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Induced breeding of African Jewelfish *Hemichromis bimaculatus* (Gill, 1862) using gonadotropic signaling molecular analogue WOVA-FH

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Abstract
The present experiment was conducted to breed African Jewelfish, *Hemichromis bimaculatus* Gill, 1862 (Family Cichlidae) in captivity through GnRH based gonadotropic signalling molecular analogue WOVA-FH. All the sets induced at different dosage were bred except low dosage and control sets. Spawning success rate varied on the rate of inducement. WOVA- FH at a dose of 0.5 mL kg^{-1} for female and 0.3 mL kg^{-1} for male was found to be effective with 79-80% fertilization and 85-86% hatching rate from 500 to 550 eggs/female. Dose of hormone apparently affected the latency period (6-12hrs), egg output (180-520), fertilization (31-79%) and hatching rate (68-86%). Administering an over-dose of the inducing agents resulted in poor fertilization (31±2.2%) and under-dosing caused no inducement in animals. The present breeding protocol is simple and can be taken up by small breeders. It will be helpful in development of aquariculture and conservation of *H. bimaculatus*.

Keywords: Induced breeding, Jewelfish, WOVA-FH, breeding technique

1. Introduction
Cichlids have a wide geographical distribution and are natives of Africa comprising about 150 genera and 1300 species, [1] making it the second largest perciform family. Cichlids are found in fresh and brackish waters of southern India and Sri Lanka. Cichlids are an important group of relatively large and often colourful aquarium fishes [2]. They exhibit a broad range of morphological, ecological and behavioural variation. It is certainly possible to find quite a few cichlids that can legitimately be characterized as large, aggressive and predatory [3]. However, there are many cichlids whose size and temperament definitely qualify them as excellent ornamental animals [4].

India has vast potential of ornamental fishes. About more than 288 exotic species and 250 indigenous fresh water fish species and 150 marine water species have been reported as potential and suitable climatically in India [5]. The export of ornamental fishes started somewhere in 50's, till 1980's the earning through export was negligible (Rs. 18-20 Lakhs). The value of international trade has increased steadily, touching US$ 350 million in 2007. The total value of ornamental fishes exported from India is Rs. 3.00 crores [6]. Out of the 2500 species are traded and a few 30-35 species of fresh water fish dominate the market. Very few of freshwater fish are captive bred while only 25 out of nearly 8,000 marine ornamental fishes are bred in captivity. According to the report of tenth 5 year plan in India, export is almost entirely dependent on freshwater species with the major exporting centres being Kolkata, Chennai and Mumbai [7].

Hemichromis bimaculatus Gill, 1862 (Family Cichlidae) is also known as African jewelfish or jewel cichlid originated in Northern Africa [8], associated with forested biotopes. It is considered as the true 'jewelfish' of aquarists and is also used as an experimental animal by physiologists and ethologists [9]. Jewelfish are beautiful creatures popular among fish keepers. The short identification description of the species includes 14-15 dorsal spines, 10-12 dorsal soft rays, 3 anal spines, 8-9 anal soft rays, 3 black blotches on sides, first on opercle, second at midpoint of body and third on caudal-fin base. The upper profile of snout is convex [10]. Fluorescent dots on red with yellowish tinge body, wide attractive fins and incredible nature of living together in aquarium fetching high market demand. There is, however, no information

on captive breeding of *H. bimaculatus* [11]. According to the IUCN Red List of freshwater fish in Northern Africa, this species is critically endangered [12]. Hence, the present study was undertaken to conserve as well as to meet the demand of aquarium fish trade by developing of captive brood stock and artificial breeding of *H. bimaculatus.*

2. Materials and Methods

2.1 Broodstock Collection and Maintenance

The captive breeding experiment was conducted at the Advance Research Farm Facility (ARFF), Fisheries College & Research institute, Tamil Nadu Fisheries University, Madhavaram campus, Chennai, India research centre. *H. bimaculatus* brooders (n = 100) were collected from ornamental fish market located at Kolathur, Chennai and transported alive to the research centre in oxygenated polyethylene bags. The fishes were given $KMnO_4$ dip treatment before releasing them into circular Fibre Reinforced Plastic (FRP) tanks (2000L capacity) at 50 nos./tank for a period of 2 months. During this period, they were fed with supplementary feed @ 7% of body weight twice daily. Animals were also fed with Rotifers, Moina, Cyclops, sludge worm (*Tubifex tubifex*) and earth worms (*Eudrilus Eugeniae*) once in two days. The fishes were monitored regularly for morphological indicators of maturation.

