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ORIGINAL RESEARCH ARTICLE

First record of induced breeding and fry production techniques of Pialy fish, *Aspidoparia jaya* (Hamilton, 1822) in Bangladesh

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ABSTRACT

This experiment was conducted for the development of induced breeding techniques of *Aspidoparia jaya* using the pituitary gland (PG) extract at the Floodplain Sub-station, BFRI, Santahar, Bogura, Bangladesh. Three breeding trials were conducted in May, June and July, 2021 where in May, none of the fish was ovulated. But breeding response was observed in June and July when we applied 8 mg (T_1), 10 mg (T_2) and 12 mg (T_3) PG/kg body weight of female, and 5 mg (T_1), 6 mg (T_2) and 7 mg (T_3) PG/kg body weight of male fish. Among all the treatments, significantly *(P<0.05)* highest breeding performance in terms of ovulation rate (78.87±1.71%), fertilization rate (79.39±1.40%) and hatching rate (86.98±1.20%) were observed in T_3 treatment in the month of July when injected with single dose of 12 mg and 7 mg PG/ kg body weight of female and male, respectively. Significantly (*P<0.05*) higher values of mean gonado-somatic index were observed during June, July, August, December and January for females where July (10.15±1.50%) and January (9.55±1.30%) showed the highest peaks which indicated that they might be spawn twice in a year (from May to August and from December to January). The results from the present experiment reveals that induced breeding of *Aspidoparia jaya*, using PG extract is successful which might be helpful for the large-scale seed production of this species for the aquaculture as well as to conserve the species from being extinct from the biodiversity.

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INTRODUCTION

Fisheries sector playing a significant role in the national economy of Bangladesh for the last few decades and it is the most productive and dynamic sector because of its vast inland, coastal and marine water resources which makes the Bangladesh one of the top fish-producing country in the world (Ghose, 2014; Shamsuzzaman *et el*., 2017; Sunny *et el*., 2020; Sunny *et al*., 2021; Sunny *et al*., 2021; Sunny *et al*., 2021a; Rashid and Mithun, 2020). Due to favorable geographic position, Bangladesh has diversified fisheries resources with 266 species of freshwater fishes and 475 species of marine water fishes (FRSS, 2018). Among 266 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). Pialy fish (Scientific name: *Aspidoparia jaya;* Common name: Jaya) is one of the endangered freshwater SIS in Bangladesh which belongs to the family Cyprinidae and genus *Aspidoparia*. Among the small indigenous species the Pialy fish (*A. jaya*) has been considered as one of the most admired edible fish species due to good taste and high market demand. Along with *A. jaya*, SIS has been considered as an excellent source of essential macro and micro-nutrients which can play an important role in the elimination of malnutrition problem in Bangladesh

(Zafri and Ahmed, 1981). According to IUCN Red List (2015), among 266 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 in Bangladesh (IUCN, 2015). Once these SIS were available in rivers, floodplains, inundated swamp fields, ditches, canals and other freshwater areas of Bangladesh. For many reasons, the Jaya fish or Pialy are in endangered condition due to habitat degradation, water pollution, degradation of breeding and feeding ground, construction of dams in the floodplain area, use of insecticides and pesticides in the agriculture field (Rahman, 2005). The species *A. jaya* (known as Pialy or Jaya) is native to Bangladesh, India, and Nepal (Talwar and Jhingran, 1991). In Bangladesh, it is commonly found in floodplain, river, beel, haor, baor etc. This fish species has high nutritional value and rich in protein, fat, vitamins and minerals. *A. jaya* is an important species for capture fisheries in our country but no culture practice introduces yet due to the lack of fry availability. But this species has commercial importance and can be cultured in the pond with other species or as a single species for local consumption or exported as a delicious expensive fish. It is crucial to gather knowledge about the food and feeding behavior, reproductive biology, fecundity, breeding behavior and season of this fish for the development of induced breeding technique to ensure the stock of quality seeds. But till now, no systematic research works have been done to breed this species artificially through establishment of induced breeding. So, this present study has been carried out to build up an appropriate induced breeding technique of *A. jaya* that will be helpful for mass production of quality seeds of this species, which will not only facilitate to aquaculture production but also will save this endangered species from being extinction.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in the earthen pond of the Floodplain Sub-station (Bogura, Bangladesh) for rearing the brood fishes and the induced breeding was done in the Hatchery & Breeding Complex of the Floodplain Sub-station, Bangladesh Fisheries Research Institute, Santaher, Bogura, Bangladesh.

