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First Record of Induced Breeding of Indian Potasi, *Neotropius atherinoides* (Bloch 1794) in Bangladesh

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ABSTRACT

This experiment was conducted for the development of induced breeding techniques of *Neotropius atherinoides* using pituitary gland (PG) extract at the Floodplain Substation, BFRI, Santahar, Bogura, Bangladesh. Three breeding trials were conducted during the months of April, May, and June where fifteen pairs of males and females were used for each trial. During the first trial in April, PG extract was used at a rate of 25, 30, and 35mg PG/ kg body weight of the female fish; however, no fish individual was ovulated. Then, two more breeding trials were conducted in May and June using 12 mg (T_1), 13 mg (T_2), and 14 mg (T_3) PG/ kg body weight of female and 6 mg (T_1), 7 mg (T_2), and 8 mg (T_3) PG/ kg body weight of male fish. During this time, the ovulation rates were recorded as 63.12±1.21, 65.21±2.13, and 69.14±1.59% in May and 65.23±1.62, 68.59±1.80, and 71.87±1.91% in June under T_1, T_2 and T_3 treatments, respectively. Fertilization rates of eggs were estimated as 59.24±1.12, 61.36±1.31, and 62.34±1.21% in May and 62.85±1.30, 64.74±1.51, and 66.39±1.40% in June, respectively. Hatching rates of eggs were recorded as 75.54±2.11, 79.64±1.62, and 80.33±1.10% in May and 80.89±1.30, 82.48±1.12, and 86.98±1.90% in June under T_1, T_2 and T_3 treatments, respectively. Significantly ($P<0.05$) higher values of the mean gonado-somatic index were observed during June, July, and August for females, and July showed the highest value. The female and male showed the best breeding performance in June treated with the dose of 14mg and 8mg PG/ kg body weight, respectively. Results from the present experiment revealed that induced breeding of *N. atherinoides*, using PG extract is successful which might be helpful in mass seed production of this species for aquaculture as well as conserving the species in nature.

INTRODUCTION

Bangladesh is one of the top fish-producing countries in the world, and it is the most productive and dynamic sector because of its vast inland, coastal and marine water resources which played a significant role in the country's economy for the last few decades **(Ghose, 2014; Shamsuzzaman *et al.*, 2017; Sunny *et al.*, 2020)**. Bangladesh

has diversified fisheries resources with 260 species of freshwater fishes and 475 species of marine water fishes for its favorable geographic position **(FRSS, 2020)**. Among 260 freshwater species in Bangladesh, about 143 species are called Small Indigenous Species (SIS) which maximum length becomes 25 cm at their mature stage **(Felts *et al.*, 1996)**. According to IUCN Red List (2015), among 253 freshwater species, about 64 freshwater species are under threatened condition of which 9 species (3%) are critically endangered, 30 species (12%) are endangered and 25 species (10%) are in vulnerable condition **(IUCN, 2015)**. A total of 55 catfish species are found in inland waters of Bangladesh **(Rahman & Akhter, 2019)**, of which Indian Potasi, *Neotropius atherinoides*, (Bengali name: Batashi) is one of the endangered freshwater SIS, which belongs to the family Schilbeidae and genus *Neotropius*. The Indian Potashi or Batashi is in endangered condition due to habitat degradation, water pollution, degradation of breeding and feeding grounds, construction of dams in the floodplain areas, and use of insecticides and pesticides in the agriculture field **(Rahman, 2005).** *Neotropius atherinoides* is distributed in Bangladesh, India, Pakistan, Nepal, and Myanmar. In Bangladesh, it is commonly found in floodplains, rivers, beels, haors and baors **(Rahman, 2005).** Like other SIS, this fish species has high nutritional value and rich in protein, fat, vitamins and minerals **(Bogard *et al.*, 2015)**. *Neotropius atherinoides* is an important species for capture fisheries in our country but no culture practice introduced yet due to lack of availability of fry and fingerlings. However, this species has commercial importance and can be cultured in the ponds with other species or as a single species for local consumption or exportation. It is important to gather knowledge about the food and feeding habits, reproductive biology, fecundity, breeding behavior, and season of this fish for the development of induced breeding technique to ensure the supply of quality seeds. However, till now no systematic research works have been done to breed this species artificially through establishment of induced breeding technique.

Therefore, the present study was carried out to build up an appropriate induced breeding technique of *N. atherinoides* that might be helpful for mass seed production, which will not only facilitate to aquaculture production but also will save this endangered species from being extinction.

MATERIALS AND METHODS

1. Experimental site

Brood fishes were reared in earthen ponds of the Substation and induced breeding activities were undertaken in the Hatchery & Breeding Complex of the Floodplain Substation, Bangladesh Fisheries Research Institute, Santaher, Bogura.

