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Comparative Study on the Effects of two Commercial Hormones on the Induced Breeding of *Channa striata* (Bloch, 1983) under Agro-climatic Conditions of Assam, North East India

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ABSTRACT

Background: Striped snakehead (*Channa striata*) is an air breathing fish with immense popularity as food. Successful breeding operation of this fish requires proper broodstock management and development of mass seed production technique. As spawning efficiency vary with species traits, climatic conditions and inducing agents for spawning, therefore, an experiment on the efficacy of two commercial GnRH based synthetic hormones *viz*., Gonopro-FH and OVAFISH on the induced breeding of *Channa striata* in captivity was conducted under agro-climatic conditions of Assam.

Methods: Three doses of Gonopro-FH and Ovafish *i.e.* 0.40 (T1), 0.60 (T2) and 0.80 (T3) ml/kg for female and 0.3, 0.45 and 0.60 ml/kg body weight for male were administered to evaluate the efficacy.

Result: Breeding aspects such as relative fecundity, fertilization rate, hatching rate, latency period and incubation period were found better with the dose of 0.45 ml/kg for male and 0.6 ml/kg for female. A higher relative fecundity was achieved by Gonopro FH administration. The results of this study may be useful in induce breeding and seed production of *Channa striata.*

Key words: *Channa striata*, GnRHa, Gonopro FH, Induced breeding, Ovafish, Striped Snakehead.

INTRODUCTION

Striped snakehead (*Channa striata*) is considered as a valuable air-breathing freshwater fish with a delicious meat, rich in nutrients and possess medicinal values (Sahu *et al*., 2012). The flesh has an appealing flavor and less intramuscular spines in comparison to other fish which imparts great economic potential particularly in Northeast India (Paray *et al*., 2013). However, the culture potential of this species is limited due to the unavailability of hatchery-reared juvenile fish, poor collection of juvenile fish from wild and bottlenecks in captive breeding (Kumari *et al*., 2018).

Proper understanding over the reproductive behavior of fish in captive conditions is vital for ensuring sustainable aquaculture production by domestication of wild fishes (Mylonas *et al*., 2010). Hormonal administration in an effective way can enhance ovulation, spermiation and augment productivity in hatchery operations (Kumar *et al*., 2021). Efficacy of various inducing agents has been studied in several fish species (Kumakura *et al*., 2003). The commonly used exogenous hormones for induction of maturation and spawning in fish are carp and salmon pituitary homogenates, GnRH analogue (GnRHa) with or without dopamine antagonist and HCG along with some other hormones (Brandao *et al*., 2020). The hormonal stimulation ensures final maturation and spawning in captivity (Kucharczyk *et al*., 2020). Moreover, exogenous hormonal implants have also been used to achieve reproductive maturity in striped snakehead reared in captivity (Kumar *et al*., 2021). To ensure maximum adoption of the breeding techniques, the hormone must be efficient, cost effective and easily available in usable scale.

There is limited studies available on induce breeding and seed production of *Channa striata* which could be a bottleneck in promoting its culture potential particularly in Northeast India. Further, developing region-specific technique of induce breeding of this commercial fish species would be useful in standardizing the package of practice (POP) for the practicing farmers. Therefore, the present study was conducted to evaluate two such commonly available commercial synthetic hormones (Ovafish and Gonopro FH) under the agro-climatic conditions of Assam,

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as there is scanty information on the captive breeding of striped snakehead. The present study assesses the efficacy of two spawning induction agents (Ovafish and Gonopro FH) for induced breeding and spawning performance of striped snakehead brooders in captive conditions under agroclimatic conditions of Assam.

MATERIALS AND METHODS

Experimental fish and ethics

Wild *Channa striata* of average weight 150 g were collected from different water bodies of Nagaon, Assam (India). Prophylactic measures for the fishes were taken using salt solution (1.5 %) (Kumar *et al*., 2021) and reared for a period of 8 months in cemented cistern of College of Fisheries fish farm. The experiment was carried out as per the guidelines of the Assam Agricultural University in compliance with the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests, Government of India.

