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Observation of hapa breeding technique of striped snakehead, *Channa striatus* (Bloch, 1793) under captive conditions

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Abstract
Hapa breeding of *Channa striatus* was investigated to address the low-cost seed production technique. The brood fish candidates of *C. stratus* were collected from wild sources for captive broodstock development in a pond. The present study was conducted with three treatments where pituitary gland (PG) hormone extract was used in treatment-1; Ovupin (compound S-GnRHa) hormone was used in treatment-2 and no inducing agent was used in treatment-3 (control). The ready brood fishes of *C. striatus* were selected from captive broodstock pond and the fishes were induced with PG and ovupin hormone. PG was used in two doses for female fish *viz*. 2.0 mg/kg (1st injection) and 6.0 mg/kg (2nd injection) and one dose of 2.0 mg/kg for male. Ovupin was used at the rate of 0.5 and 1.0 ml/kg in 1st and 2nd dose for female, respectively where single dose 0.5 ml/kg was used for male. It is indicated that the ovupin hormone treatment was performed better for hapa breeding of *C. striatus* where spawning, fertilization and hatching rate was observed 100%, 59.5% and 67.5%, respectively.

Keywords: Hapa breeding, *Channa striatus*, PG hormone, ovupin, fertilization, hatching

1. Introduction
Bangladesh is ranked third in aquatic biodiversity in Asia behind China and India [1]. The rich aquatic biodiversity of the country has been attributed to the world's one of the largest wetlands. Since time immemorial, Bangladesh is fortunate to have an extensive and diversified fisheries resource. As an agro-based country, the contribution of fisheries sector to national economy has always been important and main source of animal protein, employment opportunities, food and nutritional security, foreign earnings, aquatic biodiversity conservation and socio-economic development. Fisheries sector contributes 3.69% to GDP and 22.60% to agricultural GDP [1]. The total fish production of the country is 3.68 million tonnes of which 55.93% come from aquaculture [2]. The main cultivable species are Indian major carps (*Labeo rohita*, *Catla catla*, *Cirrhinus cirrhosus*) and some exotic fishes (Hypophthalmichthys *molitrix*, *Aristichthys nobilis*, *Ctenopharyngodon idella*, *Cyprinus carpio*, *Mylopharyngodon piceus*, *Puntius gonionotus*, *Oreochromis niloticus*, *Pangasius hypophthalmus* etc.) but there remains many other potential species yet to be brought under aquaculture [1]. The long term success of any fish culture operation, however, depends on the proper domestication of the cultured fish species. In that fact now it is important for domestication of *Channa striatus* as commercial species.
The striped snakehead *Channa striatus*, are commercially cultured in Thailand, Philippines, Vietnam, Taiwan and Cambodia [3] but in Bangladesh, it is in the stage of infancy due to the lack of seed supply and knowledge of their breeding techniques in captive conditions [4]. The *C. striatus* meat has good taste, high nutrient and also high pharmaceutical values [5, 6]. It has high market price and the market demand has been increased day by day. About two decades ago *C. striatus* were available in many waterbodies in the haors, baors, beels, rivers, ponds, ditches and even in irrigation canals of Bangladesh [7].
The natural population of this fishes is decreasing rapidly due to habitat degradation and ulcerative disease syndromes since last nineteenth century [8]. Now this species is acknowledged as an endangered fish in Bangladesh [9]. *C. striatus* generally spawn in the floodplains with low water in the monsoon with comparatively low fecundity [10]. Artificial breeding have been attempted on this species by several researchers with considerable success [11, 12]. Effects of stocking density on the growth and breeding performance of brood fish and

larval growth and survival of *C. striatus* have been conducted by Mollah *et al*. (2009) [13], which would help to move forward for future research work. However, several species of *Channa* larvae and fry have been investigated in terms of growth and survival when reared with *Artemia* nauplii, decapsulated *Artemia* cysts, *Moina micrura* etc. and interesting results have been obtained by Marimuthu and Haniffa (2007) [14]. But cannibalism appeared to be the most common problem in rearing the fry of snakeheads [6]. However, the breeding technique and culture production of *C. striatus* are yet not been established. Researchers have been attempted artificial breeding with considerable success but still some complicacies have been found. In this study, a simple seed production technique was considered using breeding hapa where selected brood fishes were stocked after injected by PG and ovupin hormone. Generally, breeding hapas have been used for breeding of major and minor carps, and so far no attempts have been made to spawn *C. striatus* in the hapa. The present experiment is the first kind for the assured supply of *C. striatus* seed for culture by farmers. Considering the economic as well as biological importance of *C. striatus*, the present study was undertaken to develop a simple hapa breeding technique under captive condition for seed production.