Collection of brood fishes

The sexually matured, strong and diseased free broods of Pialy fish (*A. jaya*) were collected from the Atrai River, Jamuna River and Roktodaho Beel under Naogoan, Sirajgonj and Bogura districts of Bangladesh (Figure 1) and stocked in the ponds for domestication for breeding purposes. Before stocking into the pond, the brood fishes were bathed into 1.5-2 ppm potassium permanganate for disinfection and then 3 to 4 g weighed fish were stocked in the pond at a rate of 250-300 broods/decimal. Regular water supply and optimum water quality parameters were maintained in the pond for the growth and development of the brood fishes.

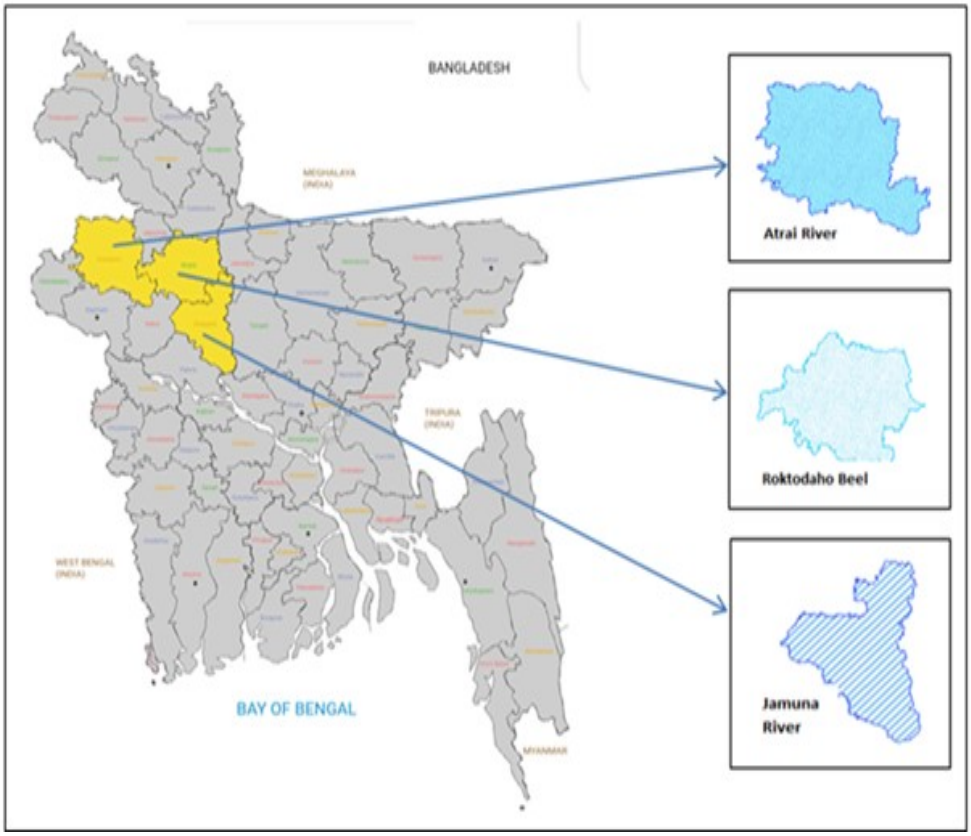

Figure 1. *Location of the capture area of A. jaya.*

Pond preparation and rearing of brood fish

For the preparation of brood pond, we eradicated the predators and unwanted fishes from the pond by dewatering and 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, organic fertilizer was applied at the rate of 5-7 Kg/decimal and inorganic fertilizers such as Urea and TSP were also applied at the rate of 200 g and 100 g per decimal, respectively for enhancing the natural food (phytoplankton and zooplankton) in the brood pond. After seven days of fertilization, fishes were released in brood pond with intensive care and fed on a special diet enriched with protein and vitamin E at the rate of 6-7% of body weight per day. During the rearing of brood fishes, proper water quality parameters were maintained for the proper growth and maturation of the fishes. Measured values of water quality parameters are shown in the following (Table 1).

