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INDUCED BREEDING AND EMBRYONIC DEVELOPMENT OF ROSY BARB, *PUNTIUS CONCHONIUS* (HAMILTON, 1822) USING SYNTHETIC HORMONES UNDER CAPTIVE CONDITION

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ABSTRACT : *Puntius conchonius* is a potential candidate species in aquariculture. Recently, catch of this species has been declined due to overfishing and various ecological changes. The present experiment was conducted to breed Rosy barb, *Puntius conchonius* (Hamilton, 1822) in captivity through intra-peritonial administration of GnRH based gonadotropic signaling molecular analogues WOVA-FH and ovatide. Spawning success rate varied with the hormone and rate of inducement. Ovatide at a dose of 0.3 mL kg^{-1} for female and 0.1 mL kg^{-1} for male was found effective and resulted 500-600 egg production with 76.0±5.12% fertilization and 82.25±1.49% hatching. Dose of hormone apparently affected the latency period (6-8hrs), egg output (300-600), fertilization (43-79%) and hatching rate (68-84%). Administering an over-dose of the inducing agents showed abnormal behaviour while under-dosing caused no inducement in animals. Again the results verified that morula, blastula, gastrula, bud formation and hatching of these species ended at 1:45-, 2:45-, 5:30-, 11:00- and 26:00 hrs post-spawning, respectively. The protocol and chronological development data will be helpful in development of aquariculture by small breeders and conservation of the species.

Key words : Conservation, *Puntius conchonius*, embryonic development, induced spawning.

INTRODUCTION

Globally, there is a steady increase in the ornamental fish trade (14%/anum) due to enhanced popularity (Dey, 2016). The ornamental fish is wealth generated source and hence have gained great deal of attraction with remarkable increase of demand in recent years. However, the aquatic habitats especially in the developing countries have witnessed rapid changes in their environment that has caused greater habitat degradation during past few decades (Geist and Hawkins, 2016). Like in other parts of the world, the fish habitats of our part of the globe also degraded as a consequence of natural and ever increasing human interferences (Umesh *et al*, 2012 and Stevens *et al*, 2017). The development of this segment of the aquaculture depends on availability of seed either in nature or in hatchery.

The rosy barb, *Puntius conchonius* (Hamilton, 1822) is a member of the family Cyprinidae and native to rivers and fast flowing streams of India, Afghanistan, Pakistan, Nepal and Bangladesh. The fish is very popular throughout the world as an efficient, peaceful and attractive ornamental fish and also becoming a potential experimental fish model for biological and biotechnological research due to its short generation (Kirankumar *et al*, 2003). In recent, it has been widely used in ecotoxicological studies (Gill *et al*, 1991; Adam *et al*, 1995 and Kirankumar *et al*, 2003).

Induced spawning in fish, employing hypophysation as a tool, was first developed by the Brazilians (Von Ihering, 1937). In many cases, much better results in controlled breeding were obtained when pure or synthetic forms of hormones were used for reproductive stimulation (Motilan *et al*, 2014). Therefore, the aim of present study was to standardizeminimum effective dosage for induced breeding and chronological embryonic development of *P. conchonius*.

MATERIALS AND METHODS

Broodstock collection and maintenance

The captive breeding experiment was conducted at the Aquatic Rainbow Technology Park (ARTP), Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Madhavaram campus, Chennai, India. *P. conchonius* brooders (n = 100) were collected from Arini River

(Lattitude 13°21'31.75" N and Longitude 80°15'17.98" E) using drag net of 5 mm mesh (1 × 6m) and transported alive to the research centre in oxygenated polyethylene bags and were stocked in circular Fibreglass Reinforced Plastic (FRP) tanks (2000L capacity) at 50 nos./tank for a period of 2 months. During this, fishes were fed with Rotifers, Moina, Cyclops, sludge worm (*Tubifex tubifex*), earth worms (*Eudrilus eugeniae*) and supplementary feed @ 5-8% of body weight twice in a day. The fishes were sampled periodically for morphological indicators of maturation.

