

Article

## Spawning and developmental biology of endangered Pabda catfish, *Ompok pabda* (Hamilton, 1822)

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**Abstract:** Pabda catfish, *Ompok pabda* was collected from the Old Brahmaputra river and successfully induced to breed by injecting pituitary gland extract to evaluate spawning performances and developmental stages under a photo microscope from February 2018 to August 2018. In this study, absolute fecundity of the *O. pabda* females varied from 6,676 to 20,410 eggs per fish. The GSI value of male and female was lower during September and gradually attained its maximum level in June with the highest average value  $6.75 \pm 0.2$  and  $1.053 \pm 0.2$  in female and male, respectively. The average fertilization and hatching rate of eggs were found  $78.67 \pm 1.33\%$  and  $70.21 \pm 0.73\%$ , respectively at  $24.5 \pm 1.0^\circ\text{C}$ . Fertilized eggs were brownish in color, very transparent and adhesive in nature with 1.0 to 1.2 mm in diameter. Cleavage stages were completed at 1.50 h of post fertilization and it eventually took 23.00 h for hatching. Larva was appeared with prominent three pairs of barbells after 12.00 h of hatching. It took 3 d post hatching to absorb their yolk sac and feeding exogenously. The present work supplemented to the deficient information on detailed spawning and developmental biology of *O. pabda* in order to save this species from extinction.

**Keywords:** *Ompok pabda*; spawning; embryonic development; larval development

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### 1. Introduction

Pabda catfish, *Ompok pabda* is a freshwater catfish out of 158 inland species (Rajagopal and Davidar, 2013) belonging to the family Siluridae of the order Siluriformes. *O. pabda* dwells and breeds in the rivers and reservoirs (Mukherjee *et al.*, 2002). It has a wide geographical range in India, Bangladesh, Nepal, Sri Lanka, Afghanistan, Pakistan, China, Thailand and Myanmar (Mukhopadhyay and Ghosh, 2007) and can thrive in all types of freshwater habitats, especially in rivers, canals, beels, swamps, floodplains and ponds. This fish species has high preference as a table fish to the people of Bangladesh, East India and North India as well (Jhingran, 2004) due to its delicious taste and high nutritional value with good amount of lipoprotein, protein and mineral content in its flesh (Banik *et al.*, 2012). Recently, it has made its entry in the international ornamental fish market and has high acceptability among the fish hobbyists (Gupta and Banerjee, 2013; Gupta, 2018). Due to high market value and consumer demand *O. pabda* bears exceptional criteria in aquaculture but the biggest constraint is lack of adequate supply of stockable fry. At present, this fish species is facing high risk of extinction in nature (Hossen *et al.*, 2015) as over the last 10 years its wild population has undergone a steady decline (>50%) due to loss of its population which attributes degradation of breeding and feeding ground, pollution, over-exploitation, poor nutrition, extensive use of pesticides and insecticides in agricultural fields along with non-judicious land use pattern and most importantly poor breeding and rearing management (Chakrabarty *et al.*, 2006; Purkayastha *et al.*, 2012). Poor domestication of *O. pabda* in culture system due to insufficient knowledge on breeding and farming technique is also a reason behind this declination (Chakrabarty *et al.*, 2006). Due to reduced abundance in nature, it has already been enlisted as an endangered species both in India (CAMP, 1998) and Bangladesh (IUCN, 2015). The detailed knowledge about spawning and

developmental biology could be the first priority that will restrict the declination of *O. pabda*. Moreover, to ensure the availability of fry as well as to prevent this fish species from extinction it is important to establish a dependable induced breeding and larvae rearing technique. Although some workers (Banik *et al.*, 2012; Sarma *et al.*, 2012) had previously reported successful induced breeding of pabda, but this had not been standardized to be recommended at farmer's level for commercial seed production. Embryonic development and larval development which provides remarkable information in itself that are imperative and consequential to the successful rearing of larvae for large scale seed production (Marimuthu and Haniffa, 2007). Optimization of mass seed production and rearing management much rely on early life history information which is very much important to optimize larval growth and survival. Earlier few studies have been carried out on food, feeding habit and reproductive biology of *O. pabda*; but consolidated report on these aspects so far is not available. The developmental biology of *O. pabda* is an indispensable aspect which may be considered as base line information for the researchers and fish culturists, who are engaged in the seed production of the species (Chakrabarti *et al.*, 2008). Therefore, the present study has been conducted to provide detailed information about onset of spawning, the early embryonic and larval development of *O. pabda*.

