Original Research Article

Reproductive Biology of Pialy fish, *Aspidoparia jaya* (Hamilton, 1822) in Bangladesh

ABSTRACT

The present study was conducted to investigate the reproductive biology of *Aspidoparia jaya* in Bangladesh Fisheries Research Institute, Floodplain Sub-station, Santahar, Bogura, Bangladesh. A total number of 80 fish samples were collected on monthly basis from Atrai River and Jamuna River during the period from January 2022 to December 2022. The highest mean value of the gonado-somatic index (GSI) was recorded in July (11.37±1.75%) and January (9.55±1.35%), whereas highest individual fecundity of 6990±1280 and 5755±1050 and ova diameter of 0.55±0.05 and 0.52±0.03 mm were also observed during these months. From the histological observation of the ovary, early perinucleolar stage, late peri-nucleolar stage, yolk vesicle stage, yolk granule stages were identified where the highest percentage of mature oocytes were observed in July and January. Based on the GSI, fecundity, and gonadal histology, two breeding season of *A. jaya* was observed from May to August and from December to late January with a remarkable peak in July and January. In case of length weight relationship (LWR), the coefficient of determination value (r^2) was found 0.95 and slope was found b= 2.02 which indicated the pattern of negative allometric growth of this species as $b<3$. In contrast, an increase was recorded in the fecundity associated with the rise of total length, body weight, and gonad weight, showed a significant linear relationship. This study would assist in the development of induced breeding techniques and provide valuable information for the sustainable management of this population in the inland open ecosystem.

Keywords: *Aspidoparia jaya*, Bangladesh, Fecundity, GSI, Gonadal histology, Length-weight-fecundity relationship.

INTRODUCTION

Bangladesh is dominated mostly by the three rivers like Ganges (Padma) Brahmaputra-Yamuna, and Meghna Rivers in which floodplains cover approximately 2.64 million ha **(DOF, 2022)** which plays a significant role as breeding and nursery grounds for a substantial percentage of hatchlings and adolescent fish **(Welcomme, 1985).** Besides fisheries sector is considered as one of the most productive and dynamic sector in Bangladesh because of its vast inland, coastal and marine water resources which made it a top fish producing country and played a significant role in the country's economy for the last few decades **(Ghose, 2014; Shamsuzzaman *et al.,* 2017 and Sunny *et al.,* 2020)**. Due to favorable geographic position, Bangladesh has diversified fisheries resources with 260 species of freshwater fishes and 475 species of marine water fishes **(FRSS, 2020).** Among all freshwater species in Bangladesh, about 143 species are called Small Indigenous Species (SIS) which maximum length at their mature stage becomes 25 cm **(Felts *et al.,* 1996).** Small indigenous species of fish contributes to feeding of millions of rural poor people and those are usually caught by subsistence fishing that provides a cushioning effect on rural poverty in Bangladesh **(FAP, 1993)** but they did not get high attention in culture systems in large scale **(Hoq, 2006).** Among 143 SIS species in Bangladesh, *Aspidoparia jaya* (Bengali name: Pialy fish; Common name: Jaya) is one of them which belongs to the family Cyprinidae and genus *Aspidoparia*. Among the small indigenous species, *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).** The availability of *A. jaya* is declining day by day 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)**. It is native to Bangladesh, India and Nepal which is commonly found in floodplains, rivers, beels, haors and baors **(Rahman, 2005).** Different natural and anthropogenic causes **(Alam et al., 2019)** is one of the major reasons attributed to decline in this population, thereby, globally it as a "Near Threatened" species in IUCN Red list **(Ng and Dahanukar, 2011)** although in Bangladesh it has been assessed as least concern recently **(IUCN, 2015).** It is an important fish 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 a great commercial and nutritional importance and can be cultured in the ponds with other species or as a single species for local consumption or exportation. Knowledge on reproductive biology of any species is a prerequisite to develop artificial breeding technique and the management of a fish stock. The most important parameters that need to be studied for the reproductive biology of any fish are the GSI, fecundity and gonadal histology, in addition to assessing the level of ripeness of the ovary **(Nandikeswari *et al.*, 2014)**. For the domestication of brood stock and the development of breeding techniques, it is important to know the different indices of reproductive biology of a fish species. Despite the enormous potentiality of *A. jaya* in freshwater aquaculture, the study of reproductive biology of this valuable species has not been addressed in Bangladesh previously. Therefore, considering the importance of this species, a thorough study was conducted to investigate the reproductive biology including gonadosomatic index, fecundity, and stages of gonadal development through gonadal histology for determining the peak breeding season of *A. jaya*, which would help in developing their artificial breeding techniques as well as their proper management in natural environment and will save this valuable species from being extinction.