Feeding

As a supplementary feeding a commercial special diet (Agatha Super Floating Premium, 0.8 mm, Code-1102) was given twice a day at the rate of 12-8% body weight per day based on gain in average body weight of fish. The proximate composition of fish feed is given below (Table 2).

Brood fish selection

For the development of induced breeding, selection of brood fish is a vital step. The size of the mature male (5-8 g) of *A. jaya* is comparatively smaller than the mature female (7-16 g) fish. The mature females could easily be identified by their whitish round abdomen and swollen urogenital papillae. On the other hand, the mature males have yellowish flat abdomens and long protruded genital papillae. A total of 38 healthy, good and sexually matured male and female broods (23 males and 15 females) were selected and kept in three cisterns for breeding purpose.

Conditioning of brood fish and preparation of PG extract

Before injecting with PG extract, the sexually matured male and female broods were weighed and kept in separate tank with continuous water flow before 5-6 hours for conditioning. Freshly prepared extract of commercially available dry pituitary glands was used for stimulating the ovulation. At first, required amount of dry pituitary gland was carefully weighed by using an electronic balance (Model FX- 300) and then homogenized with distilled water. Then the suspension was centrifuged for 8 minutes at 6000 rpm in a centrifuging machine. 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 male and female.

By using the following formula (Mollah *et al.*, 2008), the required amount and volume of PG was calculated for the injection for ovulation:

$W_t = W_b \times P_t$

Where, W_t= Weight (mg) of PG; W_b= Total body weight (kg) of; P_t= Dose (mg) of PG to be injected per kg body weight of fish

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 May, June and July in 2021 for the development of induced breeding techniques of *A. jaya*. In the month of May during first trial, fifteen females were divided into three treatments having five females in each treatment which were designated as T_1, T_2, and T_3. Each treatment had three replications which were indicated as R_1, R_2 and R_3. Then the females were treated with PG dose at the rate of 20, 25, and 30 mg/kg body weight and the males were treated at the rate of 10, 15, and 20 mg/kg body weight under T_1, T_2, and T_3 during the month of May. Besides, two more trials were conducted in June and July, 2021 where PG extract were used at a rate of 8 mg (T_1), 10 mg (T_2) and 12 mg (T_3) PG/kg body weight of female fish and 5, 6, and 7 mg/kg body weight of male, respectively to develop the induced breeding technique of *A. jaya*. Three males were employed for two females (Female and male ratio 2:3) and in total 15 females and 23 males were used in each trial.

Table 1. Recorded water quality parameters during the rearing and breeding periods 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^{-1})	5.53 ± 0.38	5.15 – 5.91
Alkalinity (ppm)	69.69 ± 3.94	65.75-73.63
Hardness (ppm)	89.23 ± 2.39	86.84-91.62
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^{-1})	0.11 ± 0.05	0.06 – 0.16

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

Gonado-somatic index (GSI)

For observing the gonado-somatic index of *A. jaya*, a total 80 samples were collected to measure the total length and body weight of individual fish from a period of September, 2020 to August, 2021. The weights of the collected ovary were measured by a sensitive portable electronic balance (Model FX-300) and were preserved in 10% buffered formalin with labeled vials for histological study.

The formula (Lagler, 1956) for the calculation of GSI of *A. jaya* is given below:

$$GSI = \frac{\text{Gonad weight}}{\text{Body weight}} * 100$$

Fecundity

Von Vayer method was applied for estimating the fecundity of relatively large size eggs where ovaries were dissected out by a pair of scissors. The external connective tissues and moisture were removed by a blotting paper from the surface of each pair of ovaries. The weights of the ovaries were measured by an electronic balance (Model FX- 300) and then 10 mg sample from each ovary was taken separately from anterior, middle and posterior portions of each lobe accurately and then counting the number of mature and maturing eggs from each portion separately. 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.