## 2. Materials and Methods

### 2.1. Experimental site and brood fish collection

*O. pabda* (N=60) weighing average length  $21.74 \pm 0.71$  cm and weight  $56.45 \pm 1.97$  g, respectively were collected (February, 2018) from the sampling sites located in Mymensingh region of the Old Brahmaputra river, Bangladesh lying at  $24^{\circ}2'0''$  North latitude and  $90^{\circ}59'0''$  East longitude. After collection, the fishes were transported to Mini Hatchery and Laboratory Complex, Faculty of Fisheries, Bangladesh Agricultural University in oxygenated polythene bags. Upon arrival, fish were sexed and conditioned for about 15 days. The fishes were stocked in cisterns having  $1.22 \text{ m} \times 2.44 \text{ m} \times 0.46 \text{ m}$  dimension with 30 cm water depth and fed with pellet diet having 30% crude protein at 5% body weight per day for rearing up to their maturation.

### 2.2. Physicochemical condition of water

Fresh and well aerated water was used for domestication of the fish in all the cisterns. Physicochemical parameters of water in all cisterns were recorded periodically for rearing and breeding this fish species. Temperature, DO and pH, total alkalinity and total hardness were determined by using a Celsius thermometer, digital DO meter (multi 340 iset, DO-5509; China), portable digital pH meter (MICRO-TEMP, pH 500, Romania), HI3811 Alkalinity test kit and HI3812 Hardness test kit of HANNA, respectively.

### 2.3. Induced breeding, fertilization and incubation of fertilized eggs

The breeding of *O. pabda* brood was conducted in June, 2018. Good looking, healthy and sexually matured broods were selected for breeding. Mature male and female broods (2:1) were selected on the basis of secondary sexual characteristics. The mature males were identified by their flat abdomens and long protruded genital papillae. On the other hand, the females could easily be recognized by their soft swollen abdomen and round swollen urogenital papillae. Broods were collected by reducing the water of the cistern and the selected broods were kept in fiber glass tank with heavy shower for about 6 h for conditioning prior to injection with pituitary gland (PG extract) collected from M/s. M V Enterprise, Jashore, Bangladesh. Male and female fish were kept in separate tanks and constant water flow was maintained to ensure proper aeration. The females (80-100 g) were induced with an injection of 3 mg PG/kg body weight and the males (60-70 g) were induced with an injection of 6 mg PG/kg body weight as 1<sup>st</sup> dose. After 6 h, 2<sup>nd</sup> dose of PG were given at 16 mg PG/kg body weight of females and 6-8 mg PG/kg body weight of males. Doses using a 1ml graduated syringe were injected intramuscularly at an angle of  $45^{\circ}$  at base of the pectoral fin. Eggs were collected from the injected female through gentle pressure on the abdomen and milt was collected by dissecting the injected male. Sperm suspension was prepared after thoroughly maceration of testes with 0.9% saline solution. Eggs and milt were gently mixed with the help of a feather for fertilization. Fertilized eggs were collected, transferred and spread as homogeneously as possible on hatching trays ( $101.6 \times 40.6 \times 12.7 \text{ cm}^3$ ). Fertilized eggs in trays received gentle shower and dead eggs and eggs shells were removed from the incubator by siphoning within one hour of hatching to protect the hatchlings from fungal infection. The hatchlings were left in the incubator few days for post-hatching observation. The water was replaced every day to maintain good water quality and was agitated to prevent the overlapping or clumping of eggs on the bottom. Although the hatchlings of *O. pabda* get nutrition from the yolk sac up to 3 d of hatching, the larvae started first feeding from 36-72 h post-hatching at ambient temperature of 26-28°C. Hard-boiled chicken egg yolk was provided as first feed for the hatchlings up to satiation level.

#### 2.4. Sampling of eggs and larvae

The fertilized egg samples were randomly collected from the hatching trays with the help of a dropper and were taken in a petridish containing water for studying the developmental stages of *O. pabda* at every 10-15 min interval until hatching and every 30 min and 1 h interval till completion of larval developmental stages. Microscopic observation was continued till first feeding of fry. At least 8-10 sample eggs and larva undergoing developmental process were observed to obtain precise information about developmental stages.

