish developed successfully under confinement circumstances.

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