Hormonal preparation

Synthetic hormones were obtained Biostadt India Ltd., Worli, Mumbai, India. Syringe of 1 mL having 40 divisions was selected for convenience in which each unit measures 0.025 mL of hormone. Hormone was diluted in equal quantity of distilled water, which made concentration of hormone at 0.0125mL unit^{-1} suitable to inject for 4-7 gm of fish (Mangesh *et al*, 2017).

Experimental design

The present experiment followed a Completely Randomized Design (CRD) using five treatments with control in triplicates. Different hormonal doses of GnRH based synthetic hormone *viz.,* WOVA-FH were used to induce breed the fish. Mature females were injected twice while males were injected once during the resolving dose of female at the base of pectoral fin. While injecting care was taken to reduce the chance of cardiac puncture by needle. Different treatments used in the experiment along with their individual doses for male and female brooders have been summarised in Table 1.

Table 1 : Treatments based on dose applied.

Treatments	Hormonal dosage to female	Hormonal dosage to male
Control	No inducement	No inducement
T_1	0.2 mL kg^{-1}	0.05 mL kg^{-1}
T_2	0.3 mL kg^{-1}	0.1 mL kg^{-1}
T_3	0.4 mL kg^{-1}	0.2 mL kg^{-1}
T_4	0.5 mL kg^{-1}	0.3 mL kg^{-1}

Breeding performance

Effective fecundity of each female after spawning was determined through random sampling of released eggs in a 5 ml graduated measuring cylinder from the eggs released by the female. The total number of eggs in 1 ml was counted and multiplied with total volume of eggs released. The fertilization rate of eggs was determined by randomly taking a sample of approximately 50 eggs from the total eggs in a glass petri-dish; fertilized eggs having intact nucleus were only considered for calculating percentage of fertilization (Mangesh *et al*, 2017). Egg development stages were measured by trinocular microscope (NLCD-120E, Lawrence & Mayo make). One day old hatchlings were maintained in 200Ltr capacity FRP tanks with water recirculation system.

Water quality parameters for broodstock management

The water quality parameters are very important for the rearing and breeding of *H. bimaculatus*. Reverse Osmosis and U.V. filtered water after well aeration was used for domestication and breeding of the fish. In all the experimental tanks for rearing and breeding water quality parameters were maintained at pH (6.9-7.2), Total Dissolved Solids (<100 mg/L), Dissolved Oxygen (5.8-6.5 mg/L), Free CO_2 (<1.2 mg/L) and were determined periodically by Standard Methods (APHA, 2012). In the rearing tanks temperature of 21-25°C was maintained with the help of regulated water heaters (Thermostat).

Statistical analysis

The experimental data were statistically analysed using SPSS software (version 16.0 for windows). One-way ANOVA was used to compare significant differences between treatments. Significant differences between two means were tested using Duncan's multiple range tests ($p < 0.05$). The results are presented as mean ± standard error (SE).

RESULTS

The number of treatments, size of brooders, latency period, number of eggs spawned, fertilization rate, hatching rate and consequent remarks using different hormones are described in Table 2.

Breeding behavior

Brooders showed mating behavior after (2.0-2.5hrs) inducement of different hormones in most of the treatments except in the control and low dosage sets. Mating was preceded by elaborated courtship behavior. First male started chasing to the female followed by releasing milt over the eggs released by female in water. In case of T_0 and T_1, no breeding behavior was seen in all groups while in case of high dosage administration (T_4), female settled down at bottom with slow movement and stressed.

Latency period

Latency period varied significantly between the hormones and doses injected. Almost all fishes have spawned within6-8 hrs of hormonal injection. Spawning took place as early as after 6 hrs of ovatide injection in T_3, while it was delayed and seen after 8hrs of WOVA-FH injection in T_4.