2. Collection of brood fishes

The sexually matured, strong, and diseased free broods of *N. atherinoides* were collected from the River Jamuna, River Atrai & Roktodaho Beel of Sirajgonj, Naogoan, and Bogura districts of Bangladesh (**Fig. 1**) and were stocked in the ponds for domestication

for breeding purposes. Before stocking into the rearing pond, the brood fishes were bathed in 1.5-2.0 ppm potassium permanganate for disinfection. Experimental fishes were stocked at the rate of 200-250 brood/decimal averaging 3-4 g each. Regular water supply was provided to maintain required water depth in the pond. One third of the brood pond was filled with water hyacinth for making a natural vibe in the pond.

3. Pond preparation and rearing of brood Fish

Before collecting the brood fishes, the pond was prepared by eradicating predatory and unwanted fishes through dewatering followed by drying. Aquatic vegetation was removed manually and harmful aquatic insects were removed by using rotenone and phostoxin. Liming was done at the rate of 1 kg/decimal and after 7 days of liming, inorganic fertilizers such as Urea and TSP were used at the rate of 200 g and 100 g per decimal, respectively for enhancing the natural production of phytoplankton and zooplankton in the brood pond. Seven days after fertilization, fishes were released into the brood pond with intensive care. During the rearing period, water quality parameters were maintained within the optimum level for the proper growth and maturation of the fishes. Measured values of water quality parameters are shown in **Table (1)**.

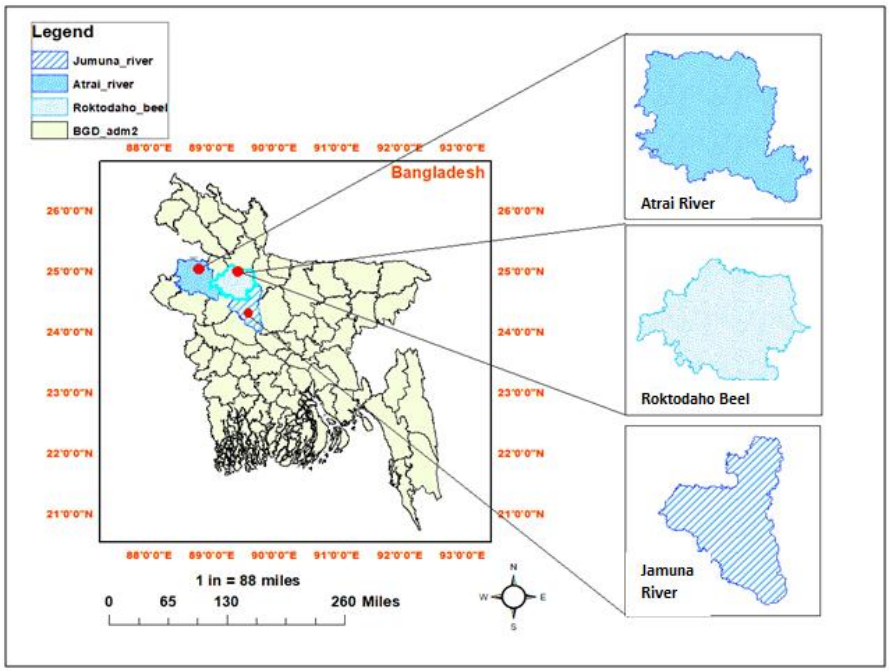

Fig. (1). Location of the capture area of *N. atherinoides*. (Images were extracted from DIVA-GIS using Geographical Information System (GIS). The map was developed by using ArcMap version 10.8)

Table (1): Recorded water quality parameters during the rearing and breeding period of the brood fishes

Water quality parameters	Values	
	Mean ± SD	Range
Water Temperature (°C)	29.91 ± 1.55	28.36 - 31.46
pH	7.82 ± 0.11	7.71 - 7.93
DO (mg/l)	5.53 ± 0.38	5.15 - 5.91
Transparency (cm)	29.12 ± 1.22	27.9 - 30.34
Free CO_2 (mg/l)	0.30 ± 0.06	0.24 - 0.36
Total ammonia (mg/l)	0.11 ± 0.05	0.06 - 0.16

4. Feeding

The fish were fed with a commercial special diet (Agatha Super Floating Premium, 0.8 mm, Code-1102) which was given twice a day at the rate of 8-10% body weight per day based on gain in average body weight of fish. The proximate composition of fish feed, according to manufacturer is presented in **(Table 2)**.

