

A Study of The Reproductive Performance of *Clarias Batrachus* Using Alpa-FH With Particular Attention to The Appearance of Morphological Defects During Early Life Stages

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Abstract

Abstract In the present investigation induced breeding of walking cat fish, *Clarias batrachus* with synthetic hormone Alpa-FH was made. An optimum result was found in induced spawning by using hormone at the dose of 0.8 ml kg⁻¹ body weight to female followed by stripping method with higher rate of fertilization (80.4%) and hatching (84.1%). Further, frequency of some common morphological deformities (1.27-3.83%) was also recorded in the induced bred *C. batrachus* hatchlings in the present study with a highest percentage at the dose of 1.2 ml kg⁻¹ body weight to female. Results of the present investigation would help mass scale quality seed production of *C. batrachus* under captive condition to augment market demand of the seed.

Keywords: *Clarias batrachus*, induced breeding, Alpa-FH, morphological deformities

Introduction

The walking catfish, *Clarias batrachus* Linnaeus, 1758 (Siluriformes; Clariidae), commonly known as “desi magur”, is a commercially important fish species in India. The fish has great market demand owing to its high consumer preference especially in North-Eastern parts of India and fetches comparatively higher price than carps [4, 11, 60]. The fish can be cultured in the low-lying paddy fields, derelict waters, swamps, oxidized ponds, seasonal water bodies or any other water receiving domestic wastes because of their hardy nature, ability to thrive under adverse water conditions and suitability to culture in limited space [4, 10, 61]. Further, culture of this indigenous fish species gets emphasis as a part of promoting diversification in culture practices in Indian aquaculture [33, 55, 65]. But the major constraint in the culture of *C. batrachus* is the non-availability of quality seeds from the natural resources. It has been declining sharply due to environmental degradation for rapid industrialization and injudicious application of pesticides, shrinkage of natural breeding ground, over exploitation and illegal killing of juveniles and brood fishes [32, 34, 60]. Induced breeding techniques for *C. batrachus* have been successfully used for seed production by few workers using various natural and synthetic agents like piscine pituitary gland extract, Human Chorionic Gonadotropin (HCG), Ovaprim, Ovatide etc. [5, 12, 21, 34, 50, 52, 53, 59]. In the present investigation, trial was made for induced breeding of magur using various doses of Alpa-FH followed by stripping method to determine its efficacy in breeding success and as well as to achieve higher rate of fertilization and hatching. Further, the deformity in larvae is

not uncommon in teleostean species for both freshwater and marine [3, 54, 56]. The causes of such deformities are invasive and may be attributed to nutritional [9, 40, 43], metabolic [38, 57], environmental [13, 17, 23, 25, 41] and genetic [35, 69] factors. Toxicological aspects like indiscriminate use of pesticides [24, 57] and non-judicious uses of heavy metals [6, 22, 38, 46, 49, 64] are also reported to have teratogenic effects on teleostean development. Deformities have also been reported as a result of traumatic injury [29], parasites [68] or by non-inheritable congenial defects [66]. Existence of unfavorable abiotic and rearing conditions may also have some crucial role in appearance of malformations in the early stages of life of fish [15, 18, 26]. There are several morphological deformities frequently observed in the hatchlings of the freshwater fish produced through induced breeding which adversely effect on their survival, growth as well as susceptibility to diseases, thus hamper quality fish seed production [8, 54]. Such abnormalities are often encountered in higher frequency during hatching in hatchery produced individuals than wild [20, 31, 58]. Teji and Thomas, 2006 [67] recorded various morphological abnormalities like under developed head and barbell, deformed trunk, enlarged yolk sac, curved tail and vertebral deformities in induced bred larvae of stringing catfish (*Heteropneustes fossilis*) and striped dwarf catfish (*Mystus vittatus*). In *C. batrachus*, abnormalities in larvae like acephala, tunicate, indeterminate growth etc. are frequently observed along with the normal larvae during breeding operation [54]. Sharma et al., 2010 [59] mentioned larval deformity in the same species induced by different doses of Ovatide. Similarly, Alarape et al., 2015 [1] recorded different organoleptic and morphological abnormalities in hatchery raised *C. gariepinus*. Fagbuaro et al. 2006 [14] observed deformation in hatchery bred *C. gariepinus* and its hybrids. The effect of various doses of Ovaprim and HCG on *C. batrachus* was observed by Sahoo et al., 2007 [51] and Sahoo et al., 2009 [52] respectively. In the present study, frequency of common morphological abnormalities among the hatchlings of *C. batrachus* as an effect of different doses of Alpa-FH, was also recorded because knowledge of early development of young fish is imperative for expansion of catfish culture [42, 67].