Number of eggs spawned

The number of eggs released varied significantly between the hormones and doses applied. Significantly highest (p<0.05) number of spawned eggs were observed in T_3 (581.50±85.8) followed by T_2 (354.5±57.89), when injected with WOVA-FH. Subsequently, ovatide inducement showed highest number of spawned eggs in T_2 (510±39.2) followed by T_3 (353±10.9).

Fertilization rate

The fertilized eggs were spherical and translucent with pale brownish colour while unfertilized eggs were pale, opaque and white in colour. Fertilization rate was significantly highest (p<0.05) at T_3 (78.50±2.72%) followed by T_2 (66.0±3.39%) when injected with WOVA-FH. Subsequently, when the fishes were injected with ovatide, highest fertilization rate was observed in T_2 (76.0±5.12%) with drastic reduction T_3 (43.5±2.7%).

Hatching rate

Twitching movement of the embryos was observed within 20:00 hours of spawning and the young ones hatched out within 24–28 hours at 26.5±2.5°C temperature. Hatching rate was significantly higher at T_3 (83.75±2.39%) followed by T_2 (75.75±2.1%), when induced with WOVA-FH. Subsequently, when fishes injected with Ovatide, T_2 showed highest hatching rate (82.25±1.49%) followed by T_3 (72.50±2.10^b%). Chronological developments of embryo of these species have been described in Fig. 1.

Larval rearing

The newly hatched larva were transparent and measures about 1.2-1.8mm in length. Yolk sac was get fully absorbed within 68-72 hrs. The spawns were reared at 30 nos.L^{-1} in RAS based nursery raceways and fed with Rotifers (*Brachionus calyciflous*) for first 10 days followed by Moina (*Moina micrura*) for further 10-25 days and then with Cyclops (*Thermocyclopshyalinus*) upto 25-35 days followed with artificial diet (50% protein).

DISCUSSION

The present study demonstrated successful breeding of indigenous ornamental fish, *P. conchonius* in captivity with the use of synthetic hormones along with its development. The results shown that Ovatide at 0.3 mL kg^{-1} for female and 0.1 mL kg^{-1} for male is sufficient to for getting higher number of eggs spawned (510±39.2), fertilization (76.0±5.12%) and hatching rate (82.25±1.49%) with 6-8 hrs latency period as compared with WOVA-FH. In similar way Motilan *et al* (2014) has successfully bred *Puntius chola* using WOVA-FH at the doses of 0.4 ml kg^{-1} (6218.75 ± 32.75). Mahapatra (2010) also succeeded in induced breeding and rearing of indigenous ornamental fish *P. shalynius* using Ovatide at 0.4ml kg^{-1}, while Udit *et al* (2014) used Ovatide at 0.2ml/200gm for induced breeding of *Puntius sarana*.

The latency period or response time is often related to the water temperature and the period, which decreases with an increase in temperature (Jamorz *et al*, 2008). Differences in the latency time of tench (*Tinca tinca*) were observed in the case of different spawning agents (Jamorz *et al*, 2008). In this study, latency period of 6-8 hrs at ambient water temperature (21-27°C) was similar to the reports of Begum *et al* (2009). Mangesh *et al*

Table 2 : Captive breeding experiments of *Puntius conchonius* with synthetic hormones.