MATERIALS AND METHODS

Sampling site and study period

For the accomplishment of this present investigation, the fish samples of *A. jaya* were collected from the fishermen of the River Jamuna, River Atrai & Choto Jamuna of Sirajgonj and Naogoan districts of Bangladesh on a monthly basis during the period from January 2022 to December 2022. During the study period, 80 fish samples were collected monthly basis from the landing centers of Sirajgonj and Naogoan districts of Bangladesh, which are usually caught using seine net and cast net to ensure the different size groups of fish.

Measurement of length and weight

A total eighty (80) live *A. jaya* was collected randomly in every month during the study period. The live fishes were collected in safety containers with good environmental condition and were brought to the laboratory of Floodplain sub-station, Santahar, Bogura, Bangladesh. The total lengths (cm) of the fish were measured in centimeter scale with an accuracy of 0.1 mm, body weight (g) and gonad weight (g) by precise digital electronic balance (FX- 300, USA).

Measurement of Gonadosomatic index (GSI) and Fecundity

For the identification of peak breeding season and gonadal cycle of *A. jaya*, 80 gonad samples of female fish were collected in monthly basis from January, 2022 to December, 2022. The value of gonadosomatic index for each fish was calculated by using the following formula **(Afonso-Dias *et al.* 2005):**

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

Maturing and mature female fish were selected for fecundity estimation, and Gilson's fluid was used to lessen the oocytes. Finally, absolute fecundity was calculated by using the description of **Rahman & Samat, (2020).**

$$F = \frac{G}{g} * n$$

where "F" denoted the Fecundity, "n" denoted the average number of eggs counted in sub-sample, "G" is net weight of the gonads, and "g" is the weight of the sub-sample

Histological observation of female gonads

The histological study was conducted in the Laboratory of Shrimp Health Management, Bangladesh Fisheries Research Institute, Bagerhat, Bangladesh using 'animal tissue technique' method **(Humason, 1972)**. The gonad samples were divided into three sub-samples: anterior, posterior, and middle and then put into a histology cassette which after dehydrated by automated tissue processor, Leica ASP300S (Leica Bio-system, Germany), with a series of increasing ethanol concentrations ranges from 70% to 100%, xylene clarification (two changes) and molten wax infiltration (two series). Paraffin-embedded blocks (2 µm thick) were cut with a rotating microtome (Leica RM2255, Leica Biosystem, Germany), and the sections were placed in a pre-heated (40^{o}C) water bath (Paraffin Bath-Leica Model HI1210, Leica Biosystem, Heidelberger, Germany). The sections were then placed on a glass slide to keep overnight. Afterwards, the sections were cleaned with xylene, rehydrated with alcoholic series stained with hematoxylin and eosin stains **(Humason, 1972).**

The stained sections were mounted with Canada balsam and covered with a cover slip. A light microscope was used to examine the slides (OLYMPUS BX 53), equipped with a camera and photographs were taken for further observation.

Relationship between different parameters

The following statistical formula were used for calculating the values of coefficient of determination (R^2) to establish the mathematical relationship of fecundity with total length, body weight and gonad weight:

$$Y = a + bX$$ **(Achakzai et al. 2013)**

where, Y = Fecundity estimates or gonad weight (g), X = total length (cm) or body weight (g) or gonad weight (g), 'a' & 'b' are regression constants.