Materials and methods

Disease free healthy gravid male (average length 26 ± 1.7 cm and average weight 142 ± 7 gm) and female (average length 24 ± 1.5 cm and average weight 225 ± 10 gm) *Clarias batrachus* were collected from the local accredited hatchery during the months of monsoon (July-August, 2017) and kept sex wise in the separate tanks with sandy bottom for acclimatization for a week prior to breeding operation filled with unchlorinated, iron free tap water (pH 7.2 ± 0.15 , CO₂ 10.22 ± 2.5 mg l⁻¹, D.O. 5.76 ± 0.36 mg l⁻¹, total alkalinity 156 ± 4.10 mg l⁻¹ as CaCO₃ and hardness 110 ± 4.50 mg l⁻¹ as CaCO₃) having aeration facilities. Aquatic macrophytes like water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) were provided in the tanks to make the habitat natural. Gravid female fish were identified by the presence of soft swollen belly with short, button shaped and slit like genital papilla. Prime maturity was determined by examining the size uniformity of the eggs released by the gentle pressure on the swollen abdomen. Fully gravid males were identified by their slender and streamlined body with conical and elongated papilla having pointed reddish tip. During breeding operation different doses of Alpa-FH [combination of 20 µg salmon gonadotropin releasing hormone analogue and 10 mg domperidone (as a dopamine receptor antagonist) dissolved in 1.5% v/v non-toxic organic solvent Benzyl alcohol, a product of Alpa Laboratories Ltd., Pigdamber, India], as a synthetic inducing agent (Batch no. S1203) were administered to separate sets of brood fishes consisting of both sexes at 1:1 ratio. The females were injected with the doses of 0.4, 0.6, 0.8, 1.0 and 1.2

ml per kg body weight to evaluate their breeding performance while male brooders were injected with a single and minimum dose of 0.2 ml per kg body weight as to trigger the response. The required doses of inducing agent were administered intramuscularly at the caudal peduncle region above the lateral line sense organ by 2 ml hypodermic syringe with a small size needle (No.24) at 45° angles during evening time.

