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Induced Breeding of *Ompok pabda* with S-GnRHa

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ABSTRACT

Ompok pabda, locally known as "modhu pabda" is a commercially important catfish for aquaculture, capture fisheries and conservation in Bangladesh. Present study was conducted with three different doses of S-GnRHa hormone (0.25, 0.5 and 1.0 mg/kg body weight) for female and the half of each dose for male to determine its effectiveness on fertilization, hatching and survival rates of hatchling of *O. pabda*. Hormone S-GnRHa of 0.5 mg/kg body weight for female and 0.25 mg/kg body weight for male was found effective to maximize the fertilization (91.00%), hatching (90.80%) and hatchling survival (76.60%) rates, and significantly differed($P < 0.05$) from the other doses. The findings of the present study will be useful for successful induced breeding and conservation of endangered *O. pabda*.

Keywords: Breeding, endangered, fertilization, hatching, *Ompok pabda*.

INTRODUCTION

Fisheries play a vital role in economic development, employment, human nutrition and for earning foreign currencies to develop our national economy. This sector contributes about 3.69% of the gross domestic product (GDP) and consists of one-fourth of the total agricultural production. About 1.92% foreign currencies come from this sector. Fish is an excellent source of protein containing all the essential amino acids in desirable concentration for human beings and available at cheaper rates than other animal protein sources. Fish plays the second important role next to agriculture in food production. Fish provides 60% of animal protein consumption for the people of Bangladesh [1]. Inland open water is the major source of fish production in the country, which consists of 7,78,055 hectors. But production from closed water bodies is increasing sharply due to dissemination of appropriate aquaculture technologies through effective extension service, which consists of 39,25,290 hectors area [2].

Ompok pabda (Hamilton) is an indigenous, small food fish in Bangladesh[3]. This freshwater catfish is belonging to the family Siluridae of the order Siluriformes. The fish is very attractive and it is silvery gray in colour, darkest on the back and fading to white on the belly with two longitudinal lighter bands above and below the lateral line. In all there is a dark oval shoulder spots [4]. Maximum length of the species was recorded up to 30 cm[5]. The species is omnivorous in nature, eating fishes, crustaceans, protozoans, algae, insects, parts of higher plants and debris [6]. The fecundity of the fish varies from 1,447 to 6,658 in the size range of 124 to 172mm [7]. It has a single spawning season during the monsoon i.e., in the month of May to July [8].

Once upon a time, *O. pabda* was abundantly found in the beels, hoars, baors, flooded water bodies, ponds, streams and rivers of Bangladesh [9]. The species is also found in India, Pakistan, Afghanistan and Burma[10]. But now-a-days, the availability of this species has declined drastically from open water bodies in Bangladesh. So, IUCN[11] has considered *O. pabda* as an endangered fish species in the country.

Due to high market price and consumer demand, fish farmers show considerable interest in its culture, but the biggest constraint is non-availability of fry. Artificial breeding is the most widely used way to increase their abundance. But of many advantages very few attempt has been made in Bangladesh to promote artificial breeding and culture of *O. pabda*. Although some workers had previously reported successful induced breeding of the species, that had not been standardized to be recommended at farmer's level for commercial seed production.

Recently, Salmon Gonadotropin releasing Hormone (SGnRHa) named ovupin is imported in Bangladesh as an inducing agent for hatchery use. This inducing agent seems to be cheaper and is available everywhere of Bangladesh even in small town surrounding fish hatcheries and farms. Ovupin is a liquid peptide preparation that contains an analog of Salmon Gonadotropin releasing Hormone (SGnRHa) and a brain neurotransmitter (dopamine) inhibitor. The SGnRHa in ovupin elicits the release of stored gonadotropins from the pituitary[12]. Hence, the present study was conducted to develop the induced breeding technique and to optimumize the dose of SGnRHa for *O. pabda* in Bangladesh.

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MATERIALS AND METHODS

Study area and period

The study was conducted in the "Alalpur Hatchery & Fisheries Limited" (Alalpur, Sombugonj, Mymensingh) from April to June, 2016.

Brood pond preparation

Ompok pabda broodstock was managed in one brood pond with an area of 20 decimal and 1m water depth. For preparation of brood pond, the predator and unwanted fishes were eradicated by dewatering and drying. Rotenone and Phostoxin were also used to kill any unwanted fish species and aquatic insects, respectively. Aquatic vegetation was removed manually. After cleaning the pond, lime was used at the rate of 1kg/decimal and 5-7 days after liming, cow dung was applied at the rate of 5-7 kg/decimal as organic fertilizer. Inorganic fertilizers such as Urea and TSP were also used at the rate of 200g and 100g per decimal, respectively. Seven days after fertilization, the brood pond was ready for brood fish stocking.