The length-weight relationship was estimated according to power equation as follows:

$$W = a \times TL^b$$ **(Froese, 2006)**

where, W= total body weight (g), TL= total length (cm), and 'a' and 'b' are constants.

Statistical analysis

To determine linear and non-linear relationship and coefficient of determination (R^2), Microsoft Excel 2013 and Statistix 10 were used with 5% level of significance.

RESULTS AND DISCUSSION

Gonadosomatic index

Seasonal changes in mean GSI values of females of *A. jaya* are presented in (Fig. 1). 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 (11.37±1.75 %) in July. Specimens collected on February, March, September, and October having small GSI varying from 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 11.37 ± 1.75 % throughout the sampling period.

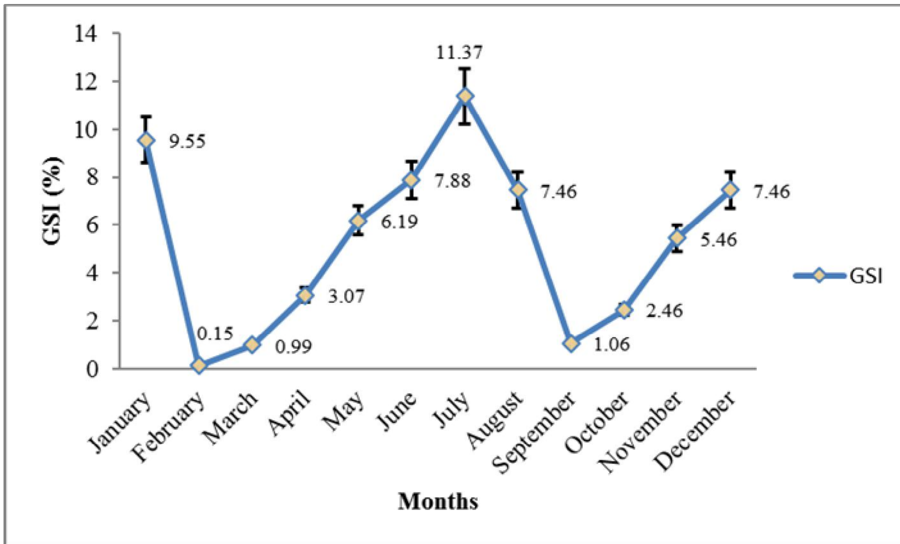

Figure 1. Monthly mean values of gonado-somatic index (GSI) of female *A. jaya*.

Fecundity

The lowest individual value of fecundity (2180) was found at the total length of 6.95 cm in August, with body weight of 5.89 g; whereas, the highest individual value (8270) was recorded at 12.45 cm total length and 11.57 g body weight in July (Table 1). Another higher individual value of fecundity (6805) was also observed in the month of January which was coincide with GSI value that was found during this month. The variation in fecundity suggests that big sized fish have more energy and larger body cavity for egg production, which agrees with the finding of **Rheman *et al.* (2002)**. The variation in fecundity is common in fish **(Reddy & Rao, 1991)** because of its dependents on many factors including fish stock, nutritional condition and racial characteristics **(Das, 1977),** such as size, age, sex, environmental conditions, availability of space and food **(Hunter, 1992).**

Table 1: Fecundity and Ova diameter range of *A. jaya*

Month	No. of fish examined	Fecundity range	Mean Fecundity (Mean±SD)	Ova diameter (mm) (Mean±SD)
May	15	2985-3515	3250±265	0.25±0.04
June	15	4430-5510	4970±540	0.31±0.03
July	15	5710-8270	6990±1280	0.55±0.05
August	15	2180-2800	2490±310	0.19±0.07
December	15	4040-5280	4660±620	0.35±0.02
January	15	4705-6805	5755±1050	0.52±0.03

Relationship among different parameters

Length-weight relationship (LWR)

