

Some Biological Aspects of Cultured *Ompok pabda* (Hamilton, 1822) Collected from A Local Fish Farm in Mymensingh, Bangladesh

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Introduction

In Bangladesh, livelihoods of rural and poor people totally depend on freshwater fisheries which is very significant (Mazid, 2002). Fish farming practices have the ability to attain self-reliance in the food sector and to minimize scarcity in Bangladesh (Al-Amin *et al.*, 2012).

Fish biology is indispensable for sustainable development and protection of fish biodiversity (Solomon *et al.*, 2012). Reproductive potential of a population is one of the basic elements to entitle the entities of that population regarding their gonadal conditions (Jhingran and Verma, 1972). To understand reproductive biology of fish is important for estimating

Abstract

An experiment was conducted to observe some biological aspects including growth, sex ratio, gonadal maturity, artificial insemination, fecundity, fertilization, hatching and larval development of *O. pabda* in a private hatchery. Total weight of the sampled fish varied from 1.60 ± 0.43 to 111.12 ± 6.83 g and length varied from 3.57 ± 0.65 to 21.22 ± 1.84 cm with the SGR 2.85 ± 0.18 and ADG 0.73 ± 0.05 . The length and weight of the fish had a strong correlation (r²=0.966). Female dominance over male has been observed in the species. The male to female ratio was 1:1.48. Females were 16.8 cm in length at first maturity, while males were 16.6 cm in length. The GSI of female were ranged from 2.93 to 4.77 with the mean of 3.77 ± 0.60 during the study period. The fecundity of *O. pabda* was ranged from 5675 to 19626 with the mean of 12503 ± 4192 for the corresponding length 21.23 ± 1.71 , body weight 111.12 ± 7.6 and gonad weight 4.19 ± 0.87 . Fertilization rate, hatching rate and larval development observed during the experiment. Scattered diagram for length-weight, fecundity-length, fecundity-body weight and fecundity-gonad weight relationship shows a perfect correlation both arithmetically and logarithmically.

the viable potentialities of its stock, life history, culture practice and management of its fishery (Cortes, 2000; Soofiani *et al.*, 2006; Dopeikar *et al.*, 2015).

Ompok pabda (Hamilton-Bouchanan, 1822) is an economically important species with high market demand in Bangladesh as well as in other South East Asian countries (Talwar and Jhingran, 1991). *O. pabda* is a native fishes of Bangladesh, locally known as pabda, a small freshwater catfish classified under the family Siluridae of the order Siluriformes (Siddiqua *et al.*, 2000). It is distributed widely in Bangladesh, India, Pakistan, Myanmar and Afghanistan (Jayaram, 2009; Mukhopadhyay and Ghosh, 2007); and is frequently found in normal water bodies like flood plains, lakes,

streams and rivers (Talwar and Jhingran, 1991; Hussain, 2006; Chakrabarti et al., 2012). Some of the researcher (Chakrabarti et al., 2012; Parameswaram et al., 1971) have documented it as a carnivorous fish while some others (Shafi and Quddus, 2001) have recognized it's an omnivorous fish. It has high fondness as a table fish due to tasteful and high dietary value (Datta et al., 2003; Chakrabarty et al., 2006, 2007; Chakrabarti et al., 2009; Banik et al., 2012; Purkayastha et al., 2012); and its suitability as an attractive fish among the fish lovers has recently been conveyed (Gupta and Banarjee, 2008, 2013). The breeding season of O. pabda have reported May to July (Siddigui and Choudhury, 1996) while Akhteruzzaman et al. (1993) and Chakraborty et al. (2010) have reported May to August. Due to indiscriminate fishing, habitat degradation, widespread use of pesticides and insecticides in agricultural fields, and the depletion of spawning grounds for this fish species, the amount of fish in the natural catch has decreased in recent years (Chakrabarti et al., 2012; Chakrabarty et al., 2006, 2007; Purkayastha et al., 2012). The reasons behind the alternation of this fish species is also resulting from the lack of proper knowledge on its domestication in culture system, breeding and farming technique and proper management (Chakrabarty et al., 2006). Due to loss of habitat in nature, it has already been enlisted as an endangered species both in India (CAMP, 1998) and Bangladesh (IUCN, 2000). IUCN has been categorize this fish species near threatened of the Red List of Threatened Species. Limited studies have been conducted on food, feeding habit and reproductive biology of O. pabda; but published paper on these aspects so far is not available in Bangladesh. The present study aims to combine the already available information on its biology including reproduction, larval development, growth, feeding, culture system etc. which can be considered for its conservation and useful for fisheries and aquaculture production in future.