Table (2): Proximate composition of the fish feed

Component	% composition
Moisture	11
Protein	40
Metabolic Energy (ME):	3300 Kcal/Kg
Lysine	2
Fibre	6
Calcium	1.5-3.5
Phosphorous	0.8-1.8
Vitamin E	300 mg/kg

5. Brood fish selection

Selection and proper identification of brood fish is a vital step for any induced breeding technique. The size of the mature female *N. atherinoides* is comparatively larger than the mature male fish. The mature females could easily be identified by their round or oval shaped abdomen and swollen urogenital papillae. On the other hand, the mature males were identified by their flat abdomens and long protruded genital papillae. A total of 30 broods (healthy, strong, and sexually matured 15 males and 15 females) were selected and kept in three separate cisterns for breeding purpose.

6. Conditioning the brood fish and preparation of PG extract

For conditioning, the sexually matured male and female broods were weighed and kept in separate tanks with continuous water flow for 6-7 hours. Freshly prepared extract

of commercially available dry pituitary glands was used for stimulating the ovulation. Firstly, required amount of PG was carefully weighed by using an electronic balance. Then the weighed PG was homogenized with a small volume of distilled water and the suspension was centrifuged for 5 minute at 6000 rpm and finally the PG solution was loaded into a graduated 1.00 ml hypodermic syringe and injected on the basis of body weight of the gravid males and females.

By using the following formula, the required amount and volume of PG was calculated for the injection for ovulation:

Weight (mg) of required amount of PG (W_t) = W_b (the total body weight (kg) of all fishes to be injected) × P_t (the dose in mg of PG to be injected per kg body weight under a particular treatment) **(Mollah *et al.*, 2008)**.

The total volume of the extract required was calculated by the following formula:

Volume of extract (ml) = W_t (the weight of PG (mg)) × 1.0 (the volume of the extract in ml to be injected kg-body weight of fish) **(Mollah *et al.*, 2008)**.

7. Experimental design

The experiment was designed for three trials having three replicates each and was assigned into a Completely Randomized Design (CRD). Three trials were conducted during the months of April, May, and June in 2021 to estimate the optimum PG dose for the induced breeding of *N. atherinoides*. In the month of April during first trial, 15 females were divided into three treatments and designated as T_1, T_2, and T_3, having five females in each treatment and the females under each treatment were indicated as R_1, R_2 and R_3 kept separately in cisterns. Then the females were treated with PG dose at the rate of 25, 30, and 35 mg/kg body weight and the males were injected at the rate of 15, 20, and 25 mg/kg body weight under T_1, T_2, and T_3 treatment, respectively. Another two trials were conducted in the month of May and June in 2021 using 12 mg (T_1), 13 mg (T_2), and 14 mg (T_3) PG/kg body weight of female fish and males were injected at the rate of 6, 7, and 8 mg/kg body weight, respectively to develop the induced breeding technique of *N. atherinoides*. One male was employed for each female and in total 15 females and 15 males were used in each trial.

8. Gonado-Somatic Index (GSI)

Female gonad of *N. atherinoides* was collected from August, 2020 to July, 2021. During the experimental period, 70 samples were collected to measure the total length and body weight of individual fish. Then the ovary of each fish was taken out very carefully and preserved in 10% buffered formalin with labeled vials for further study. The weight of the ovary was measured very carefully with the help of a sensitive portable electronic balance (Model FX- 300).

GSI of *N. atherinoides* was calculated according to the formula **(Lagler, 1956)**:

GSI = (Gonad Weight / Total weight) × 100.

9. Fecundity

Von Vayer method was applied to estimate the fecundity of relatively large size eggs of *N. atherinoides*. In this method, the ovaries were dissected out by a pair of scissors. The external connective tissues were removed from the surface of each pair of ovaries. The moisture of the ovaries was removed with the help of a blotting paper. The weight of the ovaries of each fish was measured with the help of an electronic balance (Model FX- 300). Then 10 mg of each ovary was taken separately from anterior, middle and posterior portions of each lobe accurately. The number of mature and maturing eggs from each portion was found out separately by actual counting. The mean number of eggs in 10 mg was determined and then multiplied by the total weight of the ovary, which gave the total number of eggs.

10. Injecting the PG extract to the experimental fish

Firstly, the broods of *N. atherinoides* were carefully put on a soft and soaked cloth for injecting the PG extract. Then, the required volume of PG extract based on the body weight of the gravid males and females was taken in a graduated 1.00 ml hypodermic syringe. Intramuscular injection was given to the fish under the pectoral fin. The single dose of PG extract was given to the gravid females and males at a rate of 25-35 mg/kg and 15-25 mg/kg body weight of fish in April and 12-14 mg/kg and 6-8 mg/kg body weight of fish in May and June, respectively. During handling and injecting the fish, utmost care was taken and optimum water quality parameters were maintained to minimize all kinds of stress.

