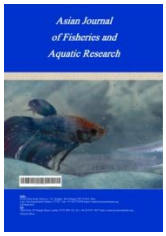

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Optimization of Hormone for Artificial Breeding and Seed Production of *Clarias batrachus* (Linnaeus, 1758) Under Captive Condition

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study aimed to develop brood stock of Magur (*Clarias batrachus*), artificial breeding and seed production under captive condition using growth hormones in north-eastern Bangladesh.
Study Design: Fifteen pairs of brood *Clarias batrachus* were used in this experiment. Brood fishes were reared up to maturation for spawning operation in the farm by providing artificial diet for 4-5 months before onset of breeding season. In the present experiment, the brood fishes were induced with three growth hormones viz., Ovupin (100 mg dompridone and 0.2 mg S-GnRHa) at the rate of 1 $ml.kg^{-1}$ body weight, Flash (20 mg S-GnRHa, 10 mg dompridone IP and propylene glycol) at the rate of 1 $ml.kg^{-1}$ body weight and Human Chorionic Gonadotropin (HCG) hormone at the rate of 2272 $IU.kg^{-1}$ body weight; respectively. The broods were injected with single injection in all treatments.

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Place and Duration of Study: The present study was conducted on induced spawning of Magur (*Clarias batrachus*) at the Reliance Aqua Farm, Boilor, Trisal of Mymensingh district from March to August, 2016.
Methodology: Sexually mature healthy, uninjured male and female fishes were collected from the broodstock ponds for induced breeding. The fishes were transferred in the clean and germ free conditioning tanks for about 6-7 hours. During conditioning, continuous splash of water was given by keeping the male and female separate. The fishes were then weighed individually and injected intramuscularly with Ovupin, Flash and HCG hormone respectively. Only females were injected at 45° angle of the caudal peduncle. About 23-24 hours of post-injection the testes from males were dissected and mixed with stripping eggs for fertilization.
Results: The incubation period for fertilized eggs were 23-24 hours for all the treatments. Fertilization rates were 78.20±0.52, 93.09±0.61 and 81.60±0.97 % and hatching rates were 54.93±0.61, 75.77±0.47 and 64.41±0.71 % in Ovupin, Flash and HCG treatments, respectively.
Conclusion: This study revealed higher fertilization (93.09 %) and hatching (75.77 %) rate was achieved with Flash hormone. Therefore, Flash hormone could be effective for the artificial breeding and seed production of Magur (*Clarias batrachus*).

Keywords: Brood; flash; HCG; ovupin; propagation.

1. INTRODUCTION

Over population together with global warming is an alarming threat to the human civilization. To defend the recent consequences, food security and its sustainable production have got considerable attention throughout the world. High production in a sustainable way can be a probable measure for recent consequences. For example, in Bangladesh fish has been a major source of protein, contributing about 60 % of the animal protein in the daily diet of the people [1]. Due to natural and manmade hazards, biodiversity of fish and other aquatic organisms in open water have been declining so much and with such rapidity that the aquatic animals, especially fish are unable to cope with [2]. That's why the dependency on hatchery produced fry has increased rapidly to protect the species from being extinct. Fin fish hatchery was first established in Jessore by Mohoshin Master in 1967. In Bangladesh both public and private hatchery produced around 6,86,754 kg hatchling [1]. Among the freshwater fishes, catfish is one of the most important groups of fish in this delta and is getting increasingly popular showing a promising future for commercial culture [3].

Magur (*Clarias batrachus*) is a freshwater species and abundance of magur is decreasing now-a-days. Growth of magur is comparatively faster compared to any catfish. Bangladesh is a densely populated country and to supply the increasing protein demand, seed production of magur by artificial propagation is mandatory. For this reason fishery scientists developed the technique of producing quality fish seed through induced breeding. As many natural and induced phenomena occurring in aquatic ecosystems, the natural breeding and feeding grounds of some of the important floodplain and riverine fishes and their habitats have been severely degraded. Open water capture fisheries are under great stress and their sustainability is in danger because of changing aquatic ecosystem, soil erosion, siltation, construction of flood control and drainage structures, dumping of agro-chemicals and industrial pollutants.