Logarithmic linear regression relationship of pooled data between total length and body weight of *A. jaya* over the study period was estimated as Log BW = 2.0214 (Log TL) + 0.089 (Fig. 2). The intercept "Ln a" was 0.089 and slope "b" was 2.0214 for this species which indicating the pattern of negative allometric growth of this species as b<3. It means that they are favor to increase in length than in mass in although variation in the Length-weight relationship may depends on the population, season and environmental conditions **(Froese, 1998). Paiboon and Mengumphan (2015)** studied on length-weight relationship of *Pangasianodon gigas* and showed negative growth pattern where b values were 2.63 and 2.03. **Chakraborty, *et al.* (2019)** estimated length weight relationship on *Mystus vittatus* in two different aquatic habitats and found a negative allometric growth. With the regression coefficient (b) of 2.71, a negative allometric growth has been reported for *M. cavasius* from natural catch **(Latif *et al.*, 2018). Mithun, *et al.* (2020)** also found a negative allometric growth pattern on length weight relationship of *Mystus cavasius* in cage culture in closed environmental condition. The coefficient of determination ’R^2’ was found 0.95 indicating a good linear regression between length and weight of the species.

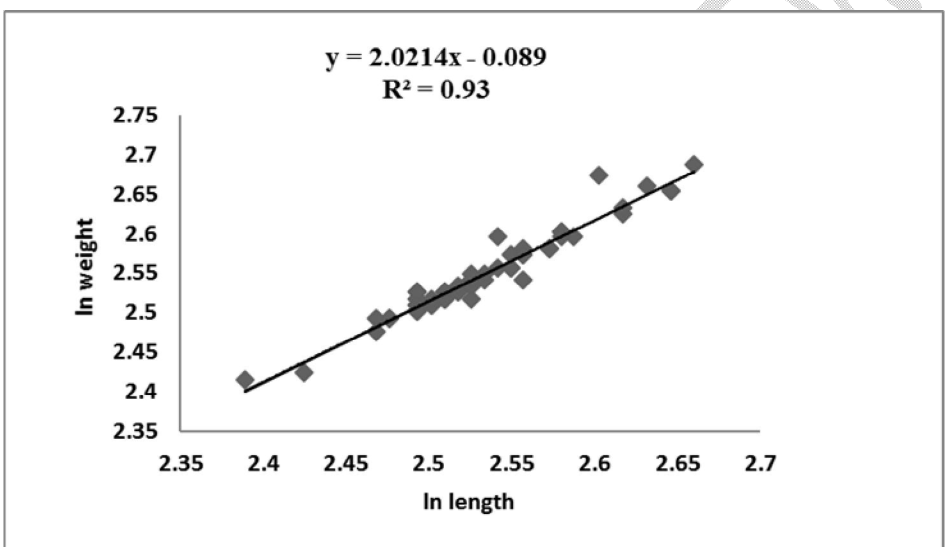

Figure 2. Relationship between total length and body weight of *A. jaya*.

Relationship of fecundity with other parameters

Linear and positive co-relationships were obtained between fecundity with total length, body weight and gonad weight. In this study, weight of fishes was recorded before removing the ovaries. The co- efficient of determination (R^2), equation of these relationships and values of ‘a’, ‘b’ are given in the (Table 2). Positive, linear and significant relationship (R^2=0.93) was observed between fecundity and gonad weight. On the other hand, the co-relation between fecundity vs. total length and Fecundity vs. body weight were found to be 0.65 and 0.82, respectively. The relationship of gonad weight with fecundity (R^2=0.95) showed high positive co- relationships; whereas, moderate positive co-relationship was observed between Fecundity and body weight (R^2=0.82) and fecundity with total length (R^2=0.65).