2. Materials and Methods

The research was carried out in the Pond Complex and Mini Hatchery of the Department of Fish Biology and Genetics, Sylhet Agricultural University, Sylhet. The hapa breeding technology of *C. striatus* was set out in earthen pond by using rectangular drag net as a breeding hapa. The research work was done during from January to May 2016. The major approaches and methodologies of the research work are as follows:

2.1 Captive Broodstock of *C. striatus*

Brood fish candidates of *C. striatus* were collected from wild sources of haor region (latitude 24°34′N to 25°12′N and longitude 90°56′E to 91°49′E) in Sylhet. These fishes were transported in open mouth plastic drum. The weight ranging of brood fish candidates was 600-900 g and the fishes were healthy, good looking, disease free. The fishes were transported to the pond complex and stocked in a pond for broodstock development after salt (NaCl) water bathing for prevention of disease.

2.2 Rearing of Brood fish

The broodstock pond size was 49 m^2 and water height was 1.5 m. A fencing was provided by synthetic net inside the lower part of the pond embankment to protect fish escaping tendency. The natural environment was created in the pond with phytoplankton, zooplankton, insects' larvae, tadpole and some aquatic macrophytes like *Eichhornia crassipes* and *Hydrilla verticillata* etc. Besides, some small live fishes such as mola (*Amblypharyngodon mola*), punti (*Puntius ticto*), darkina (*Esomus danricus*) and *tilapia fries* (*Tilapia mossambicus*) were released regularly in the pond as they fed them due to carnivorous nature.

2.3 Breeding Hapa Setting

The rectangular iron frame with covering synthetic net was used for hapa making. The breeding hapa was setting in the pond. The size of the breeding hapa was 2 m length, 2 m width and 1.5 m height (Figure 1). Iron frame was used for hapa making and bamboo poles were used for setting hapa in the pond. The base of the breeding hapa was fully submerged at the pond bottom using bricks. Fish shelter and natural environment was created in the breeding hapa through introduction of aquatic macrophytes like *Eichhornia crassipes* and *Hydrilla verticillata*. The monitoring and observation facilities of breeding hapa were provided in the pond through extra arrangement of staircase.

Fig 1: Breeding Hapa in a pond

2.4 Brood Fish Selection for Breeding

The ripe and healthy male and female brood fish were selected based on physical and visual examination of secondary sexual characteristics i.e., size, color, swollen abdomen and genital opening [15]. Cast net and seine net were used to collect brood fish whenever necessary by reducing the water of the pond. The brood fishes were selected carefully from captive broodstock hope to get positive response for breeding (Figure 2). Male brood fish were selected on the inflamed body surface. On the other hand, female brood fishes were selected by observation rounded, swollen belly, and reddish protruded genital opening. The selected brood fishes were kept into the cistern of Mini Hatchery for acclimatization and resting for inducing injection. After 4 h of resting period, finally six breeding sets of brood fishes, two (2) males and one (1) female in each breeding set, ranging between 650 g and 860 g were selected for inducing injection.

Fig 2: Brood *Channa stratus*

2.5 Experimental design

Three treatments were designed and considered as T_1, T_2 and T_3 (control). Pituitary gland hormone was used as inducing agent in treatment-1 (T_1) where ovupin (compound S-GnRHa) was used in treatment-2 (T_2) and no inducing agent was used in treatment-3 (T_3, control). Each treatment consists two breeding sets. Two males and one female of brood fish were used in a breeding hapa. The hormones were applied at two

different doses. The primer dose for female was 2.0 mg/kg and 0.5 ml/kg of PG and Ovupin, respectively. The second dose for female was 6.0 mg/kg and 1.0 ml/kg of PG and Ovupin after the interval of 6 h. The male only received the second dose of hormones at the rate of primer dose of female (Table 1).

Table 1: Doses of hormone applied to brood fish of *C. striatus*

Treatment	Hormone	Sex	Average wt. (g) of fish	Dose		Time interval
				1st	2nd	
T_1	PG (mg/kg)	Female	810	2.0	6.0	6 hours
		Male	667	-	2.0	
T_2	Ovupin (ml/kg)	Female	780	0.5	1.0	6 hours
		Male	668	-	0.5	
T_3 (control)	No use of hormone	Female	796	-	-	-
		Male	670	-	-	-

2.6 Inducing of Brood Fish and Kept in the Breeding Hapa

The selected brood fishes were injected by using inducing agents (Figure 3) at the Mini Hatchery of the Department of Fish Biology & Genetics. The inducing agent was used as per experimental design. Then the injected female and male fishes were stocked together in the breeding hapa.