Therefore, proper techniques of induced breeding and larval rearing for large scale production of fry are the most crucial factors in expanding culture practice for this species. Although, some works have previously been conducted on its induced breeding the techniques available have not been standardized to be recommended at farmer's level. In the present works some attempts have been made to focus on these aspects. Air breathing catfish of Bangladesh (*Clarias batrachus*, Clariidae) normally breeds from April to August and attains a maximum length of 35 cm and a weight of 250 g [4].

The walking catfish (*Clarias batrachus*) is a potential species for aquaculture. This species is very much popular and valuable fish in Bangladesh for several reasons. They are important part of the diet for children and lactating mothers and also prescribed as diet for the convalescents. Considering high market price and high consumer demand fish farmers show

considerable interest in its culture. Culture of magur is highly profitable due to its extreme hardiness, fast growth, high fecundity and ability to survive in poorly oxygenated water. It has been observed that the price of spawn/larvae is higher at early season than late season. Hatchery owners want to supply spawn/larvae at early season because of fry market demand to improve their socio-economic conditions.

Furthermore the species can be kept alive for long time by storing in a water container without giving any food as the species bear special accessory respiratory organ (arborescent organ). This fish is highly nutritious for food because every 100 g of its flesh contains 32.0 g protein, 2.0 g fat, 0.7 g iron, 172 mg calcium, 300 mg phosphorus and 66.3 g moisture [5]. Development of induced breeding has been a major breakthrough in promoting fish seed production. Quality of fish seed in mass scale is a must to revolutionize fish production in Bangladesh. Therefore, the present study was carried out to standardize the hormones for artificial breeding and seed production of *Clarias batrachus*.

2. MATERIALS AND METHODS

2.1 Study Site and Duration

The experiment was carried out at the Reliance Aqua Farm Hatchery, Ukilbari, Trisal, Mymensingh during the period from March to August in 2016. Reliance Aqua Farm is a leading fish hatchery of Bangladesh based in Mymensingh. It is one of the few fish hatcheries of Bangladesh that comply with the recent hatchery regulations of the government. This hatchery was founded by Ritish Pandit (Managing director) in 2004 with the aim to produce seeds of monosex Tilapia, Shing, Magur, Pabda and Koi. This occupied 20 acre area of which 1.5 acre is hatchery complex and 18.5 acre brood rearing pond area.

2.2 Broodstock Management

Magur fishes were cultured at a stocking density of 100 fish decimal^{-1} in the earthen ponds of the hatchery for broodstock development. The fishes were intensively cared by applying high protein (32 %) commercial diet enriched with vitamin (A, B, C and E) twice daily at the rate of 3-5 % body weight to make them sexually mature. Magur become sexually mature at the age of 1 year when the weight reached around 100 g.

2.3 Collection and Selection of Brood Fishes

The brood fishes were collected from the broodstock ponds in the late morning and were kept in plastic bucket containing water. Then the fishes were selected for induced breeding. It is easy to identify mature male and female broods on the basis of their secondary sexual characteristics. During breeding season (June-July) females were easily identified by their soft and swollen abdomen, robust and pigmented body, round and blunt genital opening due to full of mature eggs. Whereas, males were identified by their relatively smaller in size, normal abdomen, more translucent and less pigmented body, elongated and long protruded genital papillae. The fishes containing spot on their body and unhealthy were discarded from being selected. Only healthy, uninjured and matured fishes were selected for breeding.