Table 2. Regression equation, coefficient of determination (R^2), 'a' and 'b' values of different relationships

Relationships	Equations	a	b	R^2
Fecundity (F) vs. Total Length (TL)	F = 569.91TL + 1951.40	1951.40	569.91	0.65
Fecundity (F) vs. Body Weight (BW)	F = 931.10BW - 1359.60	1359.60	931.10	0.82
Fecundity (F) vs. Gonad Weight (GW)	F = 4452.10GW + 1478.20	4452.10	1478.20	0.95

In the present study the range of fecundity of *A. jaya* varied from 2180-8270 for a corresponding length and weight 6.95-12.45 cm and 5.89-11.57 g. The average number of eggs of *A. jaya* indicates that the fish is low fecund. During the study, it was observed that the ovaries of same the size of fishes contained different numbers of eggs. This may be due to the variations in environmental conditions and food intake by the individual. The variation in fecundity is very common in fish and has been reported by many researchers **(Das, 1977; Bhuiyan *et al*, 2006).** Numerous factors like different stock of fish, nutritional status **(Gupta, 1967)**, racial characteristics **(Das, 1977),** time of sampling and maturation stage and changes in environmental parameters **(Bhuiyan *et al*, 2006)** have so far been reported to affect the fecundity both within the species and between fish populations. So, variation in fecundities during the study was not an exception. It is familiar that the gonadosomatic index (GSI) increases with the maturation of fish, become maximum during the period of peak maturity and declining abruptly thereafter **(Parween *et al*, 1993)**. In *A. jaya*, the gonadosomatic index was higher during July and January when majority of fishes were found mature. It was found that the bigger sized fishes have higher fecundity and smaller sized fishes have lower fecundity. The regression equation (F = 569.91TL + 1951.40) representing the relationship between fecundity and total length was found as linear and the value of R^2= 0.65, which is moderately significant (Fig. 3). Variation in the fecundity of the fish in the same length class was found in the study which indicates that the fecundity of a fish is not solely dependent on its length. This comment agrees with the findings of **Doha and Hye, (1970)** in *H. ilisha*. The relationship between fecundity and body weight was significant (R^2 = 0.82) and found to be linear (F = 931.10BW - 1359.60) (Fig. 4). Positive relationships between fecundity and body weight have been reported in a number of fishes which support the present findings **(Gupta, 1967).** The relationship between fecundity and gonad weight was found to be positive, linear, highly significant (R^2 = 0.95) and the equation was F = 4452.10GW + 1478.20 (Fig. 5). Fecundity increased with increasing gonad weight. This result is also agreed with **Sultana, (2010).** Fecundity and gonad weight relationship was highly positive as the fecundity increased with the increasing of gonad weight and this was happened till maturity of the gonad.

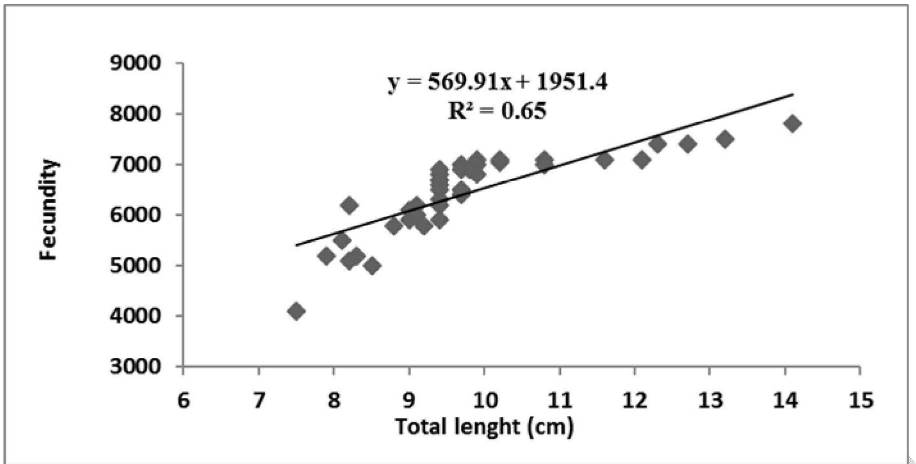

Figure 3. Relationship between fecundity and total length of *A. jaya*.