#### 2.5. Image capture and analysis

Embryonic and larval developmental stages of *O. pabda* were photographed (40x) under a binocular microscope OLYMPUS CX41RF, Tokyo, Japan with a digital camera (Pixel 5.0) OLYMPUS DP22 Tokyo, Japan.

#### 2.6. Growth and somite indices

The weight of each fish was taken with a 3 digit sensitive electric balance (AND-GULF, Model-EK600, UAE). For the estimation of gonado-somatic index, 6 fish from each cistern were taken at every month till spent period. Gonado-somatic index was expressed according to Devlaming *et al.* (1982) method for assessing the development of gonads and calculated as,

$$\text{GSI} = (\text{gonad weight/body weight}) \times 100.$$

Fecundity was determined by following the method of Hartman and Conkle (1960) using formula,

$$F = nG/g \text{ where, } F \text{ is the fecundity; } n \text{ is mean number of eggs in all samples; } G \text{ is the weight of ovary and } g \text{ is the weight of samples (eggs were collected from anterior, middle and posterior portion of entire ovaries).}$$

For calculation of fertilization and hatching rates, a portion of fertilized eggs were taken and incubated on sieve. Fertilized eggs were counted on the basis of their colour and transparency. After completion of hatching, the number of newly hatched larvae of each sieve was counted. Fertilization and hatching rates were recorded as spawning indices using the formula:

$$\% \text{ Fertilization} = (\text{No. of fertilized eggs/No. of total eggs}) \times 100$$

$$\% \text{ Hatching} = (\text{No. of hatched eggs/No. of total fertilized eggs}) \times 100, \text{ respectively.}$$

#### 2.7. Statistical analysis

Correlation analysis amongst body weight, fecundity and gonad weight and between male GSI and female GSI were calculated by MS Excel 2017. Significance level was evaluated using SPSS version 22.0.

### 3. Results and Discussion

#### 3.1. Physicochemical parameters of water

Water quality parameters are the most important factors which influence the growth, reproduction and other biological activities of fish. During the entire experimental period, temperature, pH and DO were around  $26.5 \pm 2^\circ\text{C}$ ,  $7.4 \pm 0.2$  and  $6.7 \pm 0.5$  mg/L, respectively (Table 1). Similar water quality parameters were found for the suitable health condition and survival rate of different species of fish and other aquatic organisms (Jahan *et al.*, 2020; Mollah and Hossain, 1995; Mollah and Hossain, 1998; Mollah and Sarowar, 2009; Islam *et al.*, 2018; Hossain *et al.*, 2018). Alkalinity and hardness was maintained  $85 \pm 7$  mg/L and  $56 \pm 8.3$  mg/L, respectively for broodstock management. Sarma *et al.* (2012) found similar result for incubation and nursery rearing unit of *O. pabo*.

#### 3.2. Breeding periodicity, fecundity and spawning performances of *O. pabda*

The experimental fish, *O. pabda* was found to breed in the month of June to August with spawning peak in June. Akhteruzzaman *et al.* (1993) have also documented May to August while Chakrabarty and Chakrabarti (2005) have reported early June to late July as the breeding season in West Bengal. Similarly Saha *et al.* (2014) have documented early May to early June for *Heteropneustes fossilis*. In this study, absolute fecundity of the *O. pabda* varied from 6,676 eggs (with total length of 19.9 cm and total body weight of 47.65 g) to 20,410 eggs (with total length of 25.4 cm and total body weight of 82.5 g). The average fecundity was  $11091 \pm 1876$  eggs per fish and was higher during early May to late July which sharply decreased during late August to October. Banik *et al.* (2012) have reported the range of 2,190 to 41,552 eggs for *O. pabda* while Malla and Banik (2015) have documented the absolute fecundity ranged from 2,190 to 41,552 eggs per fish for another species of pabda. The fertilization and hatching rate of eggs are important indicators of good reproductive performance. In this study, fertilization and hatching rate of eggs were found  $78.67 \pm 1.33\%$  and  $70.21 \pm 0.73\%$ , respectively at  $24.5 \pm 1.0^\circ\text{C}$  temperatures. More or less similar fertilization and hatching rate was found in different fish species (Raizada *et al.*, 2013; Hossen *et al.*, 2014c).