Materials and Methods

Study Area and Period

The study was conducted in the "Deshbandhu Matsya Hatchery and Nursury" (Shombhugonj, Mymensingh) from March to July, 2019 (Fig 1).

Brood Fish Collection

Required number of male and female broods in good physical condition were isolated (Table 1) in the prepared brood ponds. Male and female of *O. pabda* can be easily categorized observing the sex characteristics or behavioral signs which develop throughout the breeding season.

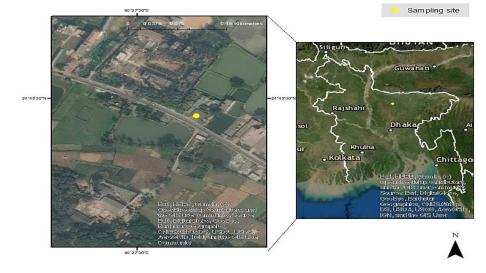
Conditioning of Brood Fish

Selected brood fish were kept in tank for about 6 hours for conditioning prior to injection with SGnRHa extract. Fishes were handled and carried very carefully to eliminate possible injury and minor infection. Males and females fish were reserved in different tanks and constant water flow was continued to maintain adequate ventilation.

Sex Ratio

The sex ratio was calculated monthly from the proportion or ratio of females to males. Sex ratio was estimated according to Pena-Mendoza *et al.*, 2015. The formula is

$$Sex \ ratio = \frac{\text{Number of males}}{\text{Number of females}}$$



Map of Deshbondhu Hatchery, Shamvuganj, Mymensingh

Table 1. Selection criteria of male and female mature breeders of O. pabda.

Criteria	Male	Female		
Size	Smaller in size	Comparatively larger in size		
Abdomen	Abdomen normal, not bulky like female	Abdomen soft, round and bulged		
Body shape	Slender in shape, more translucent and less pigmented	Body robust and pigmented		
Pectoral fin	The pectoral fin spines are relatively larger and thicker	The spines are very faint or absent		
Genital papilla	Elongated and pointed or conical shape	Large, round and fleshy with reddish vent		

Induced Breeding

Induced breeding carried out according to Bhowmick *et al.*, 2000; Chakrabarty and Chakrabarti, 2005; Sarkar *et al.*, 2005. Salmon Gonadotropin releasing Hormone (SGnRHa) named ovaprim was used as the inducing agent for induced breeding of Pabda (*Ompok pabda*). The SGnRHa elicits the release of stored gonadotropins from the pituitary (Hossain *et al.*, 2012). Both female and male fishes were injected with a constant dose (1mg/kg) of S-GnRHa.

Gonadosomatic Index

The gonads increase to mature as the fish grow. Growth of gonads with the growth of the fish is strongly related and directly proportional. GSI gradually develops till the enlargement of gonads into maturing. Gonadosomatic index (GSI) was determined following the equation of Parameshwaran (Parameshwaran *et al.*, 1971):

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

Fecundity

The fecundity is closely associated with the life history and community structure or fish population dynamics of fish (Kapoor and Khanna, 2004). Usually fecundity is defined as the number of developing eggs found in the female just before spawning (Bagenal, 1978). The fecundity of the fish was observed gravimetrically. The external connective tissues on the surface of the ovaries were carefully detached to estimate fecundity. Blotting paper was used to extract the moisture from the ovaries. Ovary weight was recorded with fine electronic balance. The fecundity of the studied fish was calculated following the formula of Le Cren (1951):

Fecundity = (number of eggs in the sample x weight of gonad) / weight of the sample.

Relationship of fecundity with different body measurements such as body length, body weight was moved into log and least squares regression analysis (Bagenal, 1978):

Where, F=Clutch size (fecundity); X=Length/Weight; a=Regression constant, b=Regression coefficient.