WOVA-FH						
Treatment	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**	***p*- value**
Male weight (g)	3.75±0.23	3.80±0.31	3.92±0.34	4.15±0.09	3.65±0.30	0.71
Female weight (g)	5.50±0.23	5.53±0.23	5.20±0.19	5.47±0.20	5.40±0.14	0.776
Latency Period (Hrs)	0^a	0^a	8.02±0.56^c	7.54±0.12^c	0^a	0. 520
Number of egg spawned	0^a	0^a	354.5±57.89^c	581.50±85.8^b	0^a	0.676
Fertilization (%)	0^a	0^a	66.0±3.39 ^{c}	78.50±2.72^b	0^a	0.588
Hatching (%)	0^a	0^a	75.75±2.1^{bc}	83.75±2.39^b	0^a	0.588
Remarks	No spawning	No spawning	Partial spawning	Complete spawning	No spawning	
Ovatide						
Male weight (g)	3.85±0.15	4.25±0.25	4.17±0.38	3.47±0.18	4.25±0.33	0.264
Female weight (g)	5.23±0.19	5.68±0.36	5.27±.0.13	5.55±0.13	5.45±0.14	0.607
Latency Period (Hrs)	0^a	0^a	7.27±0.12^b	7.12±0.20^c	0^a	0.666
Number of egg spawned	0^a	0^a	510±39.2^b	353±10.9^b	0^a	0.259
Fertilization (%)	0^a	0^a	76.0±5.12^b	43.5±2.7^b	0^a	0.732
Hatching (%)	0^a	0^a	82.25±1.49^b	72.50±2.10^b	0^a	0.546
Remarks	No spawning	No spawning	Complete spawning	Partial spawning	No spawning	

Note: values with same superscripts in a row do not differs significantly at p>0.05 (n=3).