11. Ovulation, fertilization and hatching of fertilized eggs

The males and females injected with PG extract were kept in the same cistern and their behavior of pairing or courtship was closely observed. During that time, some water hyacinth were given in the cistern for making the environment favorable and an artificial fountain system supplied water splash continuously for ovulation. After 12-15 hours of injection, both the male and female released their sperms and eggs and fertilized. Then the fertilized eggs were collected and transferred into separate plastic trays for incubation. The incubation trays were receiving gentle shower through porous PVC pipes to ensure adequate oxygen. The fertilized eggs became swell and sticky when they come into contact with water. During incubation period every two hours later, the dead eggs were removed and number of eggs recorded carefully. About 23-26 hours later, the larvae of *N. atherinoides* hatched from the eggs. After completing the hatching, the hatchling numbers were counted and recorded carefully.

The formula used for calculating percent ovulation, fertilization and hatching rate are given below:

Ovulation %= (No. of fish ovulated ÷ Total no. of fish injected) × 100

Fertilization %= {Total no. of eggs (fertilized + unfertilized) ÷ No. of fertilized eggs} × 100.

Hatching %= (Total no. of eggs ÷ No. of eggs hatched) × 100.

12. Embryonic and larval stages observation

Samples of eggs were taken prior to fertilization for further studies at every 30 minutes interval. Inside the chorion, the embryonic stage occurs which is ends with hatching. During the larval stage, their nutritive contribution occurs from yolk sac and becomes end when the larva becomes capable of exogenous feeding. The post larval stage was characterized by autonomous feeding. During that time, larvae were fed boiled egg yolk three times a day. Developmental time was rounded to the nearest minute from post fertilization until the morula stage and then to the nearest hour. The age of the larvae was denoted as hour after activation. Descriptions of the developing stages were made by examining live specimens under an Electronic microscope and microphotographs. For a clear observation, specimens were temporarily stained with methylene blue and safranin. The specimens were measured by placing them over a slide having 1.0 mm graph paper at the bottom. Ten specimens were used to describe each stage.

13. Statistical analysis

All the data collected during the experimental period were recorded and preserved on a computer spreadsheet. All the data were analyzed statistically by one-way ANOVA and DMRT (Duncan Multiple Range Test) using the statistical software (Statistix 10). If a main effect was significant, the ANOVA was followed by DMRT at 5% level of significance.

RESULTS

1. Identifying running female and oozing male

Morphological changes of brood fishes were observed regularly to assess the maturity of fishes. The matured males (4-5 g) are smaller in size than females (6-7 g). The ready to spawn females have slightly smaller spiny pectoral fins and discharges ova on applying slight pressure on abdomen, whereas males having slightly larger dull color pectoral fins, flattened body and oozing milt on applying slight pressure on abdomen.

2. Gonado-somatic Index (GSI)

The GSI values of females of *N. atherinoides* were changed from 0.46±0.11 to 12.55 ± 2.50 % with the change of seasons **(Fig. 2)**. The mean GSI value of the fish tends

to increase as the fish reach maturity and after spawning, it declines and the minimum GSI value was recorded during resting phase. In case of female *N. atherinoides*, it was found that the value of GSI gradually increased from February to April. Then it increased abruptly from April and became higher during the month of June, July, and August. Highest GSI value was recorded as 12.55 ± 2.50 % in the month of July **(Fig. 2)** which it indicated that July is the peak breeding season of *N. atherinoides*. The GSI values began to fall abruptly from August to September and gently fall down from October to January.

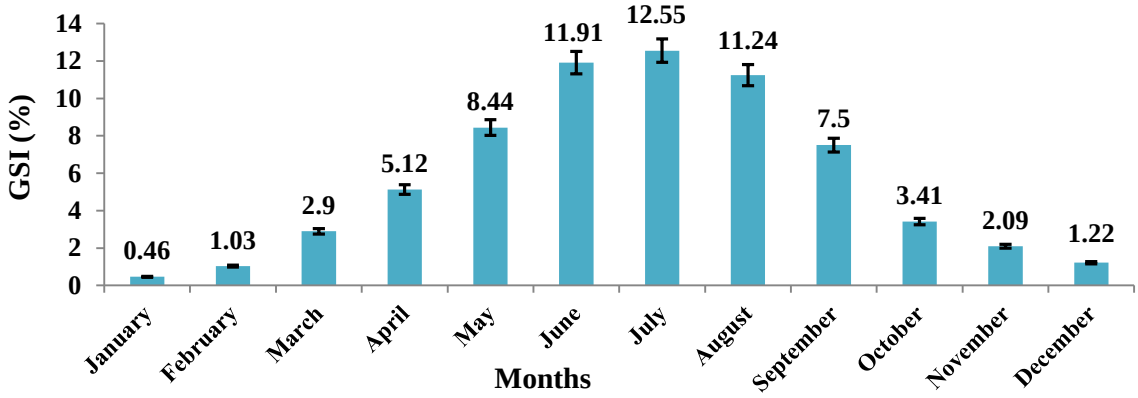

Fig. (2). Monthly mean values of gonado-somatic index (GSI) of female *N. atherinoides*

3. Fecundity

The fecundity of *N. atherinoides* was estimated through Von Vayer method. In every trial, 15 female fishes (4.14-6.65 g) were examined and the fecundity were estimated as 1210±27.59, 1800± 42.72, and 2100±98.16 in April, May, and June under the treatments of T_1, T_2, and T_3, respectively.