Water Quality Measurement

Different water quality parameters were monitored fortnightly in brood rearing ponds during the study period (Table 1).

Cleaning of the cistern

The cemented cisterns were washed by using lime and brushed. After that emery paper and water were used to clean the moss and other dirt. Finally, $KMnO_4$ and methylene blue stain were added with the conditioning water to avoid microbes.

Brood fish collection and selection

One hundred of healthy male and female *O. pabda* broods were collected from different stocking ponds of hatchery and stocked in the previously prepared brood ponds. Brood fish were caught by using gher net and separated male from female fishes based on secondary sexual characteristics, such as external features of their abdomen and pectoral fins (Figs.1and 2; Table 3).

Conditioning of brood fish

Mature males and females from the brood rearing ponds were caught and immediately carried to the hatchery. Selected brood fishes were kept in tank for about 6 hours for conditioning prior to injection with S-GnRHa (Ovupin). Handling and carrying of fish was done very carefully to avoid possible injury and secondary infection. Male and female fishes were kept in separate tanks and constant water flow was maintained to ensure proper aeration. No feed was provided during the period of conditioning.

Hormone treatment

Table 1: Water quality parameters recorded during the breeding period.

Water Parameters	Value(mean ±sd)
Temperature (^{o}C)	28±1.00
pH	7.03±0.15
DO(mg/l)	7.5±1.00
Transparency (cm)	23±1.5

Brood fish rearing

The brood fisheswere reared in brood rearing ponds at a stocking density of 25-30/decimal. The area of each pond was 20 decimal having 0.9-1.1m water depth. During rearing lime, fertilizer and cow dung were properly applied. Fertilization with urea and TSP at the rate of 200g/decimal and 100g/decimal respectively were applied at 15 days interval. Brood fishes were also fed with supplementary feed at 5-6% body weight (Table 2). Supplementary feed was used twice a day at 8.00am and 4.00pm.

The Ovupin hormone was injected into the muscular basal part of the dorsal fin. Broods were collected from cisternjust prior to injection. Single injection was given to both male and female with a specific dose of Ovupin hormone. The broods were treated with inducing agent at evening so that fish ovulate in morning. During injection brood fishes were caught by net and handled carefully. The fish was kept on foam and the head region of the fish was wrapped with a wet and soft towel or cloth. The injection administration was done very carefully with a 1 ml syringe. The needle was inserted at about 45^{o} angle to the body surface according to the body weight of

Table 2: Ingredients used in supplementary feed.

Ingredients	Amount (%)
Fish meal	30
Rice bran	20
Soybean oil cake	20
Mustered oil cake	15
Meat and bone meal	10
Wheat/ flour	4
Vitamins and minerals	1

the broods (Fig. 3). After injection male and female were kept in cistern where they released eggs automatically after 8-12 hours depending on the treated doses.

Concrete cistern having a size of 1.5×0.5×1 m^3 was used in the present study. Both female and fishes were injected with three different doses of S-GnRHa for three treatments as T_1, T_2, T_3 and distilled water for one treatment as T_4 (control) with five replications in each treatment (Table 4). The ratio of

Table 3: Criteria followed to select mature breeders of *O. pabda.*

Criteria	Male	Female
Size	Smaller in size	Relatively larger in size
Abdomen	Abdomen normal, not bulky like female	Abdomen bulging, elastic and soft
Body shape	Body slender	Body robust
Pectoral fin	Inner side rough	Inner side smooth
Gentle pressure on abdomen	Whitish milt come out through the genital pore	Eggs come out through the genital pore

Figure 1: Broods collection from brood rearing ponds using seine net

Figure 2: Male and female brood fish of *O. pabda*

Figure 3: Hormone administration by injecting

male and female was 1:1 in all cisterns. For dose optimization, ovulation, fertilization, hatching and survival rates were determined.

Table 4: Experimental layout for dose optimization

Treatment	Cistern	Hormone	Dose for female fish	Dose for male fish
T_1	Cis-1 Cis-2 Cis-3 Cis-4 Cis-5	S-GnRHa (Ovupin)	0.25 mg/kg	0.125 mg/kg
T_2	Cis-1 Cis-2 Cis-3 Cis-4 Cis-5	S-GnRHa (Ovupin)	0.5 mg/kg	0.25 mg/kg
T_3	Cis-1 Cis-2 Cis-3 Cis-4 Cis-5	S-GnRHa (Ovupin)	1.0 mg/kg	0.5 mg/kg
T_4(control)	Cis-1 Cis-2 Cis-3 Cis-4 Cis-5	Distilled water	0.5 ml/kg	0.25 ml/kg

Gonado-somatic index (GSI) of *O. pabda*

The gonado-somatic indices of *O. pabda* were estimated for three months from April to June, 2016, utilizing the following formula.

$$GSI = \frac{\text{Weight of gonad}}{\text{Weight of body}} \times 100$$

Ten female fishes were sacrificed for every month. The external connective tissues were removed carefully from the surface of the ovaries. Moisture of the ovaries was removed with the help of blotting paper. The individual weight (gm) of fish and their ovaries were recorded with fine electronic balance.