Broodstock management

Two cement cisterns (9 m × 5 m × 4 m) were prepared with 15 cm of soil base. Around 15-20% of the water spread area was covered with water hyacinth (*Eichhornia crassipes*) to support as a mesocosom. A total of 270 striped snakehead (400-500 g) in the sex ratio of 1:2 (F:M) were selected and stocked in each concrete tank. Fortnightly water exchange was done and water quality parameters were monitored periodically (APHA, 2018).

Food and feeding

The brooders were fed with a combination of live and practical feed twice in a day @3%-5% of their body weight. Practical feed was prepared by mixing dried fish meal (65%) with other ingredients like rice bran (15%), groundnut oil cake (15%), vegetable oil (4%) and 1% vitamin-mineral mixture (Agrimin Forte) using luke warm water.

Assessment of gonadal development

Periodical assessments of gonadal maturation of brood fish were done using cannulation-a flexible catheter. The fish were anesthetized using clove oil and the female brooders were cannulated for collection of ova.

Induced spawning

The effect of two different commercial hormones- Gonopro FH and OVAFISH on the induced breeding were evaluated during the month of July, 2021. Both the hormones were selected due to its wide range of commercial applications in the hatcheries of Assam, India. The chemical formulations of Gonopro FH and OVAFISH are composed of SALMON-Gn-RhA (20 mcg), Domperidone IP (10 mg), Propylene Glycol IP. Three different doses of commercial hormones (CHs) were selected based on previous research work on the species done by Kumar *et al.* (2021). The doses were 0.40 (T1), 0.60 (T2) and 0.80 (T3) ml/kg of body weight of females. Similarly, males were administered with 75% of the dose of CHs applied to female *i.e.*, 0.30 (T1), 0.45 (T2) and 0.60 (T3) ml/kg of body weight respectively.

Brooders in the weight range of 400-500 g were selected for the breeding program. The mature male and female fish were selected based on their secondary sexual characteristics which are observed prominently in females as soft and engorged abdomen with round and raised genital pore and a firm stomach with pointed genital pore in males. Total 270 fishes were selected and were kept in the replicate tanks in equal numbers (30 in each tank) in triplicate (n=9). The sex ratio for the induced spawning experiment was considered following 1:2(F:M). The fish were administered a single dose of an intramuscular injection of inducing agents (Fig 1). The injected fish were released in separate outdoor breeding tanks (cement cistern, 5 m×4 m×2 m) with floating aquatic macrophytes.

Spawning performance

The floating eggs (Fig 2a) were collected using hand scoop net. The total number of eggs from each tank was determined by counting 1 ml of the egg sample and multiplying by the total volume of eggs obtained. Fertilized eggs were distinguished from unfertilized ones on the basis of color (Fig 2b) as the unfertilized eggs were opaque white in colour. Breeding performance was assessed using the following (Kumar *et al*., 2021):

Latency period (hrs) = The period from injection till the onset of ovulation (hrs)

$$\text{Relative fecundity} = \frac{\text{Total no. of eggs released}}{\text{Total body weight}}$$

