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Captive breeding of endangered Barbs Pethia manipurensis (Menon et.al, 2000) by oral delivery of gonadotropic signaling molecular analogue WOVA-FH

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Abstract- Oral delivery of a salmon gonadotropic signaling molecules WOVA-FH @ of 0.2-0.4 $mlkg^{-1}$ to endangered Barbs *Pethia manipurensis* to induced spawning of 42-100% within 8-10 hr.

Index Terms- Ornamental fish, endemic, endangered species, artificial spawning, reintroduction

I. Introduction

Freshwater fish subfamily (Cyprininae) includes barbs which are characteristic in having large scales, bright colours, schooling behaviour and are easy to maintain and breed which made them popular in the aquarium trade (Clyde, et.al., 1997). *Pethia manipurensis* locally known as Ngaka meingangbi in Manipuri is an economically important fish for preparation of Hentak and Ngari, the two important solid substrate fermented fish products which enhance flavour of food for Manipuri (Sarojnalini and Vishwanath, 1988). The fish is having a medium value as fresh used to add flavour to vegetable curry preparation after smoking and drying. The fish also fulfils all the characters of ornamental fishes i.e., beautiful colourations, adaptability to aquarium condition, acceptable to artificial and natural food, peaceful nature etc., thus the fish becomes an ornamental species. The species is endemic to Chindwin basin (Vishwanath *et al*., 2007). The fish is catagorised under threatened according to the (IUCN 2010). In their natural habitats the species breed in ponds, lakes, paddy fields, rivers, streams and small pits. Artificial induction spawning behavior in *Puntius ganionotus* (Bleeker) was done by Liley and Tan (1895). Effect of ultrasonic sound on breeding performance of *Puntius sarna* (Hamilton) was done by Maily *et al*., (2006). Orally induced spawning of Thai carp followed by co-administration of Gly 10 (D-Arg 6) SGnRH ethmylmide and domperidone was done by Sukumaravin *et al*., (2006). The specis will be of important for the ornamental fish culture and sun dried fish for the preparation of a Hentak a fermented fish product of Manipur. Hence in order conserve the fish as well as to meet the demand of the fish for ornamental fish culture seed production of the fish is important. However, no experiment has been so far conducted on induced breeding of *P. manipurensis* with synthetic hormone WOVA-FH and any other hormones till date. The objective of the experiment is induced breeding of *P. manipurensis* by oral delivery of hormone WOVA-FH a synthetic gonadotropin releasing hormone analogue (SGnRH).

II. Material and Methods

Fishes were collected by netting from Leimeram waterfall and streams of Baruni hills Chindwin basin. Live fishes were transported to the laboratory in polythene bags partially filled with oxygen by following the method of Esther (2008). For identification of fish, Kulander and Fang (2005) were followed for measurements and counts. Measurements were taken point to point with disital calpers measuring to 0.1 mm. Count were made on the left side of the species by using stereo zoom microscope. The technique of Clyde (1997) was followed for culture in aquarium**.** Fish were culture in laboratory aquaria of size 90 × 45 × 30 cm. 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_2 (FCO_2), Carbonate (CO^-) alkalinity and Bicarbonate alkalinity (HCO_3) were measured by Welch Method (1888). Maturities of fishes were assessed on the basis of genitalia, oozing milt, swollen vent, body colouration and roughness body and pectoral fin. The experiment was conducted on 2+ year old *Pethia manipurensis* of 2-3 g body weight 20 gravid females and 40 males (n=60) in 20 aquarium sizes of 90 × 45 × 30 cm. Fish were kept starvation for one night. Artificial feed of fish were mixed with synthetic hormone WOVA-FH and fish were feed in the early morning at different doses @ 0.2-0.4$mgkg^{-1}$. 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 samples of eggs in a 10 ml graded tube. Total number of eggs in 1ml was counted and was multiplied by the total volume of eggs released. Fertilisation rates of eggs were determined by randomly taking a sample of approximately 100 eggs in a Petri dish. Only fertilized eggs with an intact nucleus were counted for the percentage of fertilization. The environmental conditions of breeding were as shown in Table 1. The significance of effects of WOVA-FH on the egg output, fertilization and hatching rate were calculated by analysis of variance (ANOVA) with a statistical software packaged SPSS version 17.0. The significance of the effects on the investigating traits was checked by F-test. A probability level of 0.05 was utilized to account for the statistical significance.