Calculation of Fertilization Rate

Total number of eggs and rate of fertilization were calculated one hour after spawning by direct counting method. After two hours of fertilization, the transparent eggs were considered as fertilized eggs whereas, the opaque eggs were considered as dead eggs. The fertilization rate was estimated according to following formula (Muir and Robert, 1985):

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

Calculation of Hatching Rate

Hatchlings were counted by naked eyes. The hatching rate was determined by following formula:

Hatching rate (%) = $\frac{No.of hatchlings}{Total no.of eggs}$ ×100 (Islam *et al.*, 2011, Roy *et al.*, 2011)

Calculation of Survival Rate

Survival rate was calculated just after the hatching. The number of hatchlings counted. The survival rate was determined by following formula (Roy *et al.*, 2019):

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

Measurement of Larvae

All morphological measurements of larvae following the method of Rahman *et al.* (2004) and McEdward *et al.* (1984) with slight modification. When hatching was completed, the observations were continued at every 6 h for the first day and thereafter at every 12 hours interval to study the each development stage, samples are collected from the hapa and rearing cistern. Larvae were stored in 10% formalin-water and were concentrated by settling of bottom of a vial. Larvae were put under an elevated cover slip on a microscope slide and finally measured by an electronic microscope (Optica) using a presetting objective micrometer and took images by Euromax camera (CMEX 10, 65 DC 10000C) with the magnification of 10XS.

Fish Sampling, Estimation of Growth

Fish sampling is an important part to estimate different growth parameters. More than 100 fish were sampled fortnightly by using a seine net. Individual fish were measured in terms of length (cm) and weight (g) using a measuring scale and a portable electronic balance. The weight of each fish was measured separately in order to determine the health and productivity of the fish. Growth in terms of weight, average daily gain (ADG), specific growth rate (SGR) and mean values (±SD) for each parameter were computed. ADG was calculated according to De Silva (1989). SGR calculated according to Brown (1957) and Ricker (1975).

Average daily gain, ADG = (Final weight W2- Initial weightW1) /Age (days)

The length-weight relationship was estimated using the linear form of the formula by Le Cren (1951),

W=a x Lb

(Where, W=weight of the fish in g; L=length of the fish in mm and 'a' and 'b' are constants) The equation has been transformed into the following logarithmic form: Log W=log a + b log L and the values of 'a' and 'b' were calculated analytically.

Statistical Analysis

MS excel was used to perform this statistical analysis. The significant (P<0.05) level was tested following Duncan's test using IBM SPSS statistics software, version 20. To tabulate the results, simple arithmetical tools such as average, range, percentage, and so on were used. A one-way analysis of variance (ANOVA) was performed to evaluate the significance (P<0.05) level of hormonal treatments. Regression equations of those relations were established. Coefficient of correlation (r) and regression co-efficient (b) were also determined.

Results

Sex Ratio

Among the 365 specimens of *O. pabda* studied, 147 were male and 218 were female (Table 2). The overall male to female sex ratio was observed to be 1:1.48. Female ascendency on male in the population of this fish species have reported by Banik *et al.* (2012).

Length and Age at First Maturity

The mature ovaries and testes in relation to length was observed. Males had a minimum length of 16.6 cm and females had a minimum length of 16.8 cm during sexual maturity. According to Banik *et al.* (2012), the male and female of *O. pabda* reach maturity at 16.3 cm and 17 cm in length respectively.

Gonado-somatic Index of O. pabda

The GSI found in the present study were ranged from 2.93 to 4.77 with the mean of 3.77±0.60 during the study period (Table 3). The highest and the lowest GSI values were in May and April respectively. The GSI values differed significantly (P<0.05) with the body length and weight of fish. Some researchers used total body weight to measure GSI of fishes, such as the cardinal fish *Apogon lineatus* (Kume *et al.*, 2000), while Brown-Peterson *et al.* (2001) and Brown-Peterson and Warren (2001) used net weight and omitted the gonad weight in their assessments.

Fecundity

Fecundity refers to egg-laying ability of fish or the amount of mature or ripe eggs released by a fish in a single spawning or breeding season. The fecundity of *O. pabda* in the present study was ranged from 5675 to 19626 with the mean of 12503±4192 for the corresponding length 21.23±1.71, body weight 111.12±7.6 and gonad weight 4.19±0.87 (Table 3). Islam

 Table 2. Variations in Sex ratio of male and female O. pabda during study period.