Injection of PG extract to the brood fish

Firstly, the broods of *A. jaya* fish 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 20-30 mg/kg and 10-20 mg/kg body weight of fish in May and 8-12 mg/kg and 5-7 mg/kg body weight of fish in June and July, respectively. During handling and injecting the fish, utmost care was taken and optimum water condition was maintained to minimize all kinds of stress.

Ovulation, Fertilization and hatching of fertilized eggs

The injected males and females were kept in the same cisterns and closely observed their pairing or courtship behavior. During that time, an artificial fountain system continuously supplied water splash for making the environment favorable for ovulation. After 6-8 hours of injection, both the male and female released their sperms and eggs and fertilized. Then the fertilized eggs were transferred into separate plastics trays for incubation that received gentle shower through porous PVC pipes to ensure adequate aeration. The fertilized eggs became swell and stickier 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 20-22 hours later the larvae of *A. jaya* hatched from the eggs. After completing the hatching, the hatchling numbers counted and recorded carefully.

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

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

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

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

Embryonic and larval stages observation

For the observation of embryonic and larval development, 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, they received nutrition from yolk sac until they become capable for exogenous feeding. The post larval stage was characterized by autonomous feeding. During that time, larvae were fed boiled egg yolk three times a day. From post fertilization to the morula stage developmental time was rounded to the nearest minute and then to the nearest hour. The age of the larvae was counted as hour during that time. 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 observed by placing them over a slide having 1.0 mm graph paper at the bottom and fifteen specimens were used to describe each stage.

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 AND DISCUSSION

Identifying running female and oozing male

Regular morphological changes of brood fishes were observed as indicators of maturation. The matured females are larger in size than males. The ready to spawn females have whitish swollen abdomen and discharged ova on applying slight pressure whereas males have slightly yellowish abdomen and flattened body but oozing milt on applying slight pressure on abdomen.

Gonado-somatic index (GSI)

Seasonal changes in mean GSI values of females of *A. jaya* are presented in (Figure 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 was recorded during resting phase. In case of female *A. jaya*, it has been found that the weight of the gonad gradually increased from April and then it increased abruptly from May and reached to a maximum value (10.15 ± 1.50 %) in July. Specimens collected on February, March, September, and October having small GSI varying from

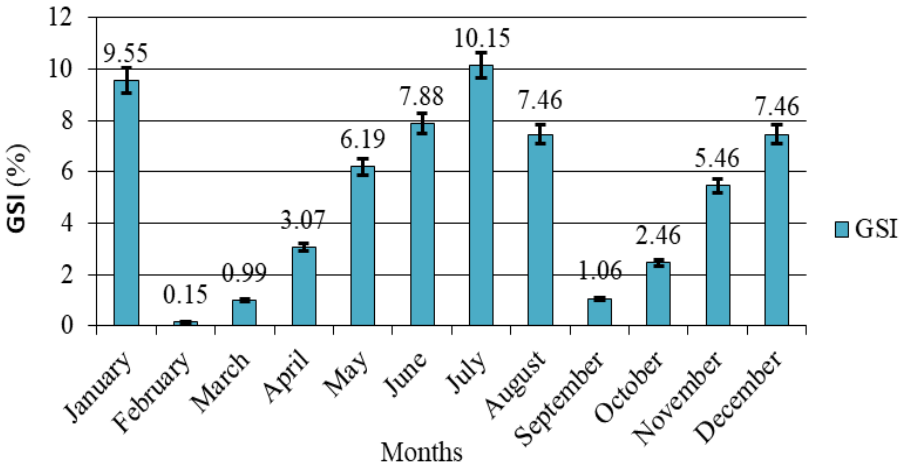

Figure 2. *Monthly mean values of gonado-somatic index (GSI) of female A. jaya.*

0.15± 0.07, 0.99± 0.10, 1.06± 0.25 and 2.46± 0.87, respectively. Specimens collected on April, May and November having medium GSI varying from 3.07± 0.99, 6.19± 2.50, and 5.46±1.57, respectively.