4. Ovulation rate

Female broods those were injected with three different doses of PG viz. 25, 30, and 35 mg/kg body weight did not respond during the month of April. However, another two breeding trials were conducted in May and June when the ovulation rates were recorded as 63.12±1.21, 65.21±2.13, and 69.14±1.59 % in May and 65.23±1.62, 68.59±1.80, and 71.87±1.91 % in June under the treatments of T_1, T_2, and T_3, respectively **(Table, 3)**. The highest ovulation rate was observed as 71.87±1.91% in June under treatment T_3, while the lowest value was recorded as 63.12±1.21% in May under treatment T_1 **(Table, 3)**. However, ovulation rate under T_3 treatment was significantly higher ($P<0.05$) than those of T_1 and T_2 treatments both in May and June. Average latency periods were recorded as 12-15 hours in May and June. In case of male fish, the PG dose of 6-8 mg/kg body weight was found optimum for inducing *N. atherinoides* both in May and June. Usually, female fishes are mainly considered for the effectiveness of PG hormone during induced breeding when they attained sexual maturity for breeding

purpose. Ovulation in the captive condition can be occurred easily if appropriate dose of PG dose is injected to matured female and male fishes.

5. Fertilization rate

Fertilization rates of eggs of *N. atherinoides* were recorded as 59.24±1.12, 61.36±1.31, and 62.34±1.21% in May; 62.85±1.30, 64.74±1.51, and 66.39±1.40% in June at 12, 13, and 14 mg PG/kg body weight under the treatments of T_1, T_2, and T_3, respectively. The highest fertilization rate was recorded as 66.39±1.40% in June under treatment T_3 whereas the lowest value was recorded as 59.24±1.12% in May under treatment T_1 **(Table, 3)**. However, fertilization rate under the treatment T_3 was significantly higher than that of T_1 and T_2 ($P<0.05$) in both May and June.

Table (3): Details of induced breeding of *N. atherinoides* by applying PG doses

Trial	Treatment	Weight of brood fish (g)		PG dose (mg/kg BW)		Latency Period (h)	Ovulation rate (%)	Fertilization rate (%)	Incubation period (h)	Hatching rate (%)
		Female	Male	Female	Male					
1 (April)	T_1	4.4±0.26	4.1±0.19	25	15	-	No	-	-	-
	T_2	4.2±0.21	3.9±0.18	30	20	-		-	-	-
	T_3	4.6±0.25	4.3±0.20	35	25	-		-	-	-
2 (May)	T_1	5.2±0.16	4.5±0.24	12	6	12 - 15	63.12±1.21^{b}	59.24±1.12^{b}	23 - 26	75.54±2.11^{b}
	T_2	5.5±0.20	4.3±0.21	13	7		65.21±2.13^{ab}	61.36±1.31^{ab}		79.64±1.62^{ab}
	T_3	5.7±0.21	4.7±0.23	14	8		69.14±1.59^{a}	62.34±1.21^{a}		80.33±1.10^{a}
3 (June)	T_1	5.9±0.24	5.1±0.20	12	6	12 - 15	65.23±1.62^{b}	62.85±1.30^{b}	23 - 26	80.89±1.30^{b}
	T_2	6.2±0.26	5.3±0.29	13	7		68.59±1.80^{ab}	64.74±1.51^{ab}		82.48±1.12^{b}
	T_3	6.4±0.25	5.7±0.23	14	8		71.87±1.91^{a}	66.39±1.40^{a}		86.98±1.90^{a}

6. Hatching rate

Hatching rate of eggs were observed as 75.54±2.11, 79.64±1.62, and 80.33±1.10% in May; 80.89±1.30, 82.48±1.12, and 86.98±1.90% in June under the treatments of T_1, T_2, and T_3 treatments, respectively. The highest hatching was observed as 86.98±1.90% in June under treatment T_3 and the lowest was recorded as 75.54±2.11% in May under T_1 **(Table, 3).** The hatching rate was significantly ($P<0.05$) higher in treatment T_3 than those of other treatments. Hatching time was ranged from 23 to 26 hours in all treatments.