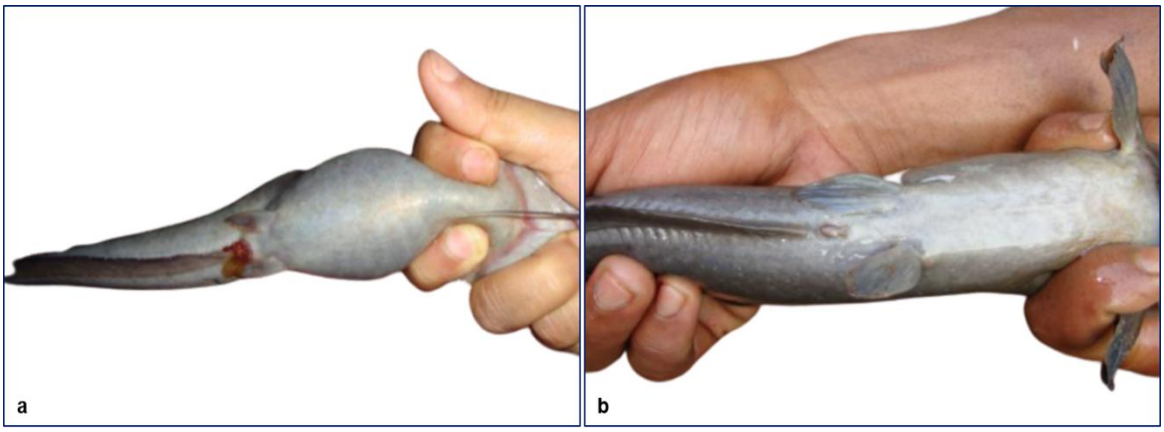

Fig. 1. Mature brood (a) Female with swollen; and (b) male with normal abdomen

2.4 Conditioning of Brood Fish

Before conditioning the tanks were washed properly with lime and potassium permanganate at the rate of 10 ppm and filled with clean water. The brood fishes were transferred in the clean and germ free conditioning tanks for 6-7 hours. During conditioning, continuous splash of water was given by keeping the male and female separate. No feed was given to the brood fishes during conditioning.

2.5 Preparation of Inducing Agent and Hormone Administration

All the hormones were collected from local market and kept in ambient temperature. Each vial of Ovupin (100 mg dompridone and 0.2 mg S-GnRHa) hormone was dissolved in 8ml saline water and Flash (20 mg S-GnRHa, 10 mg dompridone IP and propylene glycol) hormone was prepared by mixing with 1ml distilled water. However, human chorionic gonadotropin (Chorionic epithelioma, proprietory HCG active-8 complex, L-orthinine, L-cartinine, beta- alanine and adrenalinum) was packed in the vial as powder form. Each vial contains 5000 IU which was mixed with 2.5 ml distilled water. After hormone preparation, the brood fishes were handled smoothly. During injection, the fishes were kept on foam and the head region of the fish was wrapped by a wet and soft towel. Then, Ovupin (1 ml.kg-1 body weight), Flash (1 ml.kg-1 body weight) and HCG (2272 IU.kg-1 body weight) hormone were administered intra-muscularly to the females at about 45° angle of the caudal peduncle. After the injection was done, females were transferred to the breeding tank.

2.6 Spawning, Fertilization and Hatching

In this experiment fishes were allowed to spawn artificially. The females were collected from the breeding tank for stripping. The mature eggs were stripped out on a bowl. The testes from males were dissected out, cutting into pitches and mixed with mature eggs for fertilization. Total number of eggs and rate of fertilization were calculated by direct counting method. Three trays with a capacity of 1.50 liter were used for this purpose. For determination of fertilization rate, approximately 300 eggs were placed in each tray. Then the eggs were observed under a magnifying glass and fertilized eggs were counted. After 12-15 hours of fertilization, the transparent eggs were considered as fertilized eggs whereas, the opaque eggs were considered as unfertilized. The fertilization rate was determined by following formula:

Fertilization rate (%) = (Number of fertilized eggs/ Total number of eggs (fertilized + unfertilized)) × 100

The eggs were kept under shower of water by piercing a slender pipe and water run through it for the incubation and hatching. After 22-24 hours of incubation the eggs were hatched and the hatchling came out from the eggs. The hatchlings were counted by visual observation. At first the hatchling was taken in a white enamel bowl by siphoning method. From the counting of hatchlings, the rate of hatching was calculated by the following formula:

$$\text{Hatching rate (\%)} = \frac{\text{Number of hatchlings}}{\text{Total number of fertilized eggs}} \times 100$$

2.7 Data Analysis

Collected data were put in the spread sheet in Microsoft Excel (version 2016) and analyzed as mean±standard deviation (SD) for all the treatments. Number of egg released, fertilization rate (%) and hatching rate (%) of *Clarias batrachus* under different treatments were displayed in tables and figures. In contrast, few statistical tests were done on the basis of the objectives of the study using another computer program named, Statistical Product and Service Solutions (SPSS) ver. 20. One-way ANOVA and Duncan's Multiple Range Test (DMRT) were employed to observe the differences in number of egg released, fertilization and hatching rate among the treatments at a significance level of $P<0.05$.