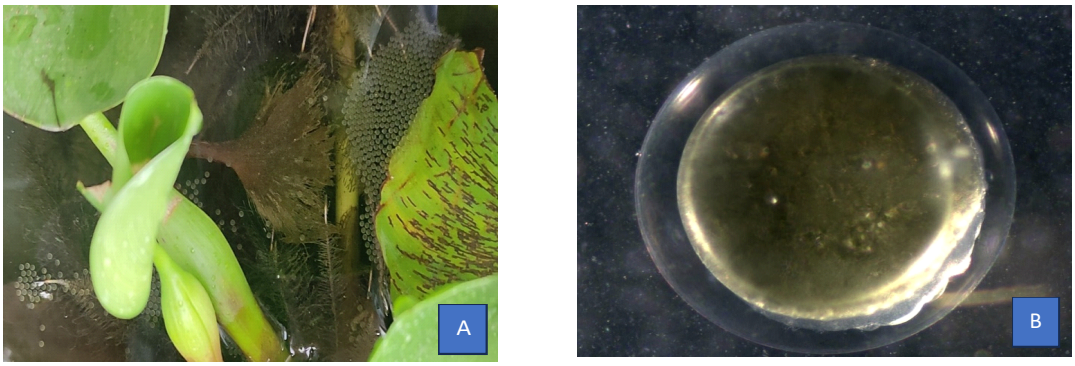
$$\text{Fertilization rate (\%)} = \frac{\text{No. of fertilized eggs}}{\text{Total no. of eggs}} \times 100$$

Incubation period (hrs) = The period of fertilization to the appearance of hatching heads

$$\text{Hatching rate (\%)} = \frac{\text{No. of eggs hatched}}{\text{Total no. of fertilized eggs}} \times 100$$



Fig 1: Administration of hormone.



a.) Clutch of fertilized eggs. b.) Fertilized egg

Fig 2: Fertilized eggs of *C. striatus.*

Statistical analysis

The Statistical Package for Social Sciences (SPSS) 26.0 was used for data analysis. The experimental data was subjected to Shapiro Wilk test for normality assumptions assessment. The data set which didn't meet normality assumptions were transformed using log transformation. The data which met the assumptions were analyzed using a two-way analysis of variance (ANOVA). The means were compared by Tukey HSD. Significance levels were set at 5% ($P \leq 0.05$).

RESULTS AND DISCUSSION

In the present study, significant ($P<0.05$) differences were observed in latency periods among the treatments. The shortest and longest average latency periods were observed in T2 (23.73 hr for OVAFISH and 21.46 hr. for Gonopro FH) and T1 (27.73 hr. for OVAFISH and 25.46 hr. for Gonopro FH) respectively which is depicted in Table 1 and 2. The duration of the latency period depends on the species and environmental parameters; mainly temperature (Brzuska, 1999).It varies with type of hormone administered and dosage of hormone (Sahoo *et al*., 2005) and ovarian maturation stage at the time of the hormone administration (Matsuyama *et al*., 1996). Short latency period might be due faster release of gonadotrophs as result of effective dose administration. Kumar *et al*., (2021) recorded longer latency period for HCG administered treatments of striped snakehead compared to CPH administered treatments. Similar results were reported in previous studies with *Barbus barbus* (Nowosad *et al*., 2016) and *Leuciscus idus* (Kucharczyk *et al*., 2020).

There were significant ($P<0.05$) differences in relative fecundities among the treatments. It was found highest in T2 (8314.33± 6.11 for OVAFISH, 9479.66 ± 16.37 for Gonopro FH) and the lowest (2225.33± 11.62 for OVAFISH, 2732.33± 6.54 for Gonopro FH) in T1 in both the hormone groups (Table 1 and 2). A greater plasma gonadotropin concentration might have stimulated final oocyte maturation and spermiation (Mylonas *et al*., 2010) which led to release of good quality gametes in the fish. The medium dose (0.6 ml/kg) gave the optimum result while lower dose (0.4 ml/kg) and higher dose (0.8 ml/kg), was not suitable for complete spawning. The partial ovulation might be due to insufficient release of gonadotropin at low dose (Sahoo *et al*., 2008) and being a batch spawner (Rawat *et al*., 2020) the fish released less eggs. At a higher dose ovulation might have decreased due to the plugging of the genital aperture and over-ripening might have led to clumping of eggs leading to low relative fecundity. Similar results were observed in *Pangasianodon hypophthalmus* (Legendre *et al*., 2000) and *Anabas testudineus* (Mandal *et al*., 2016). The relative fecundities were lower than previous study in snakehead bred with HCG and Carp Pituitary Homogenate (CPH) (Kumar *et al*., 2021), this might be due to the presence of propylene glycol, absent in HCG and CPH. A similar explanation was given in breeding trials of *Mystus gulio* (Kumar *et al*., 2021).