After injection, male and female brooders were kept separately. After a gap of 13.5 - 18.0 hours of injection the females, depending on their ambient physiological condition, were stripped for spawning and at the same time the males were also cut open. Testes were carefully removed from male in intact condition, cut into small pieces with scissor and squeezed properly. The milt was preserved in 0.9% sodium chloride (NaCl) solution. The sperms remain in dormant condition in this suspension which was used immediately. The eggs were stripped into previously washed dry and clean enamel tray. Milt suspension was added gently to the eggs by a dropper during and immediately after stripping preferably within 2-3 minutes and thoroughly mixed with the help of disinfected bird's feather. A little freshwater was added also to activate the sperms and then the tray was jerked gently for 2-3 mins for proper mixing of eggs and milt. The fertilized eggs were quickly washed and cleaned several times with fresh tap water for water hardening as well as to remove the residual milt and foam formed. Unfertilized eggs, blood clotting, muscular dirt etc. were also discarded. Finally, the transparent, fertilized eggs were transferred to a flow-through system for incubation after proper hardening. During this, the fertilized, demersal and adhesive eggs were uniformly distributed in the white enamel tray (46 cm x 30 cm x 7.5 cm) @ 3000 ± 250 eggs per tray, fitted within the high-density plastic or PVC trays (57 cm x 37 cm x 10.5 cm) having regulated water flow and continuous aeration facilities. Each PVC tray was fitted with two outlets at different levels. The outlets of the trays in each row were connected to a common drainage pipe. Water was supplied to the trays through separate taps in shower system at a regular flow rate of about 1- 1.5 l min⁻¹ as required. The water in each tray was continuously aerated lightly throughout the experiment to elevate dissolved oxygen levels to > 5 mg l⁻¹. The outlets of all the trays were covered with No.60 bolting silk cloth to prevent escape of eggs and spawns. Water level was regulated during hatching by opening and closing the outlets. Hatchlings were also reared in the same flow through system @ 2000 ± 175 fish seed per tray upto 4th day and 1500 ± 200 fish seed per tray upto 12th day until the young developing fish started vertical movement frequently. Later on, the density was reduced to 1200 ± 156 fish tray⁻¹ upto 20th day of rearing. No feed was given upto 4th day of rearing due to presence of yolk material but sieved zooplankton were supplied as preferred live feed from 5th to 20th day of rearing. Feed was given @ 2-3 ml lit⁻¹ water @ 4 hr interval upto 12th day and at 8th hr interval for the rest period of study. Water temperature during the study period ranged between 26-28°C. For assessing total number of stripped out eggs, a small portion of egg mass was randomly collected from each set and were counted by the following equation: Total number of stripped out eggs = (Total weight of stripped out eggs x N)/ Weight of the collected egg mass. [Where, N = total number of eggs in the collected egg mass]. Similarly, for the assessment of fertilization rate, a small portion of egg mass was randomly collected in a clear petridish filled with unchlorinated and iron free tap water for each set. After checking with magnifying glass, the percentage of fertilization was calculated by using the following formula: Fertilization rate = (N x 100)/Total no. of eggs in the collected egg mass. [Where, N = total number of fertilized eggs in the collected egg mass].

After hatching, the hatchlings were counted by eye estimation method and the hatching rate was recorded by the following formula: Hatching rate = (N x 100)/Total no. of eggs released in a

tray. [Where, N = total number of hatchlings obtained]. In the present study, frequency of the morphological deformities in the hatchlings of the induced bred *C. batrachus* along with their behavioral pattern in comparison to their normal hatchlings was also recorded at every set of breeding operation with scheduled doses of inducing agent to female. The whole cycle of the experiment was repeated thrice in order to overcome methodological errors. All the values in the present study are expressed as the mean \pm SD of three replicates and were statistically analyzed by one way ANOVA (analysis of variance) followed by DMRT (Duncan's Multiple Range Test) to determine significant differences between the means ^[16].