The higher GSI values were found in the month of June, July, August, December, and January. Two distinct peaks were observed during the month of July (10.15 ± 1.50 %) and January (9.55 ± 1.30 %) which indicated that they might be spawn twice in a year (from May to August and from December to January). The GSI values began to fall abruptly in September and again gradually increased from October to November and again spawn in December and January when their mean GSI value became 7.46 ± 1.20 and 9.55 ± 1.35, respectively. For female, the GSI values varied from 0.15±0.07 to 10.15 ± 1.50 (Figure 2) throughout the sampling period.

Fecundity

The fecundity was estimated soon after the ovulation of fish. In every trial, fifteen female fishes (5.86-8.18 g) were examined and the fecundity were found 2709±250, 3847± 356, and 5511±420 in May, June, and July under the treatments of T_1, T_2 and T_3, respectively.

Ovulation rate

Female brood fish those injected with three different doses of PG viz., 25, 30, 35 mg/kg body weight did not respond during the month of May. However, another two breeding trials were conducted in June and July and the average ovulation rates were recorded to be 61.12±1.21%, 66.21±1.13% and 70.14±1.09% in June and 67.23±1.62%, 72.59±1.80% and 78.87±1.71% in July under the treatments T_1, T_2 and T_3, respectively. The highest ovulation was observed as 78.87±1.71% in July under T_3 treatment, while the lowest value was recorded as 61.12±1.21% in June under T_1 treatment (Table 3). However, ovulation rate under T_3 was significantly higher ($P<0.05$) than those of T_1 and T_2 treatments in both June and July. Average latency periods were recorded as 6-8 hours in June and July. In case of male fish, the PG dose (5-7 mg/kg body weight of fish) was found to be satisfactory in both June and July months. 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 hormone is injected to matured female and male fishes by maintaining optimum water quality parameters.

Fertilization rate

Average fertilization rates of eggs were recorded to be 57.24±1.12%, 63.36±1.01% and 67.34±1.21% in June; 69.85±1.30%, 74.74±1.51% and 79.39±1.40% in July at a dose of 8, 10, and 12 mg PG/kg body weight under the treatments T_1, T_2 and T_3, respectively. The highest fertilization rate was recorded as 79.39±1.40% in July under T_3 whereas the lowest value was recorded as 57.24±1.12% in June under T_1 (Table 3). However, fertilization rate under T_3 treatment was significantly higher than that of T_1 and T_2 ($P<0.05$) in both June and July.

Hatching rate

Average hatching rate of eggs were observed to be 70.54±2.11%, 74.64±1.62% and 79.33±1.10% in June; 78.89±1.30%, 82.48±1.12% and 86.98±1.20% in July under the treatments T_1, T_2 and T_3 treatments, respectively. The highest hatching was observed as 86.98±1.20% in July under T_3 treatments and the lowest was recorded as 70.54±2.11% in June under T_1 (Table 3). The hatching rate was significantly ($P<0.05$) higher in T_3 treatments than those of other treatments. Hatching time was ranged from 20 to 22 hours in all treatments.

Embryonic and larval development of *A. jaya*

The following developmental stages were observed by examining live specimens which were temporarily stained with methylene blue and safranin for a clear view under electron microscope. The ocular micrometer and stage micrometer were fixed to motic image plus microscope connected with computer. The various embryonic developmental stages and their characteristics are given below:

Table 3. Details of induced breeding of *A. jaya* 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					
Trial 1 (May)	T_1	5.12±0.26	5.78±0.19	20	10	-	No ovulation occurred	-	-	-
	T_2	5.81±0.21	5.97±0.18	25	15	-		-	-	-
	T_3	5.54±0.25	5.61±0.20	30	20	-		-	-	-
Trial 2 (June)	T_1	6.20±0.16	6.53±0.24	8	5	6 to 8	61.12±1.21^c	57.24±1.12^c	20 to 22	70.54±2.11^c
	T_2	6.53±0.20	7.34±0.21	10	6		66.21±1.13^b	63.36±1.01^b		74.64±1.62^b
	T_3	6.78±0.21	6.93±0.23	12	7		70.14±1.09^a	67.34±1.21^a		79.33±1.10^a
Trial 3 (July)	T_1	7.94±0.24	8.10±0.20	8	5	6 to 8	67.23±1.62^c	69.85±1.30^c	20 to 22	78.89±1.30^c
	T_2	7.79±0.26	8.36±0.29	10	6		72.59±1.80^b	74.74±1.51^b		82.48±1.12^b
	T_3	7.44±0.25	8.74±0.23	12	7		78.87±1.71^a	79.39±1.40^a		86.98±1.20^a

*Mean values with different superscripts letters in the same column indicate a significant difference ($p<0.05$).

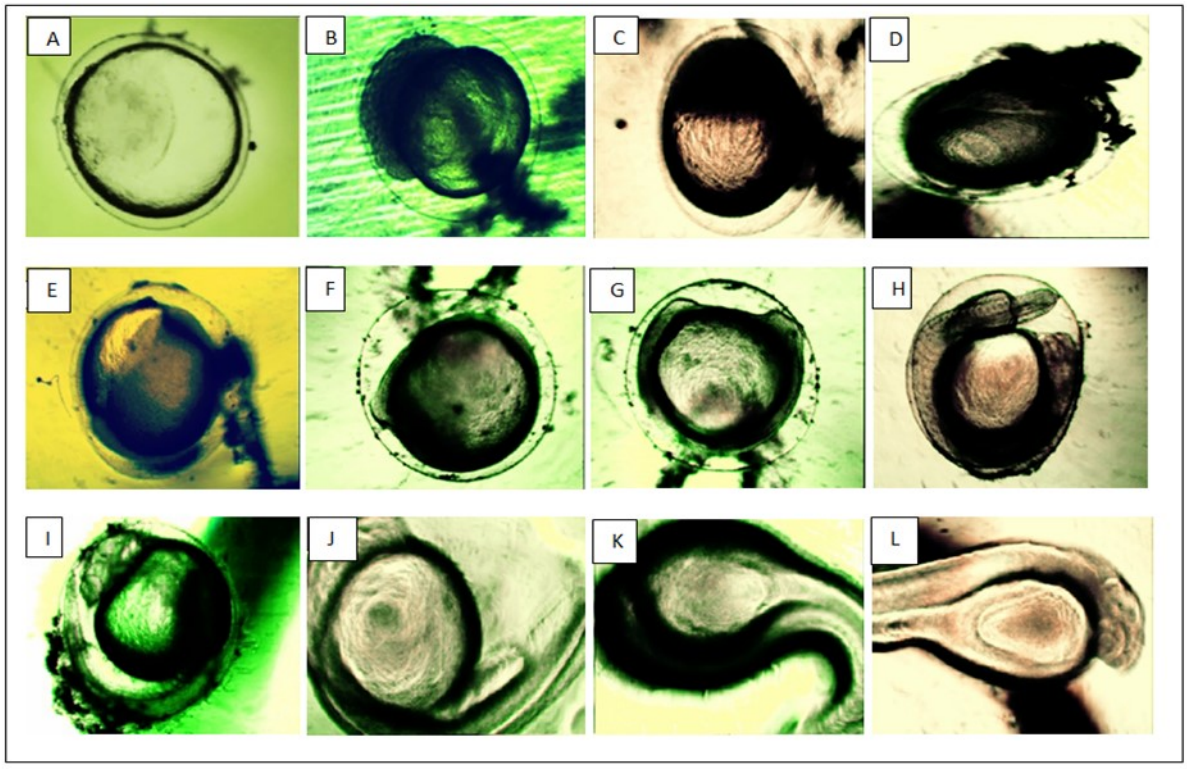

Figure 3. *Embryonic developmental stages of A. jaya. (A) Fertilized egg, (B) Morula stage, (C) Blastula stage, (D) Early gastrula stage, (E) Mid gastrula stage, (F) Late gastrula stage, (G) Yolk plug stage, (H) Organogenesis, (I) C-shaped embryo, (J) Twitching stage, (K) Hatching of embryo, (L) Newly hatched larva.*

A. Fertilized egg: The fertilized eggs of *A. jaya* were globular, adhesive and became translucent with the progress of development. The diameter of the fertilized eggs ranged from 0.50 mm to 0.65 mm (Figure 3A).