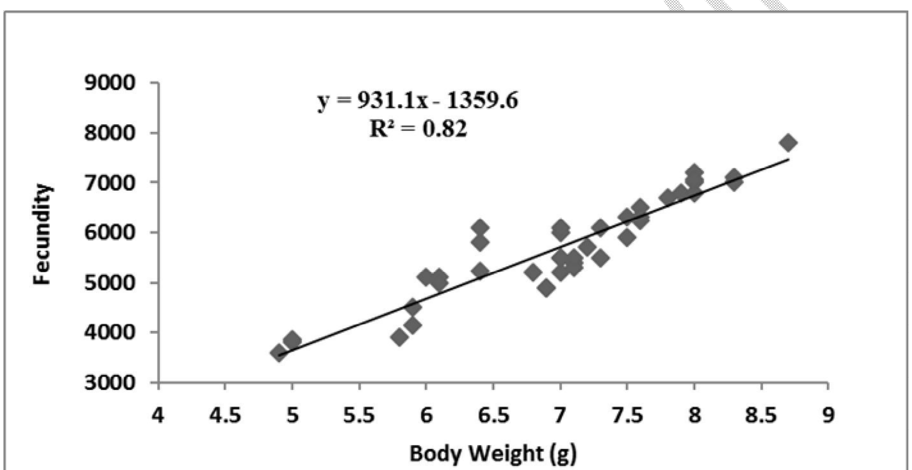

Figure 4. Relationship between fecundity and Body weight of *A. jaya*.

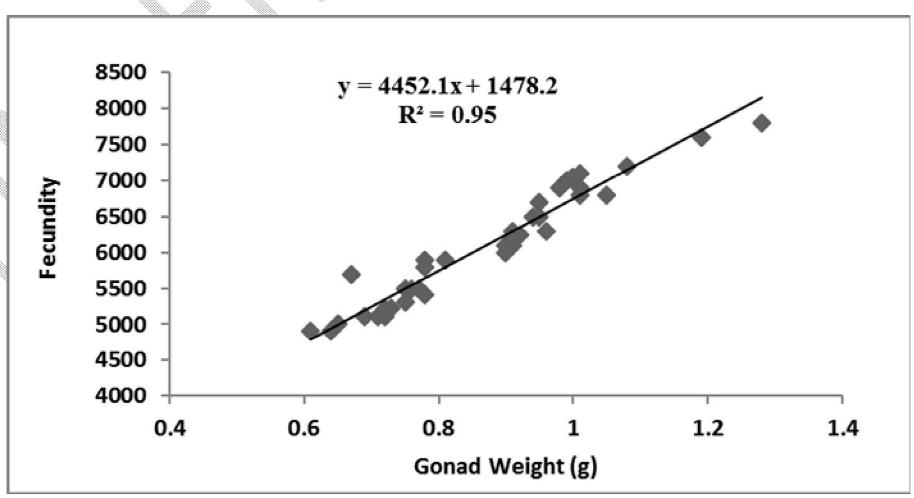

Figure 5. Relationship between fecundity and Gonad weight of *A. jaya*.

Histological observation and maturation stage of oocytes

The ovarian development of *A. jaya* was examined to investigate the development pattern and timing of the growth phase of germ cells in the gonad (Fig. 6. A-G). Histological observation of the ovaries of *A. jaya* revealed different maturity stages of this fish. The oocyte development observed throughout this study period and revealed that eggs from the ovary showed asynchronous development as oocytes of all stages are present in the ovary without dominant populations but went through discrete developmental stages, before reaching full maturity.

Seven different oocyte stages were observed in ovaries: (A) Chromatin nucleolar stage (Fig. 6 A), (B) Early perinucleolar stage (Fig. 6 B), (C) Late perinucleolar stage (Fig. 6 C), (D) Yolk vesicle stage (Fig. 6 D), (E) Early yolk granule stage (Fig. 6 E), (F) Late yolk granule stage (Fig. 6 F) and (G) Spent stage (Fig. 6 G). Ovaries can be divided into 4 phases based on the number of oocytes of each stage in the ovaries (Table 3): (1) Immature, (2) Maturing, (3) Mature and (4) Spent *A. jaya* (phase after spawning).