3. RESULTS

3.1 Breeding Behaviour

The length and weight of brood fish used in the breeding trials, egg output, fertilization, and hatching rate in response to different hormonal treatments are summarized in Table1. After 6-10 hours of hormonal administration, the brooders of *Clarias batrachus* showed various coupling behavior in all three treatments. Each female was paired with a male and the breeding progress by showing active wooing. The male followed the female when it swims around the

breeding tank. Before spawning, females were swimming up frequently at the water surface. About 23-24 hours of post-hormonal injection, eggs were collected from the female by stripping method and the males were dissected and testes were taken out from the body cavity for the collection of milt. Subsequently, the eggs and milt were macerated in 0.9 % salt solution.

3.2 Breeding Performance

The brooder size, number of egg released, fertilization rate and incubation period of all the treatments were displayed in Table 1. The total number of egg released in T2 (5543.67±79.94) was significantly higher than T1 (2326.33±24.01) and T3 (4153.33±24.66), respectively. Similarly, T3 was also significantly higher than T1. Likewise, the fertilization rate (93.09±0.61) of T2 was also higher than T1 (78.20±0.52) and T3 (81.60±0.97), respectively (Table 1). Alike number of egg released, the fertilization rate of T3 was also significantly higher than T1. Hence, the breeding performance of the different experimental treatments were as follows: T2>T3>T1, respectively.

The number of egg released per gram of body weight against the hormonal treatments were presented in Fig. 2. The highest number of egg released per gram of body weight was observed in T2 (26.49±0.90) followed by T3 (20.57±0.55) and T2 (10.93±0.31), respectively which indicates that T2 was better than other two treatments.

3.3 Hatching Rate

The hatching rate of *Clarias batrachus* under different hormonal treatments were presented in Fig. 3. The average hatching rates were 54.93±0.61, 75.77±0.47 and 64.41±0.71 % in Ovupin, Flash and HCG treatment, respectively. The highest hatching rate was found in T2 (75.77 %) which was significantly higher than T1 and T3. Similarly, hatching rate in T3 was also significantly higher than T1. Therefore, the hatching rate in different treatments were as follows: T2>T3>T1, respectively.

Table 1. Breeding performance of *Clarias batrachus* under different treatments

Treatments	Brooders size		NER	FR (%)	IP (hours)
	Female	Male			
T1	208.40±8.91	162.20±1.92	2326.33±24.01^{c}	78.20±0.52^{c}	24
T2	212.60±9.13	168.40±5.94	5543.67±79.94^{a}	93.09±0.61^{a}	24
T3	204.20±5.63	166.40±4.67	4153.33±24.66^{b}	81.60±0.97^{b}	23

Note: p<0.05; NER = Number of egg released; FR = Fertilization rate; IP = Incubation period