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Months	No. of fish	Male		Female		Ratio	Р	X ²	Significance
WOITTIS	NO. OF HSH	No	Percent	No	Percent		г	~	Significance
February	50	22	44.00	28	56.00	1: 1.27	0.396	0.72	Insignificant
March 1st	40	18	45.00	22	55.00	1: 1.22	0.527	0.40	Insignificant
March last	54	24	44.44	30	55.56	1: 1.25	0.414	0.67	Insignificant
April	56	22	39.29	34	60.71	1: 1.55	0.109	2.57	Insignificant
May	60	20	33.33	40	66.67	1: 2.00	0.009	6.67	Significant**
June	40	16	40.00	24	60.00	1: 1.50	0.206	1.60	Insignificant
July	65	25	38.46	40	61.54	1: 1.60	0.063	3.46	Insignificant
Overall	365	147	40.27	218	59.73	1: 1.48	0.0002	13.81	Significant**
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P = p-value, χ^2 = Calculated Chi-square value, ** = Significant at 1% level, * = Significant at 5% level.

At 1% level of significance, $\alpha = 0.01$

At 5% level of significance, lpha=0.05

SL.	Total Length (cm)	Body Weight (g)	Gonad Weight (g)	Fecundity	GSI
1.	16.8	98.7	3.08	5675	3.12
2.	18.5	95.4	2.92	6360	3.06
3.	19.4	97.2	3.15	7020	3.24
4.	20.3	100.1	3.06	7200	3.06
5.	19.5	111.5	3.27	8600	2.93
6.	20.4	105.2	3.62	10220	3.44
7.	20.8	117.4	3.71	11750	3.16
8.	21.6	112.05	3.67	10610	3.28
9.	21.8	114.78	3.84	12708	3.35
10.	20.7	112.3	4.01	11025	3.57
11.	21.1	115.6	4.11	12526	3.56
12.	21.8	113.7	5.03	15225	4.42
13.	22.3	119.7	4.86	14847	4.06
14.	22.0	115.6	5.13	12505	4.44
15.	22.8	113.28	4.98	15771	4.40
16.	22.9	113.15	5.01	16479	4.43
17.	23.5	119.93	5.72	18088	4.77
18.	22.8	112.6	4.67	15465	4.15
19.	23.2	118.8	4.87	19626	4.10
20.	22.4	115.6	5.18	18360	4.48
Mean±SD	21.23±1.71	111.12±7.6	4.19±0.87	12503±4192	3.77±0.60

Table 4. Measurement of different stage of embryonic and larval development.

Larval stage	Measured Length(mm)			
0 hour	1.5 mm			
6 hours	2.6 mm			
12 hours	4 mm			
24 hours	4.3 mm			
36 hours	4.6 mm			
48 hours	4.9 mm			
60 hours	5.2 mm			
72 hours	6 mm			
7 days	7.5 mm			
14 days	3.2 cm			

Table 5. Different parameters and observed values of *O. pabda* during the study period.

SI.	Parameters	Observed values (Mean±SD)	Significance	
1.	Initial length (cm)	3.57±0.65	NS	
2.	Initial body weight (g)	1.60±0.43	NS	
3.	Final length(cm)	21.22±1.84	NS	
4.	Final body weight (g)	111.12±6.83	NS	
5.	Gonad weight (g)	4.19±0.87	NS	
6.	GSI	3.77±0.6	NS	
7.	Fecundity	12503±4192	NS	
8.	Fertilization rate (%)	84.46±4.11	NS	
9.	Hatching rate (%)	71.20±1.69	NS	
10.	Survival rate (%)	60.40±0.59	NS	
11.	SGR	2.85±0.18	NS	
12.	ADG	0.73±0.05	NS	

NS= Not significant

(2011) observed the fecundity of *O. pabda* ranged from 15560±185 to 17369±213 during June to August.

Embryonic and Larval Development

The present observations on embryonic and larval development of *Ompok pabda* (Table 4 and Fig 2) were more or less similar to those reported by Sarma *et al.*, 2012; Chakrabarty and Chakrabarti, 2005. Different

length was observed in *Ompok pabda* through different hours and days, which given table 5.