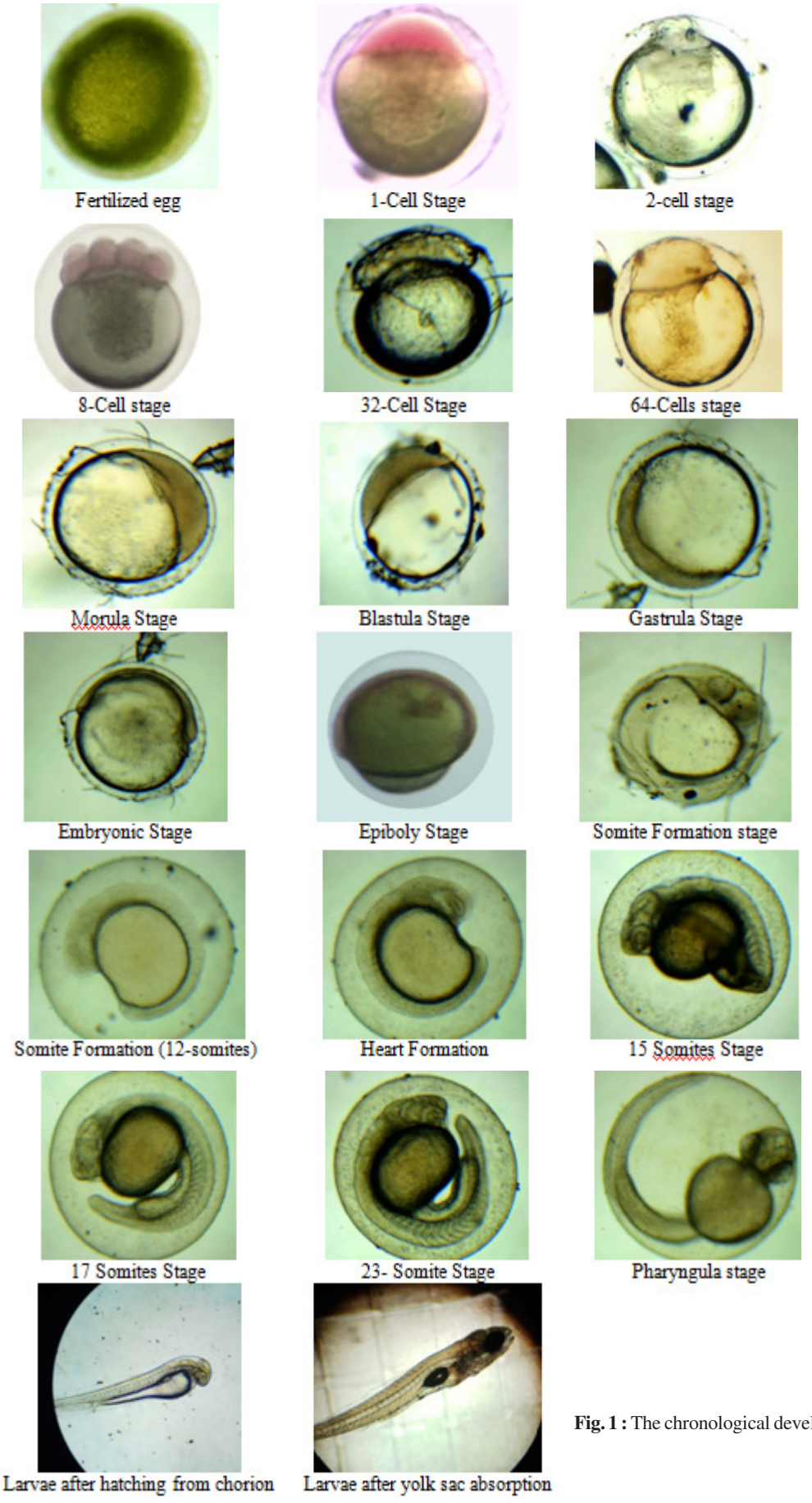

Fig. 1 : The chronological developments of embryo.

(2017) documented different latency period (6-12 hrs) at different dosage of WOVA-FH in *Hemichromis bimaculatus.* Behera *et al* (2010) also reported longer latency period in low dose of synthetic hormones, Ovaprim and Ovatide in *Labeo bata*. According to Billiard *et al* (1984), differences in dose requirement may be attributed due to varied level of dopamine activity and target organ of the hormones in fishes. Das *et al* (1994) and Chatterjee *et al* (2013) reported successful spawning of Tawes (*Puntius javanicus*) and angelfish (*Pterophyllum scalare*) using Ovatide at 0.35 mL kg^{-1} of body weight within 6-7 hrs.

In the experiment, the administered hormone and its dose apparently affected the rate of fertilization and hatching rate. Highest rate of fertilization (78.50±2.72%) and hatching (83.75±2.39%) was observed with the administration of WOVA-FH at 0.4 mL kg^{-1} for female and 0.2 mL kg^{-1} for male while in case of Ovatide inducement at 0.3 mL kg^{-1} for female and 0.1 mL kg^{-1} for male showed effective egg production (510±39.2), fertilization (76.0±5.12%) and hatching rate (82.25±1.49%). Over-dosing of the inducing agents caused early milting; resulting in poor fertilization and under-dosing caused late inducement in males. Similar findings were reported by Mangesh *et al* (2017) in *H. bimaculatus* and Routray *et al* (2007) in carps. Behera *et al* (2007) reported that the egg output per female, fertilization and hatching rate was highest using Ovaprim at a dose of 0.5 mL kg^{-1} of female and 0.2 mL kg^{-1} of male body weight, while in case of Ovatide it was of 0.4 ml kg^{-1} of female and 0.2 ml kg^{-1} of male body weight in *L. bata*. In similar manner, Sukumaravin *et al* (2007) and Motilan *et al* (2014) found increased percentage of egg production, fertilization and hatching rate in Thai carp and *Pethia manipurensis*.

Although, the majority of freshwater fish involved in the aquarium fish trade are from captive-bred sources, significant numbers are still removed from the wild (Rahdari *et al*, 2014). Therefore, captive breeding can play a great role in increasing the population of this species, which can be used by the aquarium keepers so that this species is not exploited from the wild which will help in the conservation of these fish species along with its habitat protection.

CONCLUSION

Puntius conchonius can be matured and bred successfully under captive conditions by obtaining sustainable technique and maintaining optimum water quality. The objective of the present study was fulfilled and Ovatide administration at minimum effective dosage (0.3 $mLkg^{-1}$ for female and 0.1 $mLkg^{-1}$ for male) produces the highest egg production, fertilization and hatching rate. The subject matter in this paper is useful for fish breeders and aquarium keepers for expanding aquariculture, species restoration and conservation.

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