### 3.3. Gonado-somatic index and correlation between body weight, gonad weight and fecundity

GSI gives a clear indication on the gonadal development and breeding season of a fish. During the study period, the GSI of female was relatively higher than that of males showing decreasing trend during September to March and its maximum level in June and July.. In case of female *O. pabda*, the highest GSI (%) was found as  $6.75 \pm 0.2$  in the month of June and the lowest was found as  $2.19 \pm 0.3$  in March. However in male, very low GSI (%),  $0.145 \pm 0.02$  was observed in the month of September and very high GSI (%)  $1.053 \pm 0.2$  in June (Table 2). Motin *et al.* (2013) have found the highest GSI (%) 4.06 in September and 0.76 in December for female and male of *O. pabo*, respectively. Similar GSI (%) has also been reported in *Colisa fasciatus* and *Nandus nandus* (Hossen *et al.*, 2014b; Reza *et al.*, 2013). The values of correlation coefficient (r) were established between male GSI (%) and female GSI (%) and amongst the body weight and gonad weight and fecundity. It was found that all these relationships were positively correlated and showed a straight line relationship which is expressed by an equation,  $Y = a + bX$  where, the values of intercept (a), regression co-efficient (b) and correlation co-efficient (r) and X and Y are the variables. All these correlation coefficient (r) showed significance at  $p \leq 0.01$ . Gonad weight was nearly related to the body weight (Figure 1) and thus gonad weight was directly proportional to the fecundity (Figure 2). Behera (2012) was also found positive correlation co-efficient between weight of female fish and gonad weight at  $p \leq 0.05$  for an ornamental fighting fish, *Betta splendens*. In addition, male and female GSI also showed a positive correlation ( $r = 0.951$ ;  $p \leq 0.01$ ) considering the reproductive months (Figure 3). Similar significant positive correlation ( $p < 0.05$ ) was also observed for male and female GSI in *Clarias gariepinus* (Yusuf *et al.*, 2013) and *Anabas testudineus* (Marimuthu *et al.*, 2009).