In this study, fertilization rates differed significantly ($P<0.05$) among the treatments. It was the highest (87.09 for OVAFISH, 88.68 for Gonopro FH) in T2 (0.6 ml/Kg) and the lowest (76.87 for OVAFISH, 80.30 for OVAFISH) in T1 (0.4 ml/Kg) in both the hormone groups (Table 1 and 2). The increased fertilization rates might be due to sufficient gonadotropin release for the final maturation of male and female gonads which initiated ovulations of the fully mature ova from the fish ovary. Excessive doses might have led to early milting resulting in poor fertilization in the highest dose and under-dosing caused late inducement in males (Das *et al.,* 2016). Moreover, the fertilization rates were found to be better than a previous study with *Channa striata* (Kumar *et al*., 2021) with hormonal implant and bred using HCG and where 1:1 sex ratio was maintained. This enhancement might be due to greater sperm to egg ratio attributed to the use of 1:2 (F:M) sex ratio. A similar observation was reported in *Anabas testudineus* (Mandal *et al*., 2016) and*Mystus gulio* (Kumar *et al*., 2021).

Significant ($P<0.05$) differences were observed in hatching rates among the treatments. Hatching rates were the highest (79.43 for OVAFISH, 86.06 for Gonopro FH) in T2 (0.6 ml/Kg) and the lowest (71.69 for OVAFISH, 75.79 for Gonopro FH) in T1(0.4 ml/Kg) in both the hormone

Table 1: Effects of OVAFISH on spawning efficiency of *Channa striata*.

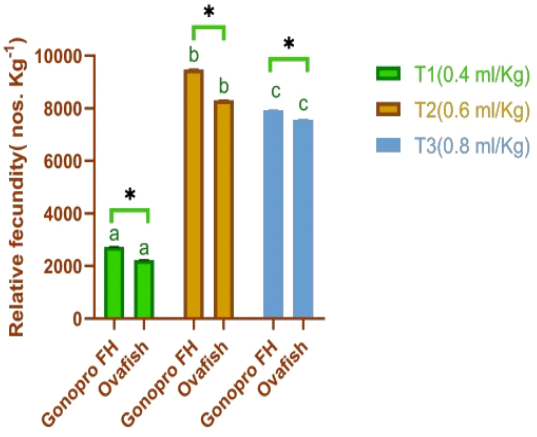
Parameters	T1 (0.4 ml/Kg)	T2 (0.6 ml/Kg)	T3 (0.8 ml/Kg)
Latency period (hrs.)	27.73±0.33^{b}	23.73±0.39^{a}	24.26±0.39^{a}
Relative fecundity	2225.33±11.62^{a}	8314.33±6.11^{c}	7561.66±10.29^{b}
Fertilization rate (%)	76.87±1.19^{a}	87.09±0.44^{c}	82.26±0.61^{b}
Hatching rate (%)	71.69±1.18^{a}	79.43±0.93^{c}	75.98±1.18^{b}
Incubation period (hrs.)	27.33±0.45^{c}	23.43±0.59^{a}	24.43±0.56^{b}

Above values are expressed as mean±SE(n=3). Mean values of all the parameters were subjected to a two-way analysis of variance. Different superscripts in the same row indicate significant difference (P<0.05) among different treatments.

Table 2: Effects of GONOPRO-FH on the spawning efficiency of *Channa striata*.