Estimation of fecundity

The total number of eggs spawned was counted by the gravimetric method [13]. In brief, approximately 1 g egg sample was weighed in triplicate times. The total number of eggs spawned was calculated by multiplying the average number of eggs from the triplicate 1 g samples with the total weight of eggs sampled.

Ovulation rate

The ovulation rate of *O. pabda* was calculated utilizing the following formula.

$$\text{Ovulation rate (\%)} = \frac{\text{No. of fish ovulated}}{\text{Total No. of fish injected}} \times 100$$

Fertilization rate

The fertilization rate of *O. pabda* was calculated utilizing the following formula.

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

Hatching rate

The number of the hatchlings was counted by visual observations. The number of spawn/larvae in each bowl was counted. The hatching rate of *O. pabda* was calculated utilizing the following formula.

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

Survival rate

The survival rate of *O. pabda* was calculated

Table 5. Gonado-somatic index (GSI) and fecundity of *O. pabda*.

Parameter	Month		
	April	May	June
No. of fish examined	10	10	10
Total length (cm)	23.20±3.01	23.30±3.13	22.60±2.84
Body weight (gm)	112.50±14.48	113.50±15.73	110.50±14.39
Gonad weight (gm)	3.55±0.50^a	11.08±1.22^c	5.30±0.72^b
GSI	3.13±0.52^a	9.50±0.30^c	4.80±0.26^b
Fecundity	9165±428^a	11368±719^c	10091±515^b

utilizing the following formula.

$$\text{Survival rate (\%)} = \frac{\text{No. of hatchlings survives}}{\text{Total No. of hatchlings}} \times 100$$

Data analysis

A one-way analysis of variance (ANOVA) was performed to determine the significance ($P<0.05$) level of hormonal treatments. MS excel was used to perform this statistical analysis. The significant ($P<0.05$) level was tested following Duncan's test using IBM SPSS statistics software, version 20 computer programmed.

RESULTS AND DISCUSSION

Gonado-somatic index of *O. pabda*

The GSI found in the present study were ranged from 3.13±0.52to 9.50±0.30 during the study period (Table 5). The highest and the lowest GSI values were in May and April, respectively. The GSI values differed significantly ($P<0.05$) in three months.

Estimation of fecundity

The fecundity of *O. pabda*in the present study was ranged from 9165±428 to 11368±719(Table 5). The fecundity of the experimental fish was significantly ($P<0.05$) highest recorded in May. Islam[14] observed the fecundity of *O. pabda* ranged from 15560 ± 185 to 17369 ± 213 during June to August. Variations in fecundity of fish species may be due to selectively different fish sample, different environmental factors such as different pond, water temperature, feeding, food abundance, species differentiation, nutritional resources etc. [15, 16]. Peak season in the fecundity of *Ompok* species coincides with the onset of rain fall and flood water (May to August)[17, 18]. Harding[19] stated that most tropical fishes were adopted to breed on the rising flood, allowing the juveniles to take full advantage of the flooded banks for smooth feeding purpose and thus protected from predation.

Breeding behavior of *O. pabda* after the S-GnRHa injection

The breeding behavior was observed continuously after the S-GnRHa injection applied to the fish. Just after injections both male and female showed normal activities and movement. At that time they stayed on the bottom at one corner of the cistern. After 6 h of injection the activities and movement of male fishes were increased. The male fish started to move around the female fish and to nudge with its snout at the ventral region of female fish after 8-9 h of injection. At that time higher rate of opercula movements was observed in female fish. The activities of female were also increased. It started to move and stay at middle of the water column. These activities of male and female and the male bent its body sideways and tried to bring its genital papilla in proximity of female's genital pore (Fig 4). On the bending condition the male brought the female fish at the surface of the water. Pressure was created on the ventral region of the male fish to the abdomen of the female. Eggs were ejected and at the same time male released milt. These activities were observed several times until the total eggs and milt was released. Fishes became calm and quite after spawning. After releasing eggs and milt, fishes were shifted in another tank. At that time they were found to stay on the bottom of the tank.

Determination of ovulation Rate

All fishes were ovulated with all treatments within 12 hours post injection but none of the fish ovulated within the specified time with T_4 (control) treatment (Table 6). When a higher dose (T_3) of ovupin was applied, many immature eggs along with mature eggs were released from the brood which negatively affected on the fertilization and hatching rates. Rahman[20] found 44.44% ovulation rate of *O. pabda* administrated with a single dose (14 mg/kg fish) of PG. However, he derived this percentage from a single breeding trail.