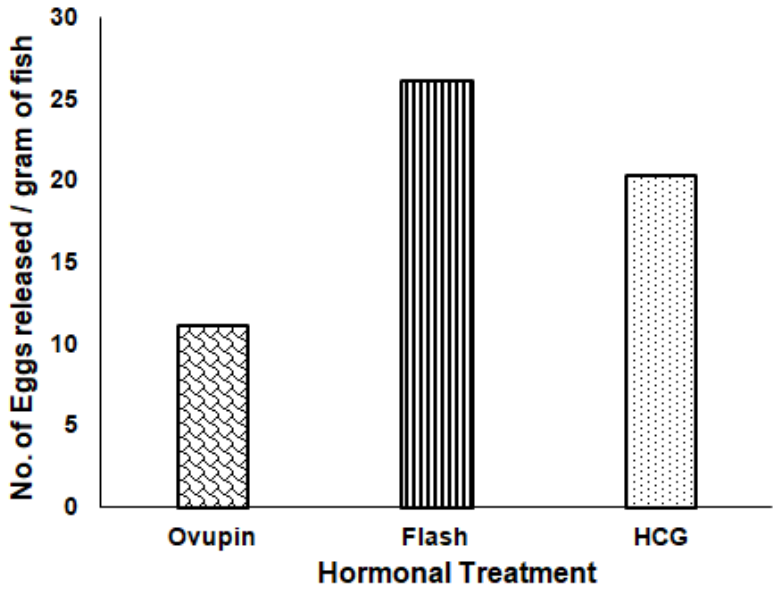

Fig. 2. Number of eggs released by *Clarias batrachus* under different hormones

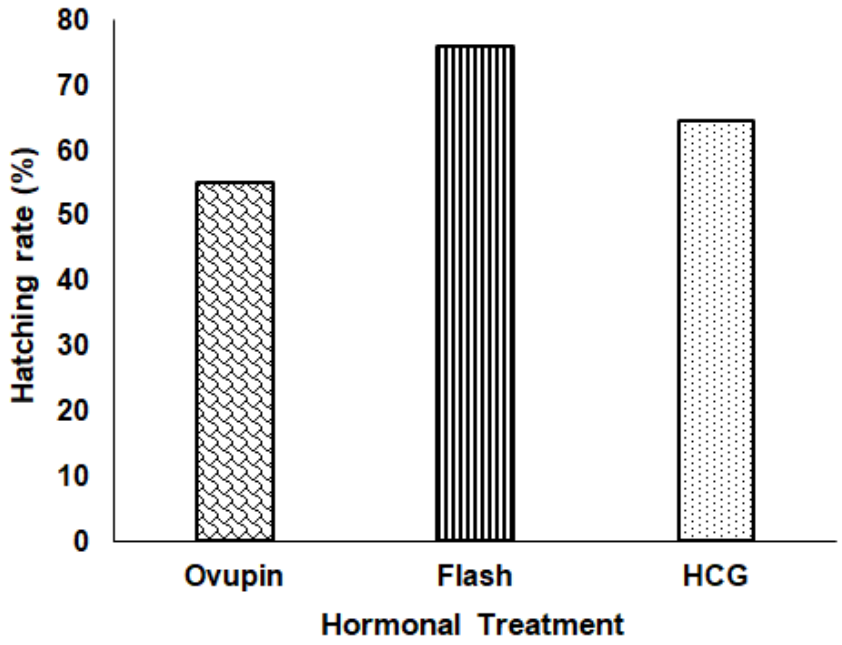

Fig. 3. Hatching rate of *Clarias batrachus* under different hormonal treatments

4. DISCUSSION

Clarias batrachus is one of the most important catfish in Bangladesh. It has been drawing the attention of fish farmers in Bangladesh day by day due to its high market values, profitable culture and hardy nature [6]. Artificial breeding of this species to obtain good quality fry become a necessary part of fry production in the hatchery. The present work on induced breeding of *Clarias batrachus* by using Ovupin, Flash and HCG have been conducted to develop a successful artificial breeding technique of the species, which will be helpful in producing good quality fry. Proper care of brood stock is very important for assuring production of good quality eggs, fry and fingerlings [7]. Broods used in the present experiment were carefully maintained in the farm providing artificial diet before onset of breeding season.

Here in the present study it was found that during April to May most of fishes become fully mature although some fish mature earlier. It is inconvenient for a person to observe the breeding activities and handling the fishes and eggs at night. So during the present experiment fish were injected at 5.30 pm to 6.00 pm. The eggs were fertilized after 14-15 hours later at 9.30 am in the morning. The fishes were stripped 20 hours after injection. In this experiment the incubation period of fertilized eggs are 23-24 hours after fertilization at 27-28°C water temperature. The hatching time of *Clarias batrachus* eggs ranges from 23 to 24 hours at 27-28°C [8]. According to [9] the incubation period varied from 16 to 19 hours at 28°- 30°C which was lower than that of the present study. This might be due to higher temperature. In the present research, *Clarias batrachus* was induced by using Ovupin, Flash (GnRHa) and HCG hormone and there were three replications for each trial. Catfishes normally do not spawn in the laboratory condition but responses to injection of fish and frog pituitary gland extract [10].