### 3.4. Early embryonic development of *O. pabda*

The embryonic development and their respective time of observation and key features of *O. pabda* are presented in Table 3. The embryonic development started soon after fertilization of eggs by the motile sperm. The unfertilized eggs (Figure 4A) were blackish in colour, demarsal, spherical and not transparent in nature. The eggs were not adhesive and were enveloped by a thick blackish chorion and had a large yolk sphere in the centre of the chorion. The sizes of the unfertilized eggs were 0.90-1.0 mm in diameter. Hossen *et al.* (2014a) also found the unfertilized eggs of *Trichogaster fasciata* floating, opaque, whitish and slightly elongated shaped with a range from 0.20 to 0.30 mm in diameter. Fertilized eggs of *O. pabda* were brownish in color and adhesive in nature and were very transparent and had a reddish brown spot on one side which is easily recognizable with naked eye. Light pinkish color and adhesive nature are similar to those of other catfish species such as *Mystus cavasius* (Rahaman *et al.*, 2004), *H. fossilis* (Puvaneswari *et al.*, 2009) and *O. malabaricus* (Vijayakumar, 2010). The sizes of the fertilized eggs of *O. pabda* were 1.0 to 1.2 mm in diameter (Figure 4B). Zadmajid *et al.* (2018) studied the embryonic and larval development in wild-caught Levantine scraper, *Capoeta damascina* and the fertilized eggs were  $3.22 \pm 0.03$  mm in diameter. Puvaneswari *et al.* (2009) showed that the fertilized eggs of *H. fossilis* were demersal, non-adhesive, spherical, brownish green in color and the average diameter of the fertilized eggs ranged from 1.30 to 1.50 mm. Soon after fertilization blastodisc (Figure 4C) just formed as a crescentic light area over one end of the massive yolk and the first cleavage (Figure 4D) took place about 35 min after fertilization which eventually followed by second (Figure 4E), third (Figure 4F), fourth (Figure 4G) and fifth cleavage (Figure 4H) at 45-50 min, 1.00-1.05 h, 1.13-1.18 h, 1.27-1.30 h of post fertilization, respectively. Chakrabarty and Mondal (2008) observed first cleavage of *O. pabda* in about 30 min after fertilization. Rakhi *et al.* (2015) have similarly found the first cleavage of *Nandus nandus* eggs at  $0.3 \pm 0.01$  h post fertilization at 26°C water temperature. According to Zhu *et al.* (2018), about 54 min after fertilization the first cleavage furrow occurred in top mouth gudgeon, *Pseudorasbora parva*. However the 64 cell stage (Figure 4I) was observed in 1.30 h and *O. pabda* took 1.50 h to complete cleavage stage besides appearing early morula stage (Figure 4J) and late morula stage (Figure 4K). According to Ferosekhan *et al.* (2015), *Pangasius pangasius* appeared 64 cell stage and morula stage at 1.27-1.30 h and  $03.43 \pm 00.33$  h, respectively. Gastrula stage (Figure 4L) was found in *O. pabda* within 4.50 to 5.00 h post fertilization. In previous study, researchers reported that *H. fossilis* (Puvaneswari *et al.*, 2009) took 7 h to reach in gastrula stage. In the current study, yolk plug stage (Figure 4M), kidney bean shaped (Figure 4N) and optical vesicle stage (Figure 4O) were observed after 6 h, 8 h and 12 h of post fertilization, respectively. Vigorous thrashing movements (Figure 4P) of the embryo were also noticed about 1-2 h before hatching (Figure 5A) and finally the embryo hatched out (Figure 5B) at 23 h. Similar result has also been reported in case of *O. pabo* and it took 22 h for hatching after fertilization (Sarma *et al.*, 2012). Similarly *O. bimaculatus* hatched out in  $21 \pm 1$  h post fertilization (hpf) and yolk-sac completely absorbed in 48 hpf (Raizada *et al.*, 2013). It was also reported that *C. batrachus* (Roy *et al.*, 2019), *H. fossilis* (Puvaneswari *et al.*, 2009), *Mastacembelus aculeatus* (Sahoo *et al.*, 2007) and *M. cavasius* (Rahaman *et al.*, 2004) took 26 h, 23-24 h, 31.45 h and 17 to 21 h, respectively for

hatching. The hatchlings of *O. pabda* were yellowish black in color and measured 2-3 mm in length. The lengths of newly hatched hatchlings of *M. montanus* were also  $3.0\pm 0.1$  mm (Arockiaraj *et al.*, 2003). Observation made on the newly hatched larvae of *H. fossilis* has been recorded with the length of  $2.5\pm 0.2$  mm, which was transparent and faintly brown in colour (Puvaneswari *et al.*, 2009). Mukherjee *et al.* (2002) reported the length 5-6 mm in *O. pabo*, while Rahaman *et al.* (2004) reported 2.59 to 2.62 mm in *M. cavasius*. Marimuthu and Haniffa (2007) revealed that length variation in different species can be related to the size of eggs.

### 3.5. Larval development of *O. pabda*

The larval developmental stages of *O. pabda* are elaborated with key features in Table 4. The newly hatched *O. pabda* larvae were slender and yellowish black in color with 2-3 mm in length whereas Marimuthu and Haniffa (2007) reported transparent and faintly brown *Channa striatus* hatchlings with  $3.4\pm 0.2$  mm in length. The hatchlings had no pigmented eyes and devoid of distinct mouth and pectoral with a small head attached to the yolk sac (Figure 5B). The 6 h old larvae (Figure 5C) were transparent with some star shaped dark black brown melanophores on both side of yolk sac and 12 h old larvae (Figure 5D) body was straight, floating on the surface of water with prominent notochord. Similar results had found in *Notopterus notopterus* (Srivastava *et al.*, 2012) with deep yellow to dark pink body. After 24 h of hatching (Figure 5E), *O. pabda* larvae remained motionless in the water surface with visible lateral line. Swim bladder and nostril were clearly visible after 48 h (Figure 5F) of hatching. The yolk sac was gradually started to disappear on the 3 d onwards (Figure 5G). At the end of this stage yolk sac was absorbed to half size, the larvae were found to swim vigorously and feeding exogenously (Figure 5H). Chakraborty *et al.* (2010) was also observed that the absorption of yolk sac in *O. pabda* from three days. In *P. pangasius* (Ferosekhan *et al.*, 2015) yolk absorption occurred at day four post-hatch. This period varies in the fish species such as 72 h for *C. batrachus* (Sahoo *et al.*, 2004) and  $64.0\pm 0.30$  h for *N. nandus* (Rakhi *et al.*, 2015). At 5 d post hatch (Figure 5H) larvae were observed free swimming with fully developed mouth, caudal fin and pectoral fin and they were capable to catch their feeds. Larvae took 5-10 days for *P. pangasius* (Ferosekhan *et al.*, 2015) and 6 days for *H. fossilis* (Puvaneswari *et al.*, 2009) to swim freely. The larvae resembled the adult in their external features and were metamorphosed to young juveniles within 20 days of post-hatching.