Results In the present study female *Clarias batrachus* injected with five doses of synthetic inducing hormone Alpa-FH (0.4, 0.6, 0.8, 1.0- and 1.2-ml kg^{-1} body weight). The breeding performance of the fish at the different doses of inducing agent in respect of stripping property and development of the respective gonads of the female brooder as well as consequent fertilization and hatching rate is presented in Table 1. Female *C. batrachus* showed smooth stripping and released cluster less fully developed ova when they were employed with 0.8 and 1.0 ml of Alpa-FH kg^{-1} body weight, while at the dose of 0.6 ml kg^{-1} body weight stripping was more or less smooth and ova were moderately developed. In case of administration of lower (0.4 ml kg^{-1} body weight) and higher (1.2 ml kg^{-1} body weight) doses, stripping was not smooth and came out in cluster. A considerable no. of ova loses their normal property in respect of shape, colour and hardyness due to over-ripeness was recorded at higher dose (Table 1). However, testes were fully developed and swollen and a significant amount of milt was obtained from male on squeezing at all the time. Depending on the optimum physiological condition of the induced female brooder, minimum latency period ranging between 13.5-15.0 hr with a mean of 14.33 ± 0.44 hr was observed when fish were injected with 0.8 ml kg^{-1} body weight (Table 1). On the contrary, 17.0-18.0 hr of latency period with a mean of 17.5 ± 0.29 hr was recorded in the females subjected to lower dose i.e. 0.4 ml kg^{-1} body weight (Table 1). However, in case of all doses, latency period as recorded in table 1 were significantly different ($p < 0.05$) from each other. After stripping significantly highest ($p < 0.05$) no. of eggs (4857.00 ± 47.27) was obtained from the female brooder fish injected with 0.8 ml kg^{-1} body weight of Alpa-FH than the other doses of stimulant (Table 1). On the other hand, significantly ($p < 0.05$) lower nos. of stripped out eggs were 3244 and 3422 when female fish were administered with lower and higher doses respectively (Table 1). Similarly, moderate nos. of eggs (4383.33 ± 68.46 and 4373.33 ± 81.99) were obtained from the stripped female brooder when induced with 0.6- and 1.0-ml kg^{-1} doses respectively which significantly ($p < 0.05$) differed from the other doses (Table 1). The rate of fertilization and hatching of eggs was significantly higher ($p < 0.05$) at the dose of 0.8 ml kg^{-1} than the other doses of inducing agent (Table 1). In the present study, the rate of fertilization of eggs released by the female injected with 0.8 ml kg^{-1} body weight was 80.4% followed by 72.9, 67.9, 49.8 and 48.8% for the doses of 1.0, 0.6, 0.4 and 1.2 ml of the stimulant kg^{-1} body weight respectively (Table 1). Similarly, higher rate of hatching success (84.1%) was recorded at the dose of 0.8 ml kg^{-1} body weight of female and lower hatching rate (52.7%) was observed at the lower dose of 0.4 ml kg^{-1} body weight (Table 1). Frequency of morphological deformities recorded in the hatchlings in every breeding set wherein female brooder magur administered with different doses of Alpa-FH is given in Table 1 which was significantly ($p < 0.05$) different from each other. Rudimentary barbels, enlarged yolk sac and curved tail were most common in the abnormal hatchlings. They were clumped together and remained in the lateral position on the bottom with least or no movement. Abnormalities in the trunk region with 'coma' shaped appearance and vertebral column impairment were also observed. Feeble tail lashing and

sometimes jerk movement were exhibited by these abnormal hatchlings. In some cases, underdeveloped dorsal and pelvic fin formation was encountered. They were shown intermittent rotational movement. Deformation was significantly ($p < 0.05$) higher in the hatchlings produced from the brooder female injected with lowest (0.4 ml kg^{-1} body weight) and highest doses (1.2 ml kg^{-1} body weight). The frequency of the abnormalities observed in the hatchlings was ranged between 1.27 - 3.83% with a highest frequency at the dose of $1.2 \text{ ml stimulant kg}^{-1}$ body weight and lowest at the dose of $0.8 \text{ ml stimulant kg}^{-1}$ body weight (Table 1).

Table 1: Breeding performance of female *Clarius batrachus* at the different doses of Alpa-FH. Values (Mean±SD) for different parameters within rows indicated by different superscript letters (a, b, c, d, e) are significantly different (DMRT, $p < 0.05$) but indicated by same superscript letters are not significantly different (DMRT, $p > 0.05$).

