

## Induced Breeding of Giant Zebra Fish *Devario acquipnnatus* (Mc Clelland, 1839) by Oral Delivery of Synthetic Gonadotropin Releasing Hormone Analogue (SALMON-Gn-RhA) GONOPRO-FH



### Zoology

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### ABSTRACT

*Devario acquipinnatus* were collected from hill streams of Leimatak and Tuivai River (Barak Basin) of Manipur and acclimatized in the laboratory aquaria. Synthetic hormone GONOPRO-FH oral delivery with feeds at the rate of 0.5mlkg<sup>-1</sup> successfully spawns. The species will be of model organism of the future research due to the transparent nature of eggs during its developmental periods.

### Introduction

Production of animals for the aquarium hobbyist trade is rapidly growing sector of aquaculture industry, and it will continue to become more important as restrictions are placed on collected animals from the wild (Tlustý, 2002). Most of the aquacultural production of ornamental fish focuses on freshwater species. Approximately 90% of freshwater ornamental fish are captive bred (Dawes, 1998). Giant Zebra fish *Devario acquipinnatus* one of the excellent candidate species of ornamental fish trade has silvery white body, golden and olivaceous on mid dorsal and pale ventrally and is also fulfilled all the characters of ornamental fish. The species was first described by Mc Clelland, in 1839 from Assam under the genus *Perilampus*. Nowadays the fish is valid under the genus *Devario* as described by Fang, 2001. The species is widely distributed in hill streams of Ganga-Brahmaputra basin (Vishwanath *et al.* 2007). The fish also occurs in school at the surface in small high-gradient upland stream (Vishwanath *et al.* 2011). When a species is discovered by the aquarium trade, the sudden interest often leads to decline in wild populations (Tlustý, 2002). Thus for sustainable utilization of the fishes, captive breeding is essential. Thus the objective of the research is to study the induced breeding technique by oral delivery of GONOPRO-FH a synthetic gonadotropin releasing hormone analogue (SGNRH).

### Materials and methods

Fishes were collected from Leimatak and Tuivai River during November 2011 and December 2011 respectively by electro-fishing equipments. Live fishes were transported to laboratory aquarium by partially filled with oxygen following the methods of Esther (2005). For identification of fish count and measurements follows Kullander & Fang, 2009. Collected fishes were cultured at Laboratory aquaria of Manipur University, by feeding with artificial market feed, bloodworms, *Artemia*, *Tubifex* worms and, phytoplankton's, zooplanktons, etc. Temperature of air and water were measured using a mercury thermometer. The pH of water was measured by digital pH meter and dissolved oxygen (DO) was measured by Winkler's Method (1948). Free CO<sub>2</sub> (FCO<sub>2</sub>), Carbonate (CO<sup>-</sup>) alkalinity and Bicarbonate alkalinity (HCO<sub>3</sub><sup>-</sup>) were measured by Welch Method (1888). Maturities of fishes were assessed from distinct sexual dimorphism viz., body form, colouration, shape of abdomen etc. The breeding experiment was conducted on 1+ year old *D. acquipinnatus* of 4-6 g body weight. 40 gravid males and 20 females (n=60) were collected from culture aquarium. Five sets of experiment were conducted in aquarium size of 90 x 45 x 30 cm. Fish were kept starvation for one night. Artificial feed of fish were mixed with synthetic hormone GONOPRO-FH and fish were feed in the early morning at different doses @ 0.3-0.5mgkg<sup>-1</sup>. After feeding the hormonal feeds, the brooders were randomly distributed into different aquaria at the male to female ratio of 2:1. After spawning, the fecundity of each female was determined

by randomly taking a sample of eggs in a 10 ml graded tube. The fertilization rates of eggs were determined by randomly taking a sample of approximately 100 eggs in a Petri dish. Only fertilized egg with an intact nucleus were counted in percentage of fertilization. The environmental conditions during the experiment were table 1. The significance effects of GONOPRO-FH on the egg output, fertilization and hatching rate were calculated by analysis of variance (ANOVA) with statistical software packed SPSS version 16.0. The significance of the effects on the investigating traits was checked by F-test. A level of 0.05 was utilized to account for the statistical significance. Larvae were reared by feeding *Tubifex* worm powder and some live life feeds such as zooplanktons.

### Result

#### Determination of optimum doses of GONOPRO-FH

A varied degree of responses was observed in relation to the different doses of hormone. Male brooders showed a chasing behavior after 4-5 hr. of feeding of GONOPRO-FH mixed feeds. None of the control fish spawned, however all the groups oral delivery of hormones mixed with feeds @ 0.3, 0.4 and 0.5 mlkg<sup>-1</sup> spawn successfully. All females were found to be paired with a single more aggressive male.