**Table 1. Physicochemical parameters managed during entire experimental period (values are presented with the mean and standard error of samples).**

Physicochemical parameters	Range
Temperature (°C)	26.5±2.2
pH	7.4±0.2
Dissolved oxygen (mg/L)	6.7±0.5
Alkalinity (mg/L)	85±7.1
Hardness (mg/L)	56±8.3

**Table 2. Male and female gonadosomatic index (GSI) during the reproductive months of *O. pabda* (values are presented with the mean and standard error of samples).**

Months	Female GSI	Male GSI
March	2.19±0.30	0.17±0.02
April	3.27±0.30	0.24±0.03
May	3.83±0.10	0.53±0.25
June	6.75±0.20*	1.05±0.20*
July	5.14±0.20*	0.83±0.03*
August	4.29±0.18*	0.23±0.28
September	2.30±0.23	0.14±0.02

\*Values are significantly different ( $P < 0.01$ ).

**Table 3. Early embryonic developmental features of *O. pabda* reared at 26-28 °C.**

Time after spawning	Stages of development	Key features	Plate No.
0 min	Unfertilized eggs	Eggs were blackish in color, not transparent, not adhesive and enveloped by a thick blackish chorion.	4A
0 min	Fertilized eggs	Fertilized eggs were brownish in color, adhesive in nature, very transparent and had a reddish brown spot on one side.	4B
15 min	Blastodisc just formed	Crescentic light area over one end of the massive yolk of fertilized eggs were formed.	4C
35 min	2 cell stage	First cleavage with formation of blastoderm over one end of yolk.	4D
45-50 min	4 cell stage	Second cleavage	4E
1.00-1.05 h	8 cell stage	Third cleavage	4F
1.13- 1.18 h	16 cell stage	Fourth cleavage	4G
1.27- 1.30 h	32 cell stage	Fifth cleavage	4H
1.50 h	64 cell stage	Sixth cleavage	4I
1.50- 2.00 h	Early Morula stage	Repeated cleavage resulted into early morula.	4J
3.00 h	Morula stage	Blastoderm cells began involution over the yolk and eggs were with a small perivitelline space.	4K
4.50-5.00 h	Gastrula stage	The blastoderm covered about $\frac{3}{4}$ of the yolk. Gastrulation ring was formed.	4L
6.00 h	Yolk plug stage	Gastrulation spreads to the whole yolk	4M
7.00-8.00 h	Kidney bean shaped	The head and tail turned into oval shape	4N
12.00 h	Optic vesicle stage	The earliest indication of embryo was discernible and embryo reveals rudimentary optic cubs	4O
18.00 h	Twisted movement	The heart was beating actively. Tubular notochord was appeared and the embryos showed occasional faint twisting movements	4P