B. Morula stage: After 30 minutes of fertilization, cleavage started in the fertilized eggs which become restricted to animal pole of cytoplasm. After repeated successive cleavages, a large number of cells formed at animal pole which is called 'morula' (Figure 3B).

C. Blastula stage: A layer of blastoderm was formed in the developing embryo which was gradually developed into blastodisc (several layer of blastoderm) due to further cell division. At this stage a space called blastocoel was formed between yolk and blastoderm. This stage of embryo is called 'blastula' (Figure 3C).

D. Early gastrula stage: This is the beginning of gastrulation when the invading of blastoderm started and slowly spread over the yolk in the form of a thin layer (Figure 3D).

E. Mid gastrula stage: At this stage, a germinal ring by thickening the lower rim of the blastodisc which was clearly visible around the yolk (Figure 3E).

F. Late gastrula stage: At this stage, embryonic shield and optic rudiment were clearly visible when blastoderm covered more than 80 percent of the yolk (Figure 3F).

G. Yolk plug stage: At this stage, rudimentary head and tail were formed which were differentiated from the yolk and yolk invasion was completed by gradual spreading over the germ layer (Figure 3G).

H. Organogenesis: In this stage, embryos become elongated and rudiments of various organs were formed. Heart beat noticed and yolk sac was clearly visible where head and tail were clearly differentiated from yolk (Figure 3H).

I. C- shaped embryo: The elongated embryo formed into C-shaped where the differentiated head and tail become attached to the tail and head. Myotomes development was observed with occasional movement of the embryo (Figure 3I).

J. Twitching stage: In this stage, yolk sac was only restricted to the head region where the tail was completely detached. The number of myotomes increased in this stage and embryo exhibited continuous twitching movement (Figure 3J).

K. Hatching of embryo: The twitching movement was started vigorously as the embryonic development completed. The embryo was hatched out within 20-22 hours at water temperature of 27-29°C (Figure 3K).

L. Newly hatched larva: During this time, the full developed embryo moved vigorously which ultimately ruptured the egg shell and the larvae came out (Figure 3L).

In the present study, three trials were carried out to develop an induced breeding technique of *A. jaya* and to standardize the dose of PG for successful ovulation. Scientists tried several times to standardize the PG dose for many species for successful ovulation although there remains uncertainty (Mollah *et al.*, 2008; Khan and Mollah, 2004; Bhuiyan *et al.*, 2006; Bhuiyan *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 *A. jaya.*

However, recently some reproductive aspects of *Aspidoparia morar* were studied by Hossian *et al.* (2020) where the highest gonado-somatic index was observed from November to April which indicated the spawning season. But the breeding season of *A. jaya* started during the period of May and continues to the month of August with a peak in June and July. So, an experiment was done to find out the suitable doses of PG for induced breeding in *A. jaya* during the months of May, June and July 2021. The PG doses of 20 to 30 mg/kg body weight were proved to be very high as no ovulation occurred in the month of May. On the other hand, two breeding trials were conducted in June and July using the PG dose 8 (T_1), 10 (T_2) and 12 (T_3) mg/kg body weight where the average ovulation rates were recorded to be 61.12±1.21%, 66.21±1.13% and 70.14±1.09% in June and 67.23±1.62%, 72.59±1.80% and 78.87±1.71% in July under the treatments T_1, T_2 and T_3, respectively.