III. Result

Determination of optimum dose of WOVA-FH

Colouration of fishes increased after the injection of the hormones. A varied degree of hormonal response was observed in relation to different doses of the hormones. Chasing behavior was seen after 8-10 hr post oral delivery of feeds mixed with hormones. None of the control fish spawn, however all the groups delivered with WOVA-FH @ 0.2, 0.3 and 0.4 $mlKg^{-1}$ spawn successfully. One of the active male paired with female released milt, the female released eggs and external fertilization took place.

Effect of WOVA-FH on egg production, fertilization and hatching

Analysis of variance showed a significant effect($P < 0.5$) of hormonal doses on egg output ($P< 0.5$) but the rate of fertilization was not significantly different between the treatments($P> 0.5$).The lowest dose of WOVA-FH i.e., 0.2 $mlkg^{-1}$ led to 42.62 ± 3.625% spawning in females. The spawning rate and number of eggs in the fish treated either with CPE (Positive control) or with 0.4ml kg^{-1} WOVA-FH were not statistically different (Table 1).

IV. Discussion

Various environmental factors such as light, temperature, pH, DO., meteorological condition etc., are known to play important role in stimulating the release of gonadotropic hormones from the anterior lobe of pituitary glands within the organisms and thereby controlling breeding behaviors of fish (Motilan, et. al ., 2013). Secretion of gonadotropic hormone and gene expression is controlled by the environmental factors (Ralston, et. al., 2008). However, the optimum environmental condition for natural breeding is not available in the aquarium. Hence, most of the fish lost their natural breeding behaviour. Gonadotropic Releasing Hormone (GnRH) is now the best available biotechnological tool for the breeding of fish (Bhattacharya, 2002 et. al). Synthetic hormone WOVA-FH oral delivery, at the doses of 0.4 $mlkg^{-1}$ body weight by-passes the environmental factors and is sufficient to induce 100% ovulation and spawning. WOVA-FH affected the percentage of fertilization, egg production and hatching rate. Similar observation was observed by Sukumaravin *et al.*, (2006) by oral delivery (50-100 µg kg^{-1} body weight) and domperidone (25-50 $mgkg^{-1}$ body weight) to Thai carp. However no reports are available for standardizing the doses of WOVA-FH in *P. manipurensis.*

In the present observation, egg production, at the doses of 0.4 $mlkg^{-1}$ (6218.75 ± 32.75) is similar to that of crude pituitary extract (6464.25 ± 250.75). However, the egg production was significantly ($P< 0.05$) higher as compared to lower doses. This shows that the dose of 0.5 ml kg^{-1} is significant to achieve ovulation with better results than with CPE. Similar results were obtained with *Anabas testudineus* that was treated with ($0.3mlkg^{-1}$) Ovatite (Bedajit *et al*., 2011) and (0.3 $mlkg^{-1}$) WOVA-FH (Sarkar *et al*., 2005), whereas Kuldeeg *et al*., (2010) showed that higher doses of Ovaprim (1.5 $mlkg^{-1}$) is required to obtain early and extended normal spawning of *Anabas testudineus*. The objective of the present study was fulfilled and WOVA-FH administration at a dose of 0.4 ml kg^{-1} of body weight produces the highest spawning rate, egg production and hatching rate in *P. manipurensis.* The positive response of both male and female to a single dose of WOVA-FH is significant for commercial seed production and it can be utilized for species restoration, conservation. The experiment will also help in the income generation of the people.

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Fig 1. Live photo of *Pethia manipurensis*

TABLE1: Physico-chemical parameters of the aquarium system

Atm.Temp (^{o}C)	Water Temp(^{o}C)	pH	DO (ppm)	FCO_2 (ppm)	Carb.alk (ppm)	Bicarb.alk (ppm)
25 ±4.0^{o}C	23± 2^{o}C	6.8 ±0.9	7.2± 2.0	5±4	3±2	26±4

Table1. Result of induced breeding experiment of *Pethia manipurensis* by graded doses of WOVA-FH administered equally to males and femals mlkg^{-1} body weight.

Treatment (mlKg^{-1})	Spawning rate	Egg production (number g^{-1})	Fertilization rate (%)	Hatching rate (%)	Remarks
0.2	42.62 ±3.625^{a}	2213.5 ± 167.5^{a}	87.295 ± 4.675	54.975 ± 3.225^{a}	Partial spawning
0.3	88.1 ± 3.6^{b}	3210.5 ± 81.5^{b}	88.95 ± 2.15	73.7 ± 3.2^{b}	Complete spawning
0.4	100^{c}	6218.75± 32.75^{c}	89.325 ± 2.125	92.525 ± 1.965^{c}	Complete spawning
100mg CPE	100^{c}	6464.25±250.75^{c}	90.475 ± 2.075	81.525 ± 2.025^{d}	Complete spawning
0.5ml 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.