	Dose of Alpa-FH on female brooder (ml kg^{-1} body wt)					
	0.4	0.6	0.8	1.0	1.2	
Spawning response of female brooder	Effects of stripping	Stripping was not smooth; blood came out sometimes	Stripping was more or less smooth	Stripping was smooth	Stripping was smooth	Stripping was not smooth; Plugging of eggs often regulated in the way of vent
	Effects on ovary	Ova were not fully developed and came out in cluster	Ova were moderately developed; few clusters of ova were seen	Ova were well developed; no cluster was recorded	Some ova lose this normal property in relation to shape, colour and hardyness	A considerable no. of ova loses their normal property in respect of shape, colour and hardyness
Latency period (hr)		17.50 ^a ±0.29	16.67 ^{ab} ±0.33	14.33 ^c ±0.44	15.17 ^b ±0.6	16.33 ^{ab} ±0.33
Total no. of eggs stripped out		3422.33 ^c ±94.38	4383.33 ^b ±68.46	4857.00 ^a ±47.27	4373.33 ^b ±81.99	3422.33 ^c ±94.38
Total no. of fertilized eggs obtained		1704.33 ^c ±46.93	2976.67 ^c ±46.44	3905.00 ^a ±37.98	3188.33 ^b ±59.81	1985.00 ^d ±30.66
Percentage of fertilization over stripped-out eggs (%)		49.8	67.9	80.4	72.9	48.8
Total No. of hatchlings obtained		898.33 ^c ±24.63	2161.33 ^c ±33.63	3284.00 ^a ±31.9	2417.00 ^b ±45.37	1103.33 ^d ±17.07
Percentage of hatching over fertilized eggs (%)		52.7	72.6	84.1	75.8	55.6
Frequency of deformities occurred hatchlings (%)		3.23 ^b ±0.15	2.77 ^c ±0.18	1.27 ^e ±0.12	1.83 ^d ±0.09	3.83 ^a ±0.15

Discussion

In the present study, Alpa-FH has been successfully employed for induced breeding of commercially important catfish *C. batrachus*. The stripping responses as well as higher egg production, higher rate of fertilization and high hatching success were obtained at the dose of $0.8 \text{ ml stimulant kg}^{-1}$ body weight to female brooder probably due to the full maturity of ova, production of clusterless eggs in females (Table 1). The dose may properly activate Gonadotropin hormone-II (GTH-II) cell present in the Pars distalis of the induced fish which helps in the proper secretion of GTH-II. This secreted GTH-II binds to specific receptor in the granulose cells of ovary subsequently stimulates steroid hormone synthesis in these cells resulting in the better ovulation [27]. The dose (0.8 ml kg^{-1}) may be stimulated the brooder female fish effectively by contracting the smooth muscle in the gonoduct of female before ovulation resulting in higher success in the breeding performance of the fish [62]. Again, Alpa-FH @ 0.4 ml kg^{-1} female body weight may be a suboptimal dose which was not suitable for complete ovulation, thus, striping was not smooth. On the other hand, the lower responses at lower doses