7. Embryonic and larval development of *N. atherinoides*

The various developmental stages were observed by examining live specimens with ocular micrometer and stage micrometer fixed to meiotic image plus microscope connected with computer. The specimens were temporarily stained with methylene blue

and safranin for a clear view under electronic microscope. The various embryonic developmental stages and their characteristics are given below:

A. Fertilized egg: The fertilized eggs of *N. atherinoides* were adhesive, demersal, and became translucent with the progress of development. The diameter of the fertilized eggs ranged from 0.40 mm to 0.50 mm **(Fig. 3A)**.

B. Morula stage: After 30 minutes of fertilization, the cleavage started in the fertilized eggs. During that time, the cleavage furrow restricted in the animal pole and a large number of cells group at animal pole after repeated successive cleavage. This stage is called Morula **(Fig. 3B)**.

C. Blastula stage: After morula stage, a layer called blastoderm was formed by dividing the numerous cell of the development. Gradually, the blastoderm transformed into blastodisc due to further cell division. When a space between yolk and blastoderm is formed then it is called blastocoel or blastula **(Fig. 3C)**.

D. Gastrula stage: During this stage, a germinal ring was formed and the blastoderm covered with more than 80 percent of the yolk. This is called the Gastrula stage when the embryonic shield and optic rudiment was clearly visible **(Fig. 3D)**.

E. Yolk plugs stage: At yolk plugs stage, the gradual spreading of yolk invasion was completed over the germ layer and the rudimentary head and tail of embryo were formed **(Fig. 3E)**.

F. 15 hours old embryo: During this stage, the cephalic region of the embryo broadened with distinct fore brain **(Fig. 3F)**.

G. Organogenesis: The embryo was elongated with clearly differentiated head and tail from the yolk, and heart beat was noticed. Yolk sac was clearly visible. Various body organs were formed with clearly visible yolk sack at this stage **(Fig. 3G)**.

H. C-Shaped embryo: In this stage, the body of the embryo became C-shaped with elongated body and differentiated head and tail. Development of myotomes was observed and embryo started occasional movement during this stage **(Fig. 3H)**.

I. Hatching of embryo: When the embryonic development completed, the embryo moved vigorously and ruptured the egg shell. The embryo hatched out within 23-26 hours at water temperature 27-30^0 C **(Fig. 3I)**.

J. Larvae immediately after hatch: Newly hatched larvae were straight and transparent and above the yolk sac the head of the hatchling was clearly seen **(Fig. 3J)**.

K. 2 days old larvae: The larvae were transparent during this time and they have heavy ovoid yolk sac. The length of the larvae ranged from 1.2-1.8 mm **(Fig. 3K)**.

L. 3 days old larvae: During this time, the larvae freely move and successfully adhere to the cistern walls **(Fig. 3L)**.