#### Effect of GONOPRO-FH on egg production, fertilization and hatching

Analysis of variance showed a significant effect (P < 0.05) of hormonal doses on egg output (P < 0.05) and hatching rate (P < 0.05) but the rate of fertilization was not significantly different between the treatments (P > 0.05). The lowest doses of GONOPRO-FH used in 0.3 mlkg<sup>-1</sup> led to 46 ± 4 % spawning in females. The spawning rate and number of eggs in the fish treated with CPE (positive control) or with 0.5 mlkg<sup>-1</sup> GONOPRO-FH was not statistically different (Table. 2).

### Discussion

Breeding behavior of fish is controlled by various environmental factors such as light temperature, pH, DO, meteorological condition (Motilan *et al.* 2013). In the control environmental condition breeding of *Devario acquipinnatus* were not observed. Synthetic hormone GONOPRO-FH mixed with feeds, at the doses of 0.5 mlkg<sup>-1</sup> body weight by-passes the environmental factors and is sufficient to induce 100% ovulation and spawning. In present observation, egg production, at the doses of 0.5 mlkg<sup>-1</sup> (400.61±14.2) is significantly (P<0.05) similar to the crude pituitary extract group (387.71±8.4). However, the egg production was significantly (P<0.5) higher as compare to lower doses. This shows that the doses of 0.5 mlkg<sup>-1</sup> is significant to achieve ovulation with better results than the CPE. The synthetic hormonal doses of 0.4-0.5, 0.3-0.4, 0.25-0.3, 0.3-0.4, 0.6-0.8, 0.4-0.8, 0.4-0.7, 0.4-0.8, 0.4-0.5, 0.6-0.7, 0.1-0.3, 0.15-0.4 mlkg<sup>-1</sup> in case of Catla, Rohu, Mrigal, Fringe-lipped carp, Catfish, Silvercarp, Grasscarp, Bighead carp, Mahseers, male species of all carps,

males of all Catfishes respectively of WOVA-FH by Basavaraja, (2007). Mani *et al.* 2007 also observed that WOVA-FH injection at the rate of 0.2 mlkg<sup>-1</sup> at weekly intervals of 28 days during June was effective in ovarian maturity and spawning of *Heteropneustus fossilis*. Motilan *et al.* 2013 also observed 100% spawning in case of *Puntius chola*. The objective of the present study fulfilled and GONOPRO-FH administration at doses of 0.5 mlkg<sup>-1</sup> of body weight produces the highest spawning rate, egg production and hatching rate. The positive response of both male and female to single doses of GONOPRO-FH is significant for commercial seed production and it can be utilized for species restoration, conservation. The species can serve as model organism in future because of the transparent egg of the species, easy to breed and directly visible cells during development, which is the character of Zebra fish, a model organism.

**Table. 1**  
**Physico-chemical parameters of the aquarium system**

Atm. Temp (°C)	Water Temp(°C)	pH	DO (ppm)	FCO <sub>2</sub> (ppm)	Carb. alk (ppm)	Bicarb.alk (ppm)
25 ±4.0°C	23± 2°C	6.8 ±0.9	7.2± 2.0	5±4	3±2	26±4

**Table2. Result of induced breeding experiment of *Devirio acqipnnatus* by graded doses of GANOPRO-FH oral delivery equally to males and females mlkg<sup>-1</sup> body weight.**

Treatment (ml kg <sup>-1</sup> )	Egg production	Spawning Rate	Fertilisation Rate (%)	Hatching rate (%)	Remarks
0.3	326.3 ± 3.5 <sup>a</sup>	46 ± 4 <sup>a</sup>	88.7 ± 3.4	44.62 ± 3.24 <sup>a</sup>	Partial spawning
0.4	337.65 ± 3.62 <sup>b</sup>	88 ± 6 <sup>b</sup>	89.34 ± 3.24	72.28 ± 4.96	Complete spawning
0.5	400.61 ± 14.25 <sup>c</sup>	100 <sup>c</sup>	89.32 ± 2.61	90.4 ± 2 <sup>b</sup>	Complete spawning
50mg CPE	387.71 ± 8.46 <sup>c</sup>	100 <sup>c</sup>	89.12 ± 0.5	80.67 ± 3 <sup>b</sup>	Complete spawning
0.5 ml 0.7 saline	0	0	0	0	No Spawning

Values are mean ± SEM (n = 5); different subscript letters indicate significant difference (p < 0.05). Spawning rate = number of fish spawned/total number of fish injected x 100 ; Egg production = number of egg released / g body weight of female; Fertilization rate (%) = total number of eggs having faint streak / total number of eggs in sample x 100; Hatching rate (%) = total number of hatched eggs as a percentage of tail of tail bud embryos.

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