Formation of embryo and differentiation of embryo into larva closely observed during the experiment. Movement of developing embryo observed at 12-14 hours while Chakrabarti *et al.* (2008) observed it at 14 hours. The caudal region of the embryo became elongated and active within the egg membrane at 16-18 hours. In *Ompok pabda*, vigorous thrashing movements of the embryo were noticeable in 22 hours (Chakrabarti *et al.,* 2008). The hatchlings were yellowish black in colour and measured 4.3mm in length. *M. montanus* hatchlings reached 3.0±0.1 mm in length when they were newly hatched (Arockiaraj *et al.,* 2003).

Fertilization Rate (%), Hatching Rate (%) and Survival Rate (%)

The fertilization rate was calculated monthly. The number of fertilized eggs in each bowl was counted. The fertilization rate was 84.46± 4.11% (Table 5). The number of spawn/larvae in each bowl was counted. The hatching rate was 71.20±1.69%. In case of survival rate, the number of fry in each bowl was counted. The survival rate was 60.40±0.59%. However, survival and growth of fry depends on stocking densities, type and quality of supplementary feeds (Debnath *et al.*, 2016).

Growth Parameters

To assess the growth performance, the mean initial weight, final weight, average daily weight gain, specific growth rate (SGR%/day) of fishes were recorded during the study period and summarized in table 5.

Length–Weight, Fecundity-Length, Fecundity-Body Weight and Fecundity-Gonad Weight Relationship

The scattered diagram obtained for the total length and body weight, fecundity and total length, fecundity and body weight and fecundity with gonad weight relationship shows a perfect correlation. The body weight (W) of the fish was plotted against their total length (L), fecundity was plotted against total length, body weight and gonad weight on arithmetic and logarithmic scales and shows the regression to be positive as the correlation. Calculating the values of regression co-efficient, intercept and co-efficient of correlation, the regression equation of total length on body weight, fecundity on total length, fecundity on body weight and fecundity on gonad weight was established for the fish (Table 6; Fig 3, 4, 5 and 6).

Sarkar et al. (2005), Chakrabarty, Chakrabarty and Mondal (2006) successfully spawned O. pabda in captivity. The sex ratio in a population can change from year to year (Nikolsky, 1963) and it is usually similar in most of the fish species. However, the sex ratio of O. pabda showed a predominance of female, which is consistent with M gulio (Islam et al., 2008), O pabda (Gupta et al., 2014). For different length groups and different months, the Chi-square test was used to assess the relative abundance of males and females to avoid theoretical 1:1 ratio on null hypothesis (Oymak et al., 2001). There were only few males and females with mature gonads in the length group of 16-17cm in size observed. O. pabda reach matureness at the end of first year of its life cycle and males become matured earlier than females reported by Parameswaran et al. (1971), Chakrabarty and Chakrabarti (2005) Chakrabarty et al. (2007) and Chakrabarti et al. (2009, 2012). Information about gonadal development and the spawning season of a species is essential for determining the spawning frequency of its population, which is significant for its management (Hasan et al., 2018). The maximum GSI recorded in the maximum length group of fishes which is also significant.

Variations in fish fecundity may be influenced by a wide range of factors, including different fish samples, different ponds, water temperatures, feeding, food differentiation, availability, species nutritional resources, and so on (Bagenal, 1978). For Ompok pabda, Purkayastha et al. (2012) recorded fecundity ranged from 80–130 eggs per gm body weight of female, while Banik et al. (2012) reported a range of 2,190-41,552. O. pabda has a relative fecundity range of 2,00,000-2,50,000/kg body weight, according to Chakrabarti et al. (2012). From the above literature, the fecundity calculated in the present study significantly varied. The freshly hatched larva measured 4 to 5.5 mm in length was thin and translucent, with no mouth or pectoral fin (Fig. 2). There was a distinct eye. There were also signs of maxillary and mandibular barbells. The yolk sac was yellowish green in color and oval in shape. The newly

Table 6. Showing a, b, r and regression equation of total length and body weight, total length and fecundity, body weight and fecundity and fecundity and gonad weight of *O. pabda*