**Table 4. Larval developmental features of *O. pabda* reared at 26-28 °C.**

Developmental stages	Key features	Plate No.
Just before hatching	Egg membrane became decomposed and lost its shape. Vigorous thrashing movements and frequent whipping motion of the embryo.	5A
Newly hatched larvae	Lack of mouth and pectoral fin, head region was attached to the yolk sphere. Body was observed without any pigmentation.	5B
6 h old larva	Some star or branch shaped melanophores distributed on both side of the body and back of the head and somites. The body of the larva was straight, floating on the surface of the water. The brain was slightly visible. Prominent notochord was found and barbell partially appeared.	5C
12 h old larva	Three pair of barbell appeared and among them the maxillary pair was clearly visible. Heart became more distinct and blood circulation was continued.	5D
24 h old larva	Lateral line was visible. Swim bladder and nostril also formed. Larvae still remained motionless in the surface layer of water. Pigmentation extended to the yolk sac both dorsally and ventrally	5E
2 d old larva	Typical star-shaped melanophores clusters appear around the final tract of the intestine.	5F
3 d old larva	Yolk sac was gradually disappeared and was absorbed to half size. Mouth was gradually developed and feeding commenced at this stage.	5G
4 d old larva	Tail and caudal fin formation were in progress and both maxillary and mandibular barbells were formed.	5H
5 d old larva	Mouth was fully developed and functional. Yolk substances were completely absorbed. The larva was free swimming and fully capable to capture food.	5I

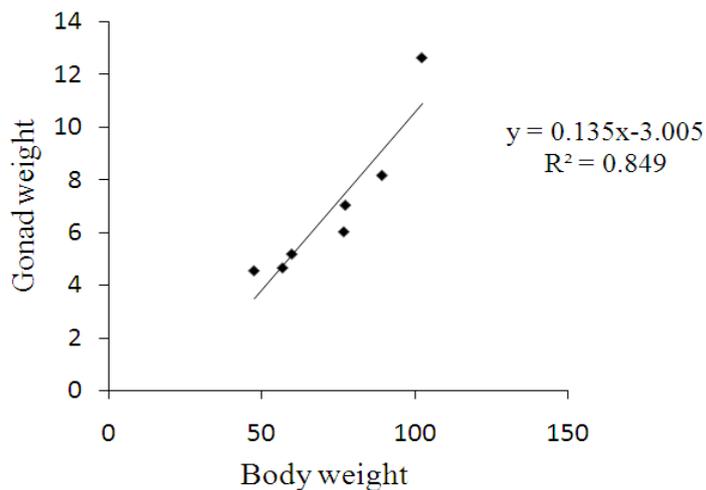


Figure 1. Correlation between body weight and gonad weight of *O. pabda*.

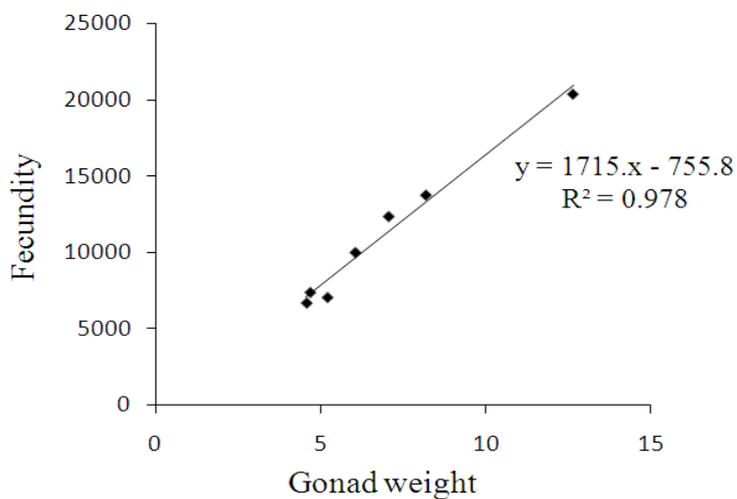


Figure 2. Correlation between fecundity and gonad weight of *O. pabda*.