2.2 Hormonal preparation

Synthetic hormone WOVA-FH was obtained from a local supplier (V.V. Health Care Pvt. Ltd., Kolathur, Chennai, India). Syringe of 1ml having 40 divisions i.e., each unit measures 0.025 ml capacity was selected for convenience. So, 1 unit of WOVA-FH is equals to 0.025 ml. After taking 5 units WOVA-FH, distilled water was added to make the volume 10 units in the vial. Now the concentration of WOVA-FH became 0.0125ml in each unit of the syringe i.e., injection of five unit of diluted WOVA-FH will contain 0.0125ml of WOVA-FH suitable for 25gm fish [16].

2.3 Experimental design

The present experiment followed a Completely Randomized Design (CRD) using five treatments in addition to a control performed in triplicates. Different hormonal doses of GnRH based synthetic hormone *viz.,* WOVA-FH (Biostadt India Limited, Mumbai) were used to induce breed the fish. Mature animals were segregated based on their morphological character which has been given in table 1. Mature female 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 2.

Table 1: Morphological characterization of *Hemichromis bimaculatus* male and female

Sl/No.	Character	Male	Female
1	Size	Smaller	Larger
2	Body colour	Bright	Slightly dull
3	Fin colour	Full red with yellow base	Whitish with reddish base
4	Belly shape	Slender and straight	Bulgy and concave
5	Belly texture	Slightly hard	Soft

Table 2: Treatments based on dose applied

Treatments	Hormonal dosage to female	Hormonal dosage to male
Control	No inducement	No inducement
T1	0.1 mL kg^{-1}	0.1 mL kg^{-1}
T2	0.3 mL kg^{-1}	0.2 mL kg^{-1}
T3	0.5 mL kg^{-1}	0.3 mL kg^{-1}
T4	0.7 mL kg^{-1}	0.3 mL kg^{-1}
T5	0.9 mL kg^{-1}	0.5 mL kg^{-1}

2.4 Breeding performance

Effective fecundity of each female after spawning was determined through random sampling of released eggs in a 10 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 [13]. 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 [14]; fertilized eggs having intact nucleus were only considered for calculating percentage of fertilization. Ova diameter and 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.

2.5 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 (7.2-7.6), Total Dissolved Solids (30-100 mg/L), Dissolved Oxygen (5.8-6.5 mg/L), Free CO_2 (1.2-1.6 mg/L) and were determined periodically by Standard Methods (APHA, 2012). In the rearing tanks temperature of 21-25ºC was maintained with the help of regulated water heaters (Thermostat).

2.6 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).

3. Results

3.1 Broodstock management

Broodstock was managed in FRP tanks to promote gonad development. Morphological indicators of maturation of females were observed by bulgy abdomen. During maturation it was observed that males were smaller in size compared to females [15]. A common secondary sexual character was the brighter body colour of the male than that of the female fish. In male fins were full red with yellow base during the full maturity, while such colour was absent in female. During the breeding period, the ripe male oozed out milt when slight pressure was applied on the vent. Eggs oozed out with slight pressure on the abdomen of ripe female. Detail of sexual dimorphism has been explained in Table 1.

A varied degree of response of administered hormone was observed at different doses. Fertilization rates, latency period, egg output and hatching rate in response to different treatments have been summarised in (Table 3). The response to hormone inducement was very high; out of 06 female

selected for induced breeding, 04 responded positively and produced viable eggs. Predictably, no spawning took place in control and low dosage sets.

Table 3: Results of captive breeding experiments of *H. bimaculatus* with WOVA-FH

Treatment	Size of Female (g)	Size of Male (g)	Latency Period	Number of eggs/female	Fertilization Rate (%)	Hatching (%)	Remarks
T0	27.35±0.5	21.23±0.5	0^{a}	0^{a}	0^{a}	0^{a}	No Spawning
T1	26.14±.0.5	22.10±0.5	0^{a}	0^{a}	0^{a}	0^{a}	No Spawning
T2	27.40±0.5	20.36±0.5	12^{c}	$380±25^{c}$	$68±1.2^{c}$	$78±1.2^{dc}$	Partial spawning
T3	27.12±0.5	22.50±0.5	8^{b}	$520±30^{d}$	$79±1.5^{c}$	$85±1.6^{d}$	Complete spawning
T4	28.14±0.5	22.58±0.5	6^{b}	$510±48^{d}$	$74±2.1^{c}$	$86±1.4^{d}$	Complete spawning
T5	28.20±0.5	23.24±0.5	0^{a}	$180±35^{b}$	$31±2.2^{b}$	$68±1.8^{b}$	Partial spawning
p- value	0.4142	0.0679		0.4142	0.0679	0.1573	