In the present study, the highest average fertilization rate (79.39±1.40%) was recorded during the month of July in T_3 treatment where *A. jaya* broods were injected with a dose of 12 mg PG/kg body weight, whereas the lowest value (57.24±1.12 %) was recorded during June in T_1 treatment, where the fishes were administered with 8 mg PG/kg body weight. Similarly, the highest hatching rate was observed as 86.98±1.20 % during the month of July in T_3 treatment and the lowest value was recorded as 70.54±2.11 % during June in T_1 treatment. Incubation period ranged from 20-22 hours in all treatments under the ambient water temperature of 27-31°C. In the present study, females treated with 12 mg PG/kg body weight in July showed the best performance in terms of ovulation, fertilization and hatching rates, while treated with 8 mg PG/kg body weight of female showed the lowest ovulation, fertilization and hatching rates of *A. jaya* eggs. The difference in the ovulation, fertilization, hatching rate may be occurred due to the variation in PG doses, maturation due to month since the broods were reared in the same management. When the trials were conducted using the dose of 8, 10, 12 mg PG/kg body weight, the 12 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, and fertilization of the brood pond, maturity of brood fish, brood fish condition, sex ratio and doses of PG hormone, egg quality and the ripeness of oocytes in the female fish (Bromage, 1998; Springate *et al.*, 1985; Nandeesha *et al.*, 1990; Pillay, 1964; Marimuthu *et al.*, 2009; Marimuthu *et al.*, 2015). Hormone doses varies from species to species (Hoq, 2006; Rahman *et al.*, 1993; Rahman *et al.*, 2006) 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 and 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*, Saha *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* used 6 mg PG/kg body weight was found to be most efficient for induced breeding of this species during the peak month of June (Bhuiyan *et al.*, 2006). Four PG doses viz., 80, 100, 120 and 140 mg/kg body weight were used in female *Rita rita* and among them 100 mg/kg body weight of female was found to be more effective for induction of ovulation, but higher and lower doses than the optimum had no effect on ovulation which indicates that dose optimization is essential for induced breeding (Mollah *et al.*, 2008). Similarly, in the present experiment, the higher doses of PG such as 20, 25 and 30 mg/kg body weight had no effect on ovulation of *A. jaya*. The latency period of *A. jaya* in the present study was found 20-22 hours during June and July month where the latency period of *Heteropneustes fossilis* were found to be 22-25 hours (Kohli and Goswami, 1987), 16-20 hours for *Clarias gariepinus* (Munshi and Hughes, 1991) and 30 hours for *C. stiriatus* (Marimuthu *et al.*, 2001).

Reproductive cycle of indigenous *A. jaya* was examined through observing the values of GSI as it is increases with the maturation of fish, which became highest during the period of peak maturity and immediately after spawning it declined abruptly (Le Cren, 1951). The results of the present experiment showed

the two distinct peaks of GSI of *A. jaya* during the month of July (10.15 ± 1.50 %) and January (9.55 ± 1.30 %) which indicated that they might be spawn twice in a year (from May to August and from December to January). The increasing GSI value of *A. jaya* suggested that the highest percentage of yolk laden ripe eggs in ovary were found in July and January. Their breeding season started during the period of May and continues up to the month of August with a peak in July and again started during the month of December and January with a peak in January. Observations on embryonic and larval developments of *A. jaya* are found similar with those of native Pangas, *Pangasius pangasius* and *Puntius sarana* (Rahman *et al.*, 2006; Udit *et al.*, 2014)

The results in the present study revealed that artificial breeding of *A. jaya* 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 observed in T_3 treatment at a dose of 12 mg PG/ kg body weight of female and 7 mg PG/ kg body weight of male.

Conclusion

Aspidoparia jaya has high nutritional value but due to environmental degradation and destructive 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 extinct in near future. So, mass seed production in the hatchery through induced breeding technique is the only way to protect them from being extinction. In the present study the induced breeding of *A. jaya* through PG extract was successful and upon all considerations, injection of PG extract at a dose of 12 mg PG/kg body weight of female *A. jaya* in July showed the better results in captive condition. This success 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.

Conflict of interest

We confirm that there are no conflicts of interest among the authors or between the authors and other people, institutions or organizations.

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