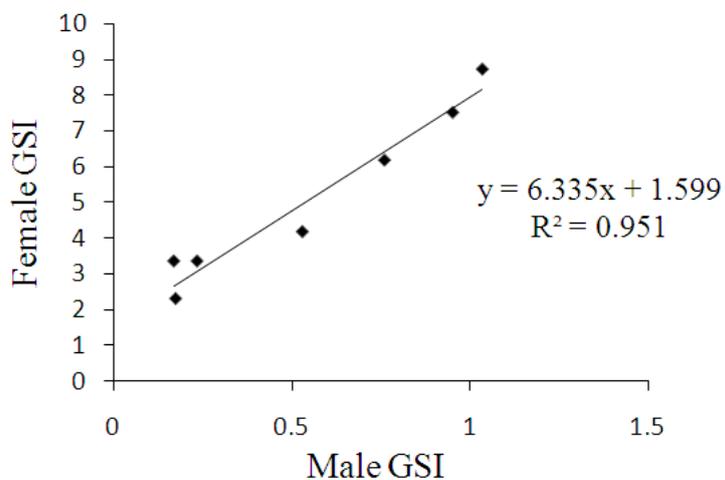
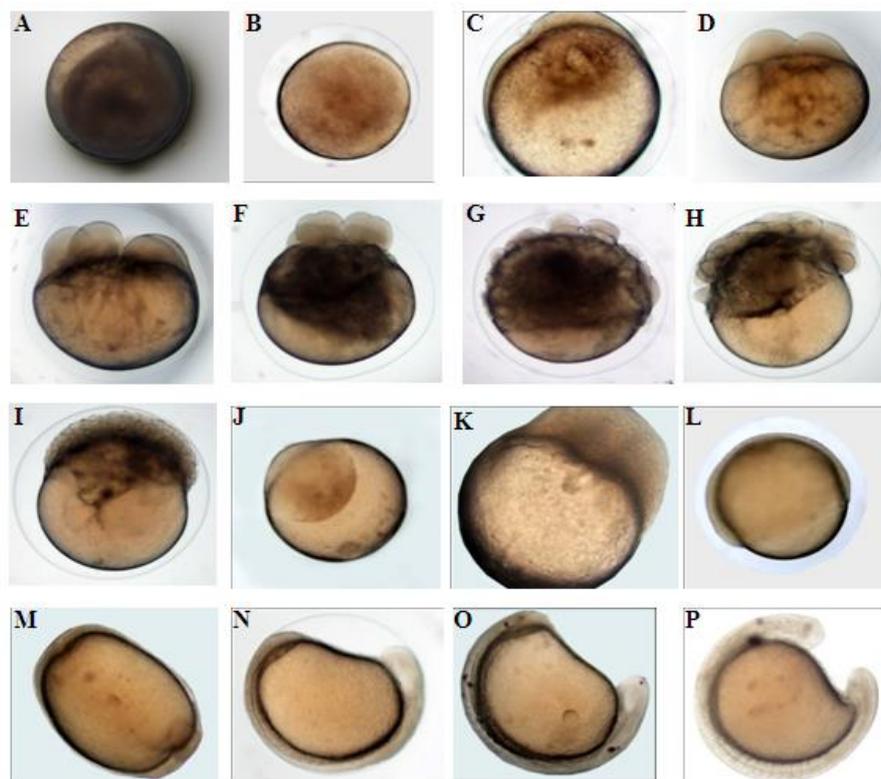


Figure 3. Correlation between male GSI and female GSI of *O. pabda*.



**Figure 4.** Early embryonic developmental stages of *O. pabda*, A. Unfertilized egg (0 min), B. Fertilized egg, C. Blastodisc just formed (15 min), D. 2 cell stage (35 min), E. 4 cell stage (45-50 min), F. 8 cell stage (1.00-1.05 h), G. 16 cell stage (1.13-1.18 h), H. 32 cell stage (1.27-1.30 h), I. 64 cell stage (1.50 h), J. Early morula stage (1.5 h to 2.0 h), K. Morula stage (2.0 h to 3.0 h), L. Gastrula stage (4.50 h to 5.00 h), M. Yolk plug stage (6 h), N. Kidney bean shaped (7 h to 8 h), O. Optical vesicle stage (12 h) and P. Twisted movement (18 h).



**Figure 5.** Larval developmental stages of *O. pabda*, A. Just before hatching (23.00 h), B. Newly hatched larva, C. 6 h old larva, D. 12 h old larva, E. 24 h old larva, F. 48 h (2 d) old larva, G. 72 h (3 d) old larva, H. 96 h (4 d) old larva and I. 120 h (5 d) old larva.

#### 4. Conclusions

The findings of the present study supplements detailed spawning and developmental biology of *O. pabda* which is now at threat of extinction due to poor breeding and rearing management. This study documents spawning indices along with captive breeding strategy, embryonic and larval development till it completely resembles the adult fish. These findings would be useful for aquaculture entrepreneurs, fish breeders, aquarium keepers and those interested in spawning and developmental study of *O. pabda* to mitigate the probable threats from extinction.

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#### Conflict of interest

None to declare.

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