of stimulant in the present study may be due to insufficient secretion of gonadotropin leading to ovulation failure or blocking of ovipore by disintegrated ovarian tissue and egg bunches results into asynchrony between maturation and ovulation^[48, 50]. Similar results were also reported by Mahapatra et al., 2000^[34] and Sharma et al., 2010^[59] using Ovaprim and Ovatide respectively as the stimulants. Further, higher dose may result in early ovulation and the ovulated eggs remained in the ovarian lumen in a hypoxic condition for longer time leading to over ripeness and subsequently resulting to lower success of fertilization and hatching^[19, 28, 39]. This was in agreement with the present study. Zonneveld et al., 1988^[70] opined that right combination between dose of inducing agent and latency period is significant for optimum quantity of spawning egg in catfish. In present study, latency period within 13.5 to 15.0 hr was congenial for smooth release of eggs, better fertilization rate and hatching success. Such period for proper spawning was also almost in agreement with some earlier workers^[12, 44, 50]. Morphological deformity in hatchlings is not uncommon in induced bred freshwater catfish^[14, 51, 52, 54, 67]. The deformities in hatchery bred larvae of *C. batrachus* ranged from 7-8% during pre and post monsoon, and 3-4% during monsoon^[54]. Linhart and Billard, 1995^[30] noted the quantity of deformed larvae in *Silurus glanis* as high as 50%. The occurrence of deformity in *C. batrachus* larvae was 10-11% at the dose of 1.5-2.0 ml of Ovaprim at 23hr latency period^[51]. Similar higher percentage (more than 11%) of deformed larvae was observed in the same species induced with HCG at the dose of 5000 IU at the 20-23hr of latency period^[52]. Normally ovum maturation and ovulation in fish is controlled by episodic release of gonadotrophins but the surge of gonadotrophic hormones due to stimulation of inducing agents might be one of the reasons for the production of deformed hatchlings^[67]. In the present study, deformation in the total population of induced bred *C. batrachus* hatchlings ranged between 1.27-83% (Table 1). This is almost in conformity with the data recorded by Sahoo et al., 2004^[54] on *C. batrachus* induced to breed during monsoon. Observation of more frequency (3.83%) in the hatchling deformity at highest dose (1.2 ml of the body weight) in the present study may be due to the fertilization of unripe and over-ripe eggs^[59]. Perhaps

ovulation of the over-ripe ova may cause due to stimulation of inducing agent and ova come out during stripping. These ova were viable and able to fertilize, but probably led to impaired embryogenesis which results into the production of deformed hatchlings^[36, 37, 45, 49, 54]. Besides, Lam et al., 1978^[28] opined that over-ripe eggs did not form a perivitelline space when placed into fresh water, suggesting that there might be a reduction in the permeability of the chorion. Such reduced permeability of the chorion to water may adversely effect on the utilization of yolk materials and subsequently leads to retarded or abnormal embryogenesis in the over-ripe eggs^[59, 63]. Similarly, release of unripe eggs cannot be ruled out during the stripping of incomplete ovulated female brooder. So, the frequency of occurrence of deformed larvae (3.23%) at lower dose (0.4 ml of the body weight) of inducing agent may be resulted from the fertilization of unripe ova during induced breeding operation in the present study supporting the previous reports on *Clarias* sp.^[47, 51, 52, 53]. The deformity of larvae is originated due to poor egg quality as a result of fertilization of unripe or over-ripe ova or as an impact of several unfavorable biotic and abiotic factors during hatchery operation like inbreeding, scarcity of nutrients, parasitic infestation, and oxygen depletion, water current, sudden changes in temperature etc^[52, 54, 56]. In the present study, management of water quality and supplement of food was maintained at optimum level. Al-Hassan, 1982^[2] suggested low dissolved oxygen level in the water during spawning and developing stages may be responsible for deformities in fish. But aeration was continued during the hatching and rearing process to maintain optimum dissolved oxygen contents in the present study. Further, there was no scope of inbreeding depression. Thus, such

factors cannot be limiting factors for the present study. Though the further study is needed to find out the exact reason of deformities in *C. batrachus* larvae as a function of Alpa-FH but the poor egg quality may be logically supposed to be a significant reason for that.

Summary and Conclusion

Alpa-FH, an inducing synthetic hormone consisting GnRH analogue in combination with dopamine antagonist, can be effective in controlled spawning of *Clarias batrachus*. Most successful result in respect of their egg production (4857 ± 47.27 nos. per female), rate of fertilization (80.4%) and hatching (84.1%) was recorded when the female brooder injected with the inducing agent @ 0.8 ml kg^{-1} body weight during the present breeding operation. This breeding operation may provide some valuable information in the development of breeding technology of *C. batrachus* and be helpful for the fish breeders for its minimum dose efficiency. Further, the observation on the frequency of abnormal deformities in the hatchlings in the present study may also supplement some helpful information in the genesis of knowledge to overcome constrains during the early development of *C. batrachus* seeds and thereby enhance quality seed production during induced breeding.

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