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

3.2 Breeding behaviour

Brooders showed mating behaviour after hormonal injection in all the treatments (except in the control and low dosage sets). Each female was paired with one male. Mating was preceded by elaborated courtship behaviour. We observed that male started chasing and releasing milt over the eggs laid by the female inside the coconut shell. Brooders showed mating behaviour after 6–8 hrs of injection in T3 and T4. It was delayed (seen after 8-10 hrs of injection) in T2. In case of T1, no breeding behaviour was seen while in case of T5, female settled down at bottom with slow movement and stressed.

3.3 Latency period

Latency period varied significantly between the doses of synthetic hormones injected. Spawning took place as early as after 6 hrs of injection in T4; 8 hrs in T3 and 12 hrs in T2. The result showed delayed latency period with administration of low dose of WOVA-FH than other groups.

3.4 Egg output

The number of eggs released by the female was significantly higher ($P<0.05$) at T3 (520±30) followed by T4 (510±48), T2 (380±25) and T5 (180±35) using WOVA-FH injection. The fertilized eggs were spherical, translucent and attached measuring 12-14µm in diameter and were adhesive. Unfertilized eggs were pale, opaque and white in colour.

3.5 Fertilization rate

Fertilization rate was significantly higher ($P<0.05$) at T3 (79±1.5%) followed by T4 (74±2.1%), T2 (68±1.2%) and T5 (31±2.2%) using Wova-FH. The results showed that fertilization rate was significantly higher with injection of synthetic hormones at 0.5 mL kg^{-1} for female and 0.3 mL kg^{-1} for male than other doses.

3.6 Hatching rate

Twitching movement of the embryos was observed within 48–60 hrs of spawning and the young ones hatched out within 70–74 hrs at 23.5 ± 1.5 ºC. Estimated hatching rate was significantly higher (P<0.05) at T4 (86±1.4%) and T3 (85±1.6%) followed by T2 (78±1.2%) and T5 (68±1.8%). Here the results showed that the highest hatching rate was observed when the hormone dose for male was increased by 0.1 unit.