Fig 3: Induced hormonal injection

2.7 Feeding and health care of Brood fish

The live small fishes like mola and darkina were supplied into the breeding hapa as feed of *C. stratus*. Water hyacinth was added into the breeding hapa to create natural environment in the hapa as well as to control jumping of fish for avoiding injuries.

2.8 Breeding and spawning situation

The brood fishes in the breeding hapa were monitored and observed minutely. Fish breeding, spawning time, fertilization rate, hatching rate were observed closely and necessary work was done carefully. The following formula was used for fertilization and hatching rate:

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

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

2.9 Measurement of water quality

The water quality parameters of the breeding hapa were recorded carefully. Water quality parameters such as temperature, pH and dissolved oxygen (DO) were measured daily. Digital pH meter (Lutron, Model- PH-222) was used for the measurement of pH, a DO meter (Lutron DO-5509) was used for the measurement of DO and a thermometer was used for recording of water temperature.

2.10 Data analysis

Data were analyzed by using the statistical package IBM SPSS version 22.

3. Results and Discussion

3.1 Maintenance of brood fishes

The brood fishes of *C. striatus* were reared for almost four months in the broodstock pond in the Departmental pond complex of Fisheries Faculty. Through the proper broodstock management, the *C. striatus* broods were found to be healthy and fully mature for ready to spawn. It was possible due to the fishes were fed regularly with some live small fishes like mola, punti, darkina and *tilapia* fries. Robert *et al*. (1982) [16] have reported that proper care of broodstock is very important for assuring good production of eggs, fry and fingerlings. Mollah *et al*. (2009) [17] have conducted an experiment where the brood fish were fed with washed and chopped poultry viscera twice a day and with live silver carp fry twice a week.

3.2 Water quality

The average water quality parameters of the breeding hapa are presented in Table 2. The water quality parameters such as temperature, pH and dissolved oxygen (DO) of water in breeding hapa under different treatments of *C. striatus* ranged between 28 to 31 ºC of temperature, 7.8 to 8.5 of pH and 4.8 to 5.6 mg/L of DO, respectively with negligible variation. The mean values of water quality parameters were not significantly (P<0.05) different among the treatments (Table 2). Mollah *et al*. (2009) has recorded the temperature, pH and dissolved oxygen ranged between 27.5 and 28.3ºC, 6.8 and 7.5, and 5.3 and 6.0 mg/L, respectively of water in raceway chambers under different treatments [17].

Table 2: Water quality parameters of different treatment for hapa breeding of *C. striatus*

Treatment	Parameters		
	Temperature (ºc)	pH	DO (mg/l)
T_1	28.50±0.50^a	8.25±0.05^b	4.90±0.10^c
T_2	30.50±0.50^a	8.20±0.20^b	5.40±0.20^c
T_3	29.50±0.50^a	8.10±0.10^b	4.85±0.05^c

(Mean ± SE); values of the parameter in each column with different superscripts (a, b, c) differ significantly (P<0.05).

3.3 Breeding behavior and spawning

The spawning pairs were seen moving together in the breeding hapa. Male showed more aggressiveness and active participation in mating. Spawning was noticed 12-14 h after administration of second dose of hormone. In T_1, spawned period was observed 12-13 h after second dose of hormone injection where it was recorded 12-14 h in T_2. No spawning was observed in control treatment (T_3) where no inducing agent was used. Paray *et al*. (2012) [3] has reported the latency or spawned period 19-29 h occurred in case of HCG hormone treated of snakehead murrel. Francis (1996) [18] has reported high latency period for *H. fossilis* and *C. batrachus* due to potency of hormone. The latency period available in the literature was 24-30 h in *Channa punctatus* [12, 19], 22-25 h *Heteropneustes fossilis* [20] and 16-20 h for *Clarias gariepinus* [21]. Hossain *et al*. (2008) [22] have stated that the ovulation was recorded after 9-12 hours of the second dose of injection

which is similar to the present findings. The difference in the latency period was irrespective of the breeding tank captivity [3]. No remarkable differences in breeding and spawning behaviour were observed in case of male, with varied doses of the hormone.