There were many works conducted regarding the standardization of pituitary gland dose for successful ovulation though there remains ambiguity among the doses reported by various workers [11]. About the induced breeding of *Clarias batrachus*, there are several references available in Bangladesh and elsewhere in the world regarding the dose optimization of the inducing agents. Therefore, the present study was conducted with a view to inducing by different hormones by which assessment of the seed production technique can be developed. A dose of 6 and 9 mg PG.kg-1 was also found to be effective to induce ovulation in *Clarias batrachus* by [12]. Hossain et al., 2006 found 10.0 mg PG/kg body weight in first dose and 45.0 mg in second dose successfully ovulation in *Clarias batrachus* [13]. On the other hand 30-60

mg/kg body weight which enhanced the breeding response of 140-260 g catfish reported by [14]. An experiment was also encountered while using HCG at the rate of 4000IU/kg of body weight during breeding operation [15].

Successful spawning was observed at 1000, 2000 and 3000 IU/kg body weight administered a single intramuscular injection of HCG to *Heteropneustes fossilis* by [16]. In this present study the fertilization rates were recorded as 82, 93 and 79 % treated with Ovupin, Flash and HCG respectively. The highest fertilization rate (93 %) was recorded with 1 ml/kg body weight of *Clarias batrachus* using Flash whereas the lowest fertilization rate (79 %) was found with dose of 5000 IU/2.2 kg body weight of *Clarias batrachus*. The fertilization rate 70.6-72.8 % was reported with 1.0-2.0 ml/kg of ovaprim in *Clarias batrachus* which was lower than the present findings [17]. Sultana, 2013 studied on induced breeding of magur (*Clarias batrachus*) by using PG and ovaprim [18]. When the male and female fishes were injected with the eggs and milt released at the contemporary times resulting highest fertilization rate (95 %). Sahoo et al., 2005 reported that 83 % fertilization rate with 1.0 ml/kg of ovatide for *Clarias batrachus* which is more or less similar to the present study [19]. The average hatching rates were recorded as 55, 76 and 64 % treated with Ovupin, Flash and HCG respectively.

The highest hatching rate (75.77 %) was recorded with 1 ml/kg of Flash whereas the lowest hatching rate (54.93 %) was found with dose of 1ml/kg treated with Ovupin. High hatching rate 81 % was reported by [20] with 1.0 ml/kg of ovatide which was higher than the present findings. [21] reported 50.6±17.7 hatching rate for pabda (*Ompok pabda*) with rajputi (*Puntius gonionotus*) in mini ponds. From the above discussion it can be concluded that the findings of this experiment were more or less similar in the findings of the others and agreed also. The present work generated some information on biology of the species, inducing agents and doses, and induced breeding technique of *Clarias batrachus*. There is a scope for further study for testing the efficacy of other inducing agents for induced breeding of this species.

5. CONCLUSION

Catfish have widely been caught and farmed for food for hundreds of years in Africa, Asia, Europe, and North America. In Bangladesh and India catfish is eaten as a favored delicacy. Due to the loss of habitat and other climatic condition and over-exploitation, abundance of *Clarias batrachus* is decreasing gradually. To address this problem the study was aimed with a view to developing an artificial breeding technique through inducing agent. At 27 to 28˚C water temperature the best result was attained for Flash hormone in terms of fertilization (93.09 %) and hatching (75.77 %) were found among the three different hormones. The highest percentage of fertilization rate and hatching rate obtained in the present experiment may be considered as satisfactory. However, there is scope for further improvement in the process. The present investigation on the induced breeding of the fish, higher percentages of fertilization and hatching were achieved from a comparatively lower dose of hormone. This can be considered as an effective means for commercial seed production in the hatcheries since cost and quality of hormone is one of the important key factors for a hatchery manager.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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