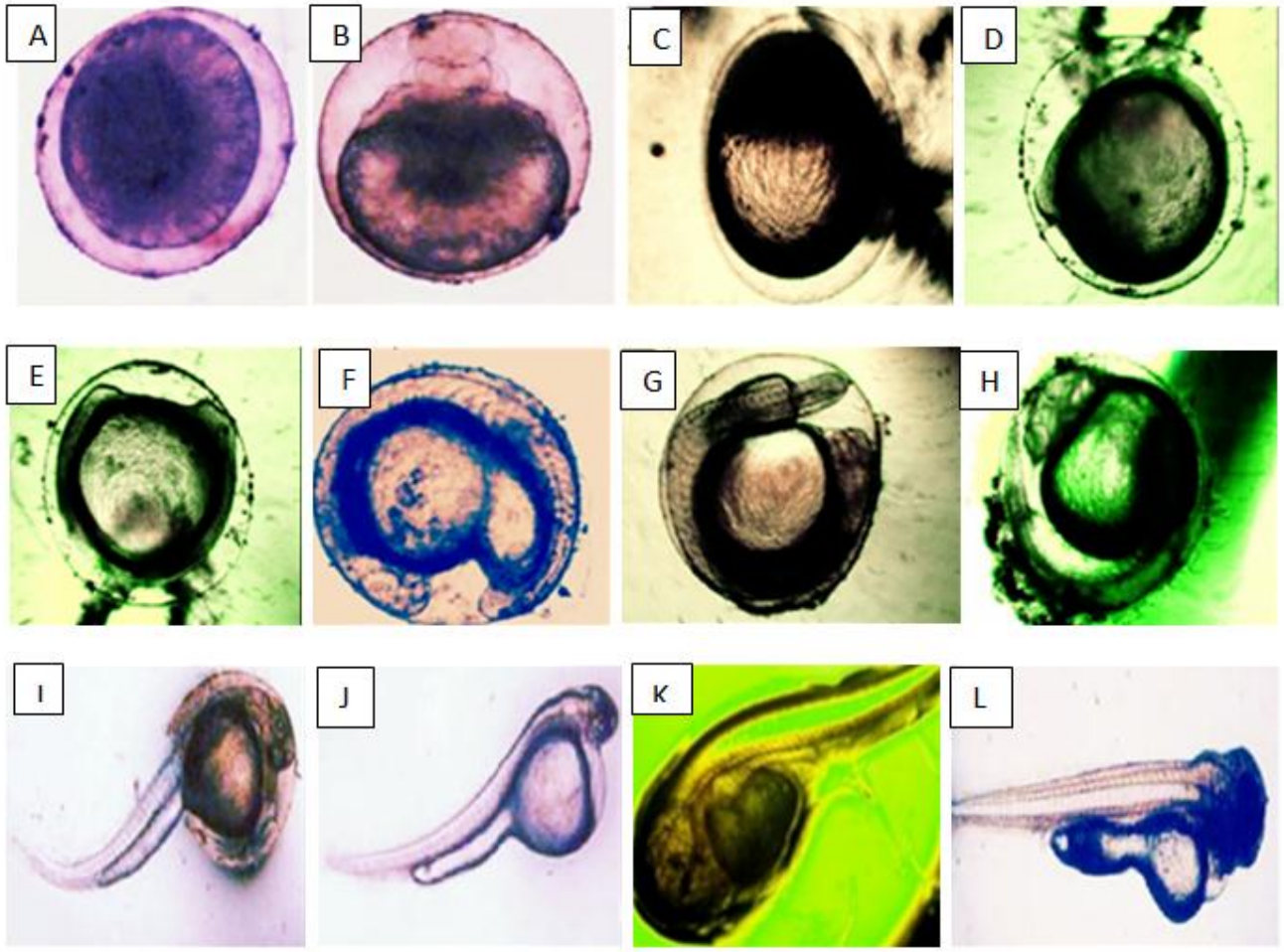

Fig. (3). Embryonic developmental stages of *N. atherinoides*. **(A)** Fertilized egg; **(B)** Morula; **(C)** Blastula; **(D)** Gastrula; **(E)** Yolk plug stage; **(F)** 15 hours old embryo; **(G)** C-shaped embryo; **(H)** Organogenesis; **(I)** Larvae come out from the shell; **(J)** Larvae immediately after hatch; **(K)** 2 days old larvae; **(L)** 3 days old larvae

DISCUSSION

In the present study, three trials were carried out to develop an induced breeding technique of *N. atherinoides* and to standardize the dose of PG for successful ovulation. Many scientists tried several times to standardize the PG dose for many species for successful ovulation although there remains uncertainty **(Khan & Mollah 2004; Bhuiyan *et al.*, 2006; Bhuiyan *et al.*, 2008; Mollah *et al.*, 2008).** This experiment was extremely crucial as there is no information available in Bangladesh or elsewhere in the world regarding the induced breeding techniques and dose optimization of PG of *N. atherinoides.*

So, an experiment was done to find out the suitable doses of PG for induced breeding of *N. atherinoides* during the months of April, May, and June 2021. The PG doses of 25 to 35 mg/kg body weight were proved to be very high as no ovulation

occurred in the month of April. It has been reported that the administration of single dose with high PG extract in female *Carassius carassius* yielded no ovulatory response where they responded well when applied the same amount of PG extract as a double dose **(Ali *et al.*, 2015)**. On the other hand, two breeding trials were conducted in May and June using the PG dose 12 (T_1), 13 (T_2), and 14 (T_3) mg/kg body weight where ovulation rates were recorded as 63.12±1.21, 65.21±2.13, and 69.14±1.59% in May and 65.23±1.62, 68.59±1.80.53, and 71.87±1.91% in June under the treatments of T_1, T_2, and T_3, respectively.