It was observed that immature stages of ovary (chromatin nucleolar stage, early and late perinucleolar stage) were mostly observed in the months of February, March and September (Fig. 6 A-C). Yolk- vesicle stage oocytes appeared in April, May and October (Fig. 6 D). Early yolk granular stages were observed in early-June and November (Fig. 6 E) where late yolk granular stages were observed in late-June and December months (Fig. 6 F). But eggs in the month of July and January contain more yolk granule in the oocyte. In August and late-January, post-ovulatory follicles and germinal vesicle breakdown stages of oocyte (Fig. 6 G) were observed and then in the later months oocytes were started to develop again in the ovary of *A. jaya*. Histological data revealed that there are two spawning season of *A. jaya* where the first one starts from May and continued till August and second season starts from December and continued till late January which was configured by the presence of a large number of mature eggs in July and January. This result was also consistent with the values of GSI as the two peak values were found in July and January. From the histological study of ovaries at successive months, it was observed that oocytes did not develop synchronously and oocytes at various maturation stages were observed in paired ovaries in the pre-breeding period.

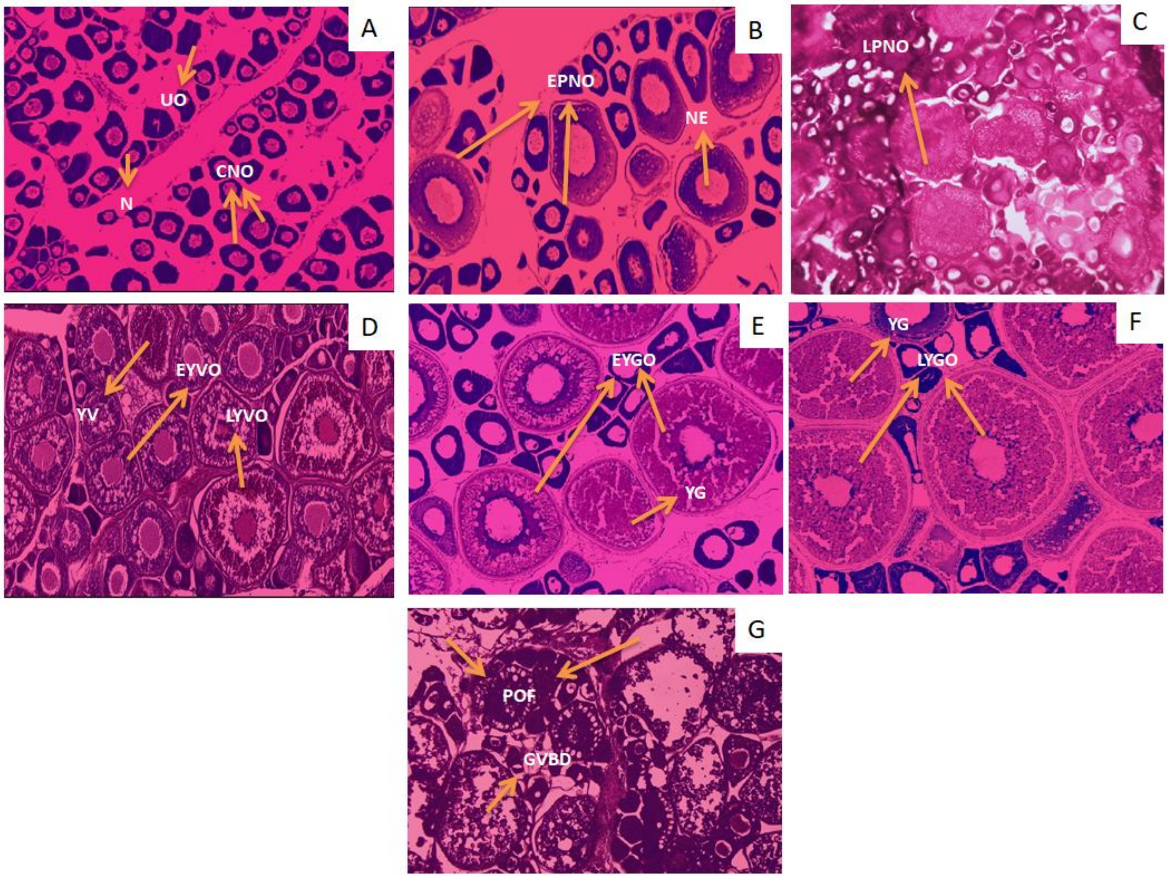

Figure 6. (A) Chromatin nuclear stage (N= Nucleoli; UO= Undeveloped oocyte; CNO= Chromatin nuclear oocyte), (B) Early Perinucleolar stage (NE= Nucleolus; EPNO= Early perinucleolar oocyte), (C) Late perinucleolar stage (LPNO= Late perinucleolar oocyte), (D)Yolk vesicle stage (EYVO= Early yolk vesicle oocyte; LYVO= Late yolk vesicle oocyte; YV= Yolk vesicle), (E) Early yolk granular stage (EYVO= Early yolk vesicle stage oocyte; YG= Yolk granule), (F) Late yolk granular stage (LYGO= Late yolk granule stage oocyte), (G) SPENT (GVBD= Germinal vesicle breakdown ; POF= Post ovulatory follicle)

Ovaries can be divided into 4 phases based on the number of oocytes of each stage in the ovaries (Table 3): (1) Immature, (2) Maturing, (4) Mature and (5) Spent of *A. jaya* (phase after spawning). In the present study, the gonadal maturity stages of *A. jaya* were identified in females based on the description mentioned by different authors with slight modifications **(Coward and Bromage, 1998; Wright, 2007)** and the knowledge on the ovarian development and peak breeding period of a species is crucial for the effective management of its population.

Table 3. Developmental stages of ovary with ova diameter of *A. jaya* with histological characteristics

Stages of ovaries	Histological characters	Months