Fertilization rate (%)

Immediate after ovulation, fertilization of the experimental fish was occurred. The fertilization rates were recorded as 66.20%, 91.00% and 77.00% with T_1, T_2 and T_3, respectively (Table 6). Fertilization rate was significantly ($P < 0.05$) highest in T_2 followed by T_1 and T_3. Present findings of fertilization rate coincide with the result of Akhteruzzamanet al[8] who recorded a fertilization rate between 55% and 75% at the doses of 10-18 mg

Figure 4: Reproductive behavior of male and female *O. pabda*

PG/kg body weight for females and 12 mg PG/kg body weight for the male *O. pabda* in cistern, among them doses of 13-16 mg PG/kg fish showed the best results.

Hatching rate (%)

The hatching rates were 78.40%, 90.80%, and 83.80%, with T_1, T_2 and T_3, respectively (Table 6). Hatching rate was significantly ($P < 0.05$) highest in T_2 followed by T_1 and T_3 treatments. Rahman[20] found a hatching rate of 95% in cistern by administration of a single PG dose of 14 and 16 mg/kg fish to female and 12 mg/kg fish to male. Akhteruzzamanet al[8] reported the hatching rate of *O. pabda* to vary from 40%-60% when treated with PG doses of 10-18 mg/kg body weight of fish. Hatching rate of *O. pabda* in the present study was much higher than those of published data so far. It might be due to effectiveness of ovupin hormone used in the present study.

Survival rate (%)

The survival rate of *O. pabda* larvae in the present experiment were 46.20%, 76.60% and 53.60% inT_1, T_2 and T_3 treatments, respectively after 3 days of experimental period(Table 6). Survival rate was significantly ($P < 0.05$) highest in T_2 followed by T_3 and lowest in T_1. Islam[14]observed the highest survival rate as 60.40 ± 0.59% in June and the lowest rate as 42.76 ± 0.55% in August, 2011 in *O. pabda*. The survival rate of *O. pabda* in the present study was also higher with ovupin injected fish.

Table 6: Performance of different treatments of ovupin on *O. pabda*

Parameter	Treatment			
	T_1 (0.25mg/kgDoses)	**T_2 (0.50 mg/kg Doses)**	**T_3 (1.00 mg/kg Doses)**	**T_4 (0.05 ml/kg Doses)**
Total length (cm)	22.40±0.89	22.20±0.84	22.20±0.84	22.20±0.84
Body weight (gm)	113.00±8.03	115.80±12.19	116.40±10.92	115.80±12.05
Gonad weight (gm)	9.67±0.31	9.58±0.16	9.58±0.35	9.58±0.16
Ovulation rate (%)	100	100	100	0
Fertilization rate (%)	$66.20±1.10^a$	$91.00±1.00^c$	$77.00±1.58^b$	-
Hatching rate (%)	$78.40±2.30^a$	$90.80±2.38^c$	$83.80±1.92^b$	-
Survival rate (%)	$46.20±0.84^a$	$76.60±1.14^c$	$53.60±1.51^b$	-

CONCLUSION

The induced breeding of *O. pabda* using compound S-GnRHa (ovupin) as the inducing agent was successful. The fish responded to hatch with a single S-GnRH (Ovupin) dose of 0.5mg/kg body weight in female and 0.25mg/kg body weight in male fish, showing the best result in consideration of fertilization, hatching and survival rates. The highest average fertilization rate was 91.00±1.00 recorded in T_2 treatment whereas lowest average fertilization rate was 66.20±1.10 recorded in T_1. The highest hatching rate was recorded as 90.80±2.38in T_2 and lowest hatchling rate was recorded as 78.40±2.30in T_1. The highest survival rate of *O. pabda* was 76.60±1.14 in T_2 whereas the lowest survival rate was 46.20±0.84 found in T_1. The highest mean GSI value was observed in May and the lowest in April during the study period. This study has determined the optimum doses, fecundity, ovulation, fertilization, hatching and survivability of *O. pabda*. The findings of the present study reflected a number of recommendations for induced breeding such as selected brood fish should be kept in tank for about 6 hours for conditioning prior to injection with S-GnRHa (Ovupin). Handling of brood fish should be done very carefully to avoid possible injury and secondary infection. The S-GnRHa (Ovupin) hormone should be injected into the muscular basal part of the pectoral fin. After fertilization, dead eggs should be removed every three hours and maintained with continuous water flow from porous pipe for aeration. Injected of over doses should be avoided and maintenance of good environment for induced breeding is essential.

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