In the present study, the highest fertilization rate (66.39±1.40 %) was recorded during the month of June in treatment of T_3 where female broods were injected with a dose of 14 mg PG/kg body weight, whereas the lowest value (59.24±1.12 %) was recorded during May in T_1 treatment, where the females were administered with 12 mg PG/kg body weight. Similarly, the highest hatching rate was observed as 86.98±1.90 % during the month of June in T_3 treatment and the lowest value was recorded as 75.54±2.11 % during May in T_1 treatment. Incubation period ranged from 23-26 hours in all treatments under the ambient water temperature of 27-30° C. In the present experiment, the fishes treated with the dose of 14 mg PG/kg body weight in the month of June showed the best performance so far as the ovulation, fertilization, and hatching rates were concerned, while the fish treated with 12 mg PG/kg body weight showed the lowest ovulation, fertilization, and hatching rates of *N. atherinoides* eggs. The difference in the ovulation, fertilization, hatching rate may be occurred due to the variation in PG doses and maturation due to different month since the broods were reared in the same management. When the trials were conducted using the dose of 12, 13, and 14 mg PG/kg body weight, the 14 mg PG/kg body weight dose showed the best performance. Behind this successful breeding many factors might be responsible like environmental conditions, good management practice, feeding, fertilization of the brood pond, maturity of brood fish, brood fish condition, sex ratio, doses of PG hormone, egg quality and the ripeness of oocytes in the female fish **(Pillay, 1964; Springate *et al.*, 1985; Nandeesha *et al.*, 1990; Bromag, 1998; Marimuthu *et al.*, 2009** and **Marimuthu *et al.*, 2015)**. Hormone doses varies from species to species **(Rahman *et al.*, 1993 ; Hoq, 2006; Rahman *et al.*, 2006a)** like **Islam *et al.* (2011)** used PG dose at the rate of 8 mg/kg body weight for female and 4 mg/kg body weight for male of *Mystus vittatus* and found 80% fertilization and 56% hatching rates whereas **Khan & Mollah, (2004)** used PG extract at the rate of 10 mg/kg body weight which resulted 100% ovulation and showed best fertilization and hatching rate of eggs. In case of *Anabas testudineus*, **Shaha *et al.* (2009)** successfully used the PG dose at the rate of 12 mg/kg body weight of female and 6 mg/kg body weight of male for the sex ratio 1:2 (female: male) and in *Puntius gonionotus* **Bhuiyan *et al.*** (**2006)** used 6 mg PG dose/kg body weight as most efficient dose for induced breeding of *P. gonionotus* during the peak month of June. Four PG doses viz., 80, 100, 120, and 140 mg/kg body weight were used in female *Rita rita* and 100 mg/kg body weight was found more

effective for induction of ovulation, but lower and higher doses than the optimum had no effect on ovulation which indicates that dose optimization is crucial to induce ovulation **(Mollah *et al.*, 2008)**. Similarly, in the present experiment, the higher doses of PG such as 25, 30, and 35 mg/kg body weight had no effect on ovulation of *N. atherinoides*.

The latency period of *N. atherinoides* in the present study was found 23-26 hours during May and June month where the latency period of other species found in the literature were 22-25 hours for *Heteropneustes fossilis* **(Kohli & Goswami, 1987),** 16-20 hours for *Clarias gariepinus* **(Munshi & Hughes, 1991),** and 30 hours for *C. stiriatus* **(Marimuthu *et al.*, 2001).**

Reproductive cycle of indigenous *N. atherinoides* was examined through observing the values of GSI as it is increases with the maturation of fish, being highest during the period of peak maturity and declining abruptly thereafter, when the fish become spent **(Le Cren, 1951)**. The monthly change of GSI reflects the ovarian activity of fish. The results of the present experiment indicated that the GSI of *N. atherinoides* is highest during July when the fish is found to be mature. The increasing GSI value of *N. atherinoides* suggested that the percentage of yolk laden ripe eggs in ovary was found in June. However, recently some reproductive aspects of *N. atherinoides* were studied by **Hossian *et al*. (2020)**, where the highest gonado-somatic index was observed in the months of April, May, and June. But in the present experiment the highest GSI value was found in the month of June, July, and August. The increasing GSI value of *N. atherinoides* suggested that the percentage of yolk laden ripe eggs in ovary was found higher in June, July, and August. Their breeding season started during the period of May and continues up to the month of September with a peak in June, July, and August. Observations on embryonic and larval developments of *N. atherinoides* are found similar with those of native Pangas, *Pangasius pangasius* (**Rahman *et al.*, 2006b**).

The results of the present study indicated that induced breeding of *N. atherinoides* was successful by using different doses of PG extract and among all trials, comparatively better performances in terms of ovulation, fertilization, and hatching rates were found in treatment T_3 when 14 mg PG/ kg body weight was applied.

CONCLUSION

Indian potashi *(N. atherinoides)* is a tasty and nutritious fish species but due to environmental and anthropogenic activities, we degraded their natural breeding and nursery grounds and made them endangered in natural habitat. Therefore, it is essential to conserve the fish from being extinction in near future. So, artificial seed production through induced breeding technique is the only way to protect them. In the present study, the induced breeding of *N. atherinoides* through PG extract was successful and upon all considerations, injection of PG extract at a dose of 14 mg PG/kg body weight of female *N. atherinoides* in June showed the better results in captive condition. This result might

be helpful towards the large-scale production of quality seeds for aquaculture production as well as conservation of this important fish species from extinction.

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