3.4 Breeding performance

The breeding performance varied with different treatments which are presented in Table 3.

Table 3: Breeding performance of *C. striatus* with different treatments in hapa breeding

Treatment	Breeding sets	Spawning performance	Hours of spawned	Fertilization rate (%)	Hatching time (h)	Hatching rate (%)
T_1	2	75%^{a}	12.5±0.50^a	44.0±4.00^b	34.5±0.50^a	61.5±1.50^a
T_2	2	100%^{a}	13.0±1.00^a	59.5±2.50^a	33.0±1.00^a	67.5±2.50^a
T_3	2	0%^{b}	0±0.00^b	0±0.00^c	0±0.00^b	0±0.00^b

(Mean ± SE); values of the parameter in each column with different superscripts (a, b, c) differ significantly (P<0.05).

In the present study, the breeding performance was found in T_1 & T_2 where no breeding performance has occurred in T_3 (control). The T_1 showed 75% spawning performance where T_2 has showed 100% spawning, and there was no significant difference in spawning performance between two treatments but showed significant difference in T_3 due to no spawning.

In this study, the egg samples were collected from the egg mass and kept inside a beaker (capacity 500 ml) to determine the fertilization and hatching rates. The fertilized eggs were adhesive and observed to the roots of the water hyacinths. The eggs were straw yellow in colour and spherical in shape. The egg fertilization rate varied from 40-62% where mean value of lower rate was recorded 44.0±4.00% in T_1 and higher rate 59.5±2.50% was in T_2. The fertilization rate was significantly higher in T_2 rather than T_1. No fertilization has occurred in T_3 where no spawning was recorded. The fertilization rate in this study was similar to the study of Paray *et al*. (2012) and Hossain *et al*. (2008) and they found the fertilization rate of 58.83% and 40-80%, respectively in their experiment [3, 22].

Fig 4: Parental care

Fig 5: Fry collection

The fertilized eggs of *C. striatus* hatched out between 32-35 h after fertilization. The mean value of egg hatching time was recorded 34.5±0.50 and 33.0±1.00 in T_1 and T_2, respectively (Table 3) and there was no significant difference between two treatments. After hatching, the hatchlings congregated near the water surface and were allowed to grow along with the parents in the breeding hapa (Figure 4). First time, the hatchlings were of black colour and after that the hatchlings were converted into the yellowish colour (Figure 5). Paray *et al*. (2012) [3] have reported that after 24 h of hatching time, fiber tanks were found full of hatchlings black in colour and they also found both the parents showed care for hatchlings by guarding them right from the stage of fertilized egg till the fry stage but male care was more dominant.

The eggs hatching rate of *C. striatus* was recorded 60-70% where the mean value of hatching rate was observed 61.5±1.50% and 67.5±2.50% in T_1 and T_2, respectively (Table 3) and there was no significant difference between two treatments. Hatching rate in treatments 1 & 2 were found to be more or less similar to the study of Paray *et al*. (2012) [3] and they recorded the hatching rate 55-80% in their experiment. Hossain *et al*. (2008) [22] have reported that the hatching rate varied from 45-82% with an average of 62.33%. No breeding activity was performed in treatment-3 (control), as because there was no use of inducing agent for breeding. As a result no spawning was recorded in control treatment. It is assumed that the inducing agent may stimulate and enhance the breeding protocol of *C. striatus* under captive condition. Though, the overall breeding performances of *C. striatus* were found to be satisfactory for providing seed production in the captive hapa breeding conditions. However, the ovupin hormone doses treatment was performed better for hapa breeding of *C. striatus*.

4. Conclusion

It is a new concept and diversification of aquaculture in Bangladesh. The simple breeding technique of *C. striatus* with the fertilization and hatching rate were viable for the seed production in captive condition. In the present study, among 6 breeding sets under the 3 treatments, the hormone using treatments (Treatment 1 & 2) were responded spawning positively. No breeding activity was found in the control treatment indicating that inducing agent is necessary for breeding under captivity. Based on our findings, ovupin hormone treated breeding could be recommended for seed production of *C striatus* under using breeding hapa in pond conditions. The successful development of protocols for captive breeding is likely to understand the way forwards commercialization of the technology for upscaling of the seed production of *C. striatus* at rural farms situation. It is hoped that the hapa breeding of this fish would reduce the dependency on the natural spawning for fry collection and may ensure the supply of fish seeds to the fish farmers.

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