Relationship	Regression constant (a)	Regression coefficient (b)	Coefficient of correlation(r)	Regression equation
Total length (L)	-60.62	8.01	0.98**	W = - 60.62 + 8.01 L
Body weight (W)	(-1.30)	(2.52)	(0.98**)	(Log W = - 1.30 + 2.52 Log L)
Total length (L) Fecundity	-34874	2231.6	0.91**	F = - 34874 + 2231.6 L
(F)	(-1.36)	(4.10)	(0.94**)	(Log F = - 1.36 + 4.10 Log L)
Body weight (W)	-38000	454.45	0.82**	F = -38000 + 454.45 W
Fecundity (F)	(-5.30)	(4.58)	(0.88**)	(Log F = - 5.30 + 4.58 Log W)
Fecundity (F)	-6105	4436.3	0.92**	F = - 6105 + 4436.3 G
Gonad weight (G)	(3.07)	(1.63)	(0.93**)	(Log F = 3.07 + 1.63 Log G)

** indicate highly significant at 1% level of significance

(If p-value < 0.01, then we reject our null hypothesis at 1% level of significance and conclude that our test is highly significant)

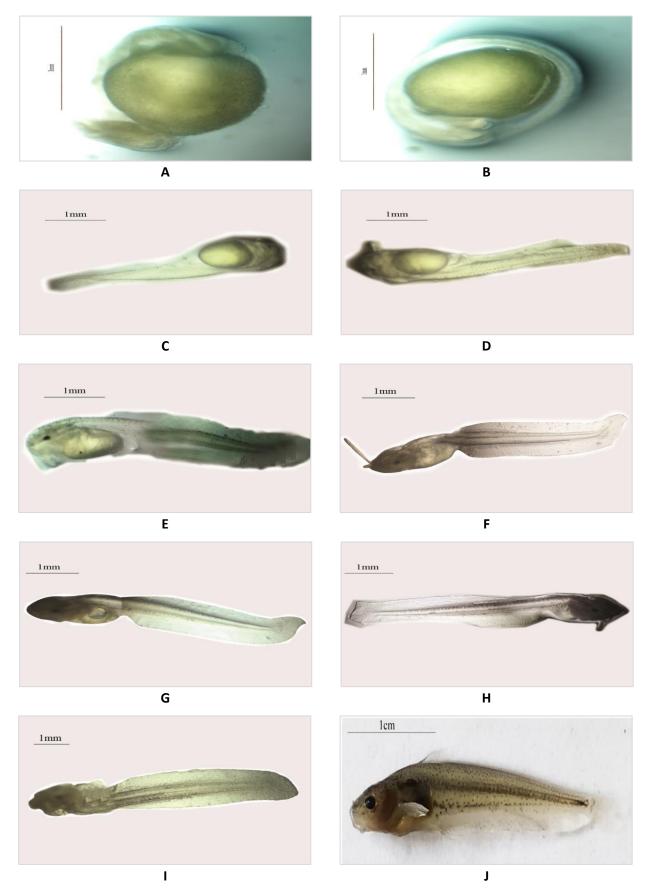


Figure 2. A. 0-hour larvae; B. 6 hours larvae; C. 12 hours larvae; D. 24 hours larvae; E. 36 hours larvae. F. 48 hours larvae; G. 60 hours larvae; H. 72 hours larvae; I.7days larvae; J. 14 days larvae

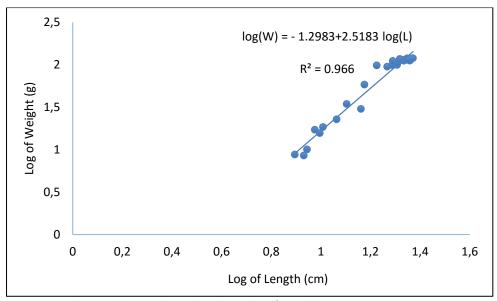


Figure 3. Regression curve showing the Length-weight relationship of *O. pabda*

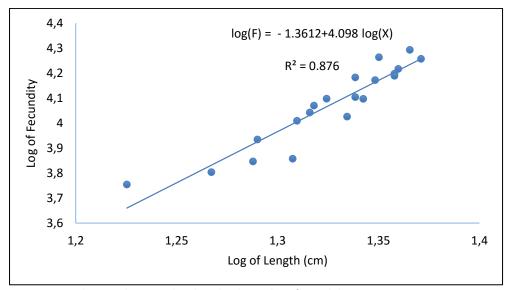


Figure 4. Regression curve showing the Fecundity- length relationship of O. pabda