Parameters	T1 (0.4 ml/Kg)	T2 (0.6 ml/Kg)	T3 (0.8 ml/Kg)
Latency period (hrs.)	25.46±0.45^{b}	21.46±0.50^{a}	22.06±0.39^{a}
Relative fecundity	2732.33±6.54^{a}	9479.66±16.37^{c}	7920.00±8.97^{b}
Fertilization rate (%)	80.30±0.51^{a}	88.68±0.66^{c}	84.316±0.74^{b}
Hatching rate (%)	75.79±1.20^{a}	86.06±0.84^{c}	80.02±0.81^{b}
Incubation period (hrs.)	25.33±0.45^{c}	22.5±0.44^{a}	23.53±0.45^{b}

Above values are expressed as mean±SE (n=3). Mean values of all the parameters were subjected to a two-way analysis of variance. Different superscripts in the same row indicate significant difference (P<0.05) among different treatments.



Different alphabets (a, b and c) indicates significant (P<0.05) differences between the different doses and asterisk (*) indicates significant (P<0.05) difference between the hormones.

Fig 3: Graph depicting significant (p<0.05) two-way interactions between the relative fecundities of different hormones and the doses.

groups (Table 1 and 2). The increase in hatching rates might be due to fertilization of more fully matured eggs which were released due to higher gonadotropin release. Similar explanation was reported in previous study on *Channa striata* (Kumar *et al*., 2021). A very high doses of hormone can have harmful effects on egg quality, especially when administered with a single injection (Gardes *et al*., 2000) which may have led to decreased hatching rate in the fish administered with the highest dose.

Incubation periods were within the previously reported range (Rawat *et al*., 2020). There were significant (P<0.05) differences in incubation periods among the treatments. Incubation period were the shortest (23.43 hr. for OVAFISH, 22.5 hr for Gonopro FH) in T2(0.6 ml/Kg) and the longest (25.33 hr for OVAFISH, 27.33 hr for Gonopro FH) in T1(0.4 ml/Kg) in both the hormone groups (Table 1 and 2). Different incubation period may be attributed to species specific (Ngueku, 2015) and the efficacy of the inducing agents. Biochemical activities are stimulated by heat energy, rising temperature fasten metabolism (Kumar *et al*., 2016), therefore, incubation periods can vary with the temperature. Shorter incubation period in the optimal dose (0.6 ml/Kg) might be due to optimal fertilization of fully mature eggs leading to faster development.

Two way significant (P<0.05) interaction was observed only between the relative fecundities (Fig 3) of the two hormones whereas there were no other significant(P>0.05) interactions between other breeding aspects of the two hormones. The variations in effective dosage among different species might be due to the different levels of dopamine activity (Peter *et al*., 1986). Relative fecundities, in this study, were higher in Gonopro FH induced fish in comparison to OVAFISH induced fish. This might be due to suitability of the synthetic hormone constituents to the snakeheads under climatic conditions of Assam. The efficacy of different inducing agents was reported by several studies. Chaturvedi *et al*. (2015) reported the superior efficacy of Ovatide over Ovaprim and Gonopro-FH for induce spawning of silver carp. In a similar study, Ovaprim was found to be better over Ovatide and carp pituitary extract in terms of hatching rate of *L. rohita* (Gurpreet and Sudhanshu, 2012). In the present study, for both the inducing hormone the optimal dose was found as 0.6 ml/Kg body weight of female to conduct the artificial breeding of *Channa striata* in captive condition.

CONCLUSION

The present study demonstrated that *C. striata* can be bred using synthetic hormones under the climatic conditions of Assam. Two hormones (Gonopro FH and OVAFISH) were found to be efficient in induce breeding of *C. striata*. In both the cases 0.6 ml/Kg and 0.45 ml/kg body weight of female and male respectively was found to be the optimal dose. Moreover, the fish induced with Gonopro FH produced better relative fecundities than the fish induced with OVAFISH. Findings of the study suggest that Gonopro-FH can be a more suitable inducing agent for mass production of *C. striata* under climatic conditions of Assam. The results may significantly facilitate quality seed production towards development of aquaculture of *C. striata*.

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Conflict of interest

The authors declares no conflict of interest.

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