Fig 1: Length-Weight Measurement of *Hemichromis bimaculatus*

Fig 2: Injecting Hormone at Pectoral Base

Fig 3: Eggs of *H. bimaculatus*

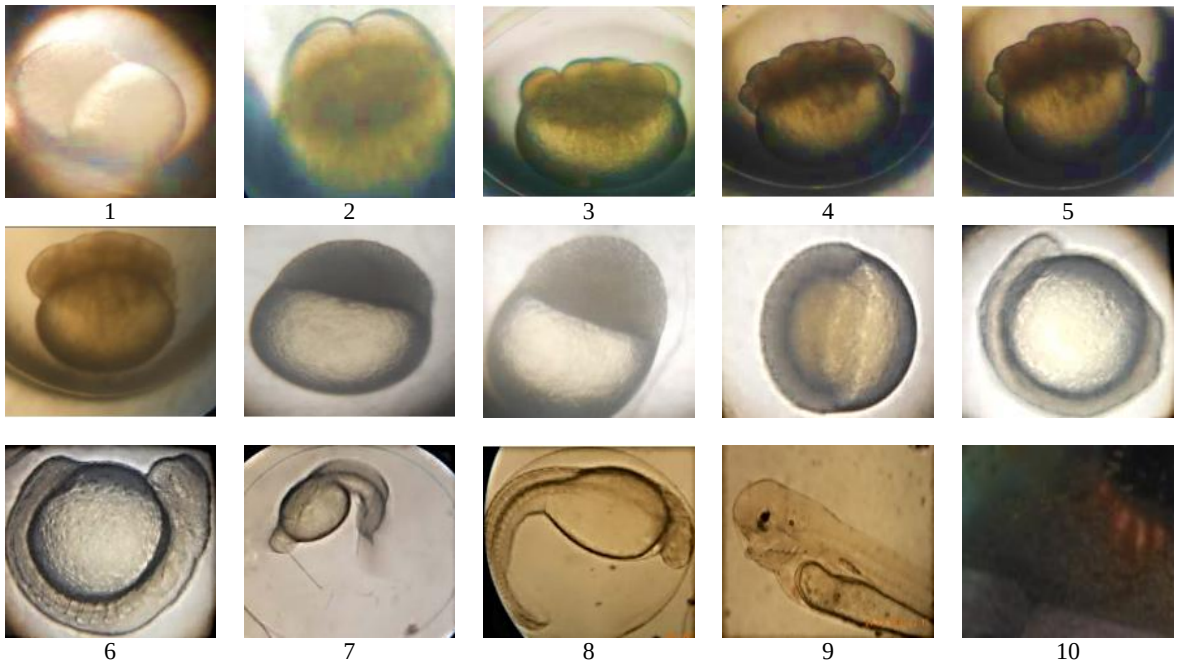

Fig 4: 1. 2 Cell Stage, 2. 4 Cell Stage, 3. 16 Cell Stage, 4. 32 Cell Stage, 5. 64 Cell Stage, 6. 128 Cell Stage, 7. Oblong Stage, 8. Sphere Stage, 9. Epiboly Stage, 10. Bud Stage, 11. Somite Stage, 12. Pharyngula, 13. C- Shape, 14. Hatchling and 15. Young Ones

4. Discussion

In present investigation, *H. bimaculatus* has successfully bred by inducing in captivity by administration of synthetic hormones like WOVA-FH. Spawning response was comparable to that with other cichlid (Angel Fish), in which 90-95% breeding response was easily achieved at 0.35mL kg^{-1} dosage of synthetic hormone [16]. In our study, WOVA-FH at a dose of 0.5 mL kg^{-1} of female and 0.3 mL kg^{-1} of male brooders were found to be effective for induced breeding in captivity. However, the efficacy of the synthetic hormones was significantly higher than that of CPE. Rath *et al.* did a comparative evaluation of CPE and 3 different GnRH based synthetic hormones on the induced breeding performance of Indian Major Carps (IMC) [17].

Difference in latency period was observed among different treatments in the present study. Spawning took place as early as after 6 hrs of injection in T4; 8 hrs in T3 and 12 hrs in T2 while lower dosage showed prolonged latency period which indicates difference in the mode of action of the hormone. Similar observation was reported by Pandey *et al.* in *L. rohita* [18]. Behera *et al.* also reported the longer latency period in low dose of synthetic hormone, Ovaprim and Ovatide, on induced breeding of *Labeo bata* [19]. According to Billiard *et al.* and Peter *et al.*, differences in dose requirement may be attributed to varied level of dopamine activity in fishes [20, 21]. Das *et al.*, and Chatterjee *et al.*, also documented injection @ 0.35 ml/kg of body weight of a mature female Tawes (*Puntius javanicus*) and Angelfish (*Pterophyllum scalare*) gave better spawning within 4-5 hrs [22, 16].

In the experiment, the administered hormonal dose apparently affected the rate of fertilization. Over-dosing of the inducing agents caused early milting; resulting in poor fertilization and under-dosing caused late inducement in males. Similar finding was reported [23]. Behera *et al.* reported that the egg output per female, fertilization and hatching rate was the highest with Ovaprim at a dose of 0.5 mL kg^{-1} of female and 0.2 mL kg^{-1} of male body weight; 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* [24]. In similar manner, Sukumaravin *et al.*, and Motilan *et al.*, found increased percentage of fertilization, egg production and hatching rate of in Thai carp and *Pethia manipurensis* using WOVA-FH [25, 26].

The dose of hormone obviously affected hatching in *H. bimaculatus*. Optimal range of water quality parameters recorded in the breeding tank also might have contributed to increased hatching rates. Behera *et al.*, Rath *et al.*, and Das *et al.*, reported, hatching rate of *L. bata*, IMC and *O. belangeri* was highest when induced with synthetic hormones along with optimum range of water quality in the experimental pond [24, 17, 27]. Rath *et al.* also reported higher hatching rate in IMC administered with WOVA-FH at 0.4-0.5 ml kg^{-1} of female body weight [17].

5. Conclusion

H. bimaculatus can be easily matured and bred successfully under captive conditions. The objective of the present study was fulfilled and WOVA-FH administration at a dose of 0.5 mL kg^{-1} for female and 0.3 mL kg^{-1} for male produces the highest spawning, egg production and hatching rate in *H. bimaculatus.* 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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