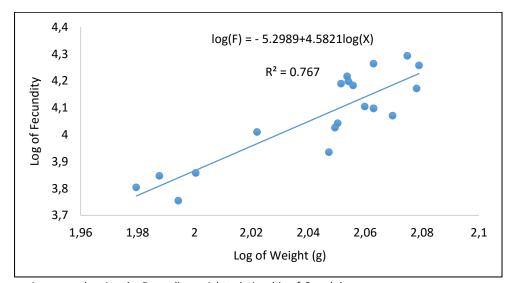


Figure 5. Regression curve showing the Fecundity-weight relationship of O. pabda

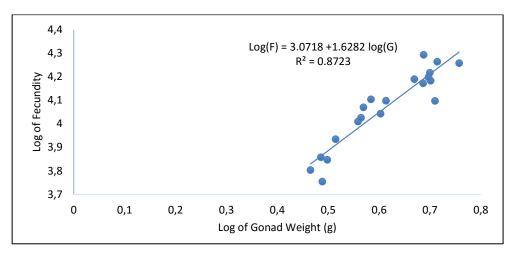


Figure 6. Regression curve showing the Fecundity- gonad weight relationship of O. pabda

hatched larvae of *H. fossilis* measured 2.5 ± 0.2 mm in length and were translucent and faintly brown in colour (Puvaneswari *et al.*, 2009). Mukherjee *et al.* (2002) reported the length of 5-6 mm in *Ompok pabo*, while Rahman *et al.* (2004) reported 2.59 to 2.62 mm in *M. cavasius*.

The fertilization rate found in *O pabda* is similar to Purkayastha et al. (2012) who reported the fertilization rate of 65.1-75.5% using different dosage of ovatid and Hossain et al. (2017) recorded as 66.20%, 91.00% and 77.00% with the three treatments of T1, T2 and T3, respectively. The hatching rate of O. pabda was estimated to range from 45% to 60.5% when treated with ovatid doses of 0.3-0.6 ml/kg body weight of fish by Purkayastha et al. (2012) which differs slightly from the current research. However, Hossain et al. (2017) recorded the hatching rates 78.40%, 90.80%, and 83.80%, in T1, T2 and T3, respectively which supports the present findings. Hossain et al. (2017) recorded the survival rate of O. pabda in three experiment were 46.20%, 76.60% and 53.60% respectively which is also almost similar to the present findings.

Different relationship such as fecundity - total length and fecundity - total body weight shows the affinity with the findings of Islam and Das (2006) for *M. cavasius*, Gupta *et al.* (2014) for *O. pabda*. The relationship between gonad weight and fecundity was found to be the most significant (p<0.01) r=0.92. This relationship is similar with other findings for *M. cavasius* (Islam and Das, 2006). Fecundity was found to be positively correlated with total length (r^2 =0.85), total weight (r^2 =0.68), and ovary weight (r^2 =0.79) in *Arius gagora* (Rahman *et al.*, 2020), which supports the current findings.

Conclusion

The findings of the research will provide useful baseline information of *Ompok pabda* in Bangladesh. The growth pattern indicated isometric growth for the species. Moreover, information on sex-ratio, length at first maturity, gonado-somatic index fecundity and larval development are observed in this experiment are very significant information regarding to commercial culture and production of this species. Fertilization rate and hatching rate were satisfactory while the survival rate is required to maximize through proper management during captive culture of *O pabda*. Finally, it can be concluded that further studies are needed to gather more information on biology of *O. pabda* which will be helpful to conserve this species and to continue its fishery for long run.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Statement

Not applicable.

Funding Information

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Author Contribution

Dulon Roy and Gulashan Ara Latifa conceived and designed the experiments; Ashish Kumar Sarker and Abu Musa Mohammad Khairul Abedin performed the experiments; Ashish Kumar Sarker, Smita Sarker, Kazi Nahida Begum and Abu Musa Mohammad Khairul Abedin analyzed the data; Dulon Roy wrote the paper. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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