1. Immature	Young females possess this stage. Undeveloped oocyte and pre mature oocyte are well-organized. Oocyte mostly in CN stage, but some in PN stage. This stage does not present follicles in vitellogenesis.	February to April
2. Maturing	Females are considered entering the reproductive cycle. Oogonia, primary growth (PG) oocytes, CA oocytes are predominant. As maturation progresses, the quantity of vtg oocytes increases.	May, June & December
3. Mature	Number of yolk granules was sharply increasing. Mature oocytes were numerous Vitellogenic oocytes predominant but some primary growth oocytes also seen behind the mature oocytes.	July & January
4. Spent	Abundant POFs and GVM follicles. Initial stages of oocyte maturation may also be present, as well as GVBD follicles.	late-August & late-January

Based on the oocyte prevalence percentage, ovarian developmental phases were classified as immature (Stage I), maturing (Stage II), mature (Stage III), and spent (Stage IV). The immature stage oocytes were found in February to April. A rapid development of oocytes with the shifting towards maturing stage was observed in May, June and December. There was a significant mature stage oocyte observed in July and January with average oocyte diameter of 0.55 mm and 0.52 mm, respectively (Table 1). The immature stage oocytes was found to be minimal at mature stage but present as they possess asynchronous ovary development while the maximum number of maturing stage and mature stage oocytes were present in July and January, respectively. The highest percentage of spent stage of ovary was observed in August and late-January. In the current study, the macroscopic and histological observations of the gonads, GSI, fecundity, and oocyte diameter showed a good agreement that there are two spawning season of *A. jaya* where the first one starts from May and continued till August and second season starts from December and continued till late January.

CONCLUSION

This study revealed that *A. jaya* has a double reproductive cycle in a year and the peak breeding seasons are July and January. The detection and characterization of various gonadal development stages, GSI, fecundity, egg diameter, and relationship of different parameters with fecundity will be serving as a benchmark to conserve and breed this valuable species in captive conditions. This study can also be helpful for sustainable fishery management of *A. jaya* in its original habitat. In addition, it would be effective for fisheries experts to implement regulations for the control of over-exploitation, which will ensure the sustainable management of this species in open water bodies of Bangladesh.

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