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SOME ASPECTS OF REPRODUCTIVE BIOLOGY OF GANGETIC AILIA, *Ailia coila* (HAMILTON, 1822) IN BANGLADESH

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ABSTRACT

The present study was conducted by Bangladesh Fisheries Research Institute, Floodplain Sub-station to investigate the reproductive biology of *Ailia coila* in the northern region of Bangladesh. A total number of 100 fish samples were collected on a monthly basis from the 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 (8.78 ± 1.95%), whereas the lowest was found in December (0.15 ± 0.09%). The highest individual fecundity (2,450 ± 570) and ova diameter (0.38 ± 0.07 mm) was also observed in July. From the histological observation of the ovary, the highest percentages (75%) of mature oocytes were observed during the month of July. Based on the GSI, fecundity and gonadal histology, the breeding season of *A. coila* was observed from June to August with a remarkable peak in July. In the case of length weight relationship (LWR), the coefficient of determination value (r^2) was found 0.96 and the slope was found b = 1.50 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: Bangladesh, Breeding season, Fecundity, GSI, Gonadal histology, Length-Weight-Fecundity relationship, *Ailia coila*.

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INTRODUCTION

Bangladesh is a riparian floodplain of the three most dominant rivers like Ganges (Padma) Brahmaputra-Yamuna, and Meghna Rivers in which floodplains cover approximately 2.64 million ha (DOF, 2022). Floodplains are nutrient rich perennial and seasonal wetland (Mondal & Pal, 2017) which plays a significant role as breeding and nursery grounds for a substantial percentage of hatchlings and adolescent fish (Welcomme, 1985). There are about 265 indigenous freshwater fish species in Bangladesh (Rahman, 2005; Shamsuzzaman et al., 2017; Newaz & Rahman, 2019) of which 143 species are called Small Indigenous Species (SIS) which maximum length at their mature stage becomes 25 cm and 55 species are catfish (Felts et al., 1996; Rahman & Akhter, 2019). Small indigenous species of fish contributes to feeding 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). Gangetic ailia, *Ailia coila*, (Bengali name: Kajuli) belonging to the family Ailiidae which is South-Asian endemic and confined in the Jamuna, Ganga, Bramhaputra and Mahananda rivers in India; Indus plains in Pakistan; Nepal; Padma-Jamuna-Meghna river systems in Bangladesh (Talwar & Jhingran, 1991; Rahman, 2005; Parween, 2007). They usually live in shoals in rivers connected to large natural water bodies with sand or mud created turbid water (Talwar & Jhingran, 1991; Ahmed, 2002; Parween, 2007) and migrate to connected water bodies during monsoon and even moves to estuarine waters in the south of Bangladesh (Rahman, 2005; Parween, 2007; Chandra, 2009; Galib et al., 2013). It is a carnivorous species that mostly feed on zooplankton but occasionally prefers algae, plant materials and debris (IUCN, 2015). There is no empirical data available regarding the decline in catch in its entire range except in studies of Patra et al. (2005) and Mishra et al. (2009), who have observed a decline in population by 30‒80% in different parts of West Bengal. Overexploitation, natural and anthropogenic causes (Alam et al., 2019) are the major reasons attributed to the decline in this population, thereby, globally it is a “Near Threatened” species in the IUCN Red List (Ng & Dahanukar, 2011) although in Bangladesh it has been assessed as least concern recently (IUCN, 2015). The captive breeding of this species is not yet standardized, so the market demand for this species is met only through the wild populations from the rivers. Morphometric and biometrics i.e fecundity (F), gonadosomatic index (GSI), hepato-somatic index (HSI) and length-weight relationship (LWR) are known as important parameters in fish biology (Zin et al., 2011), represents details regarding reproductive status and ascertaining breeding period of fish (Gupta & Srivastava, 2001; Mohan & Jhajhria, 2001; Shankar & Kulkarni, 2005). This information likewise facilitates the effective conservation and management of any species (Ghaedi et al., 2013; Rahman, 2014; Rheman et al., 2002). Despite the enormous potentiality of *A. coila* in freshwater aquaculture, the study of the 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. coila*, which would help in developing their artificial breeding techniques as well as their proper management in the natural environment and will save this valuable species from being extinction.

MATERIALS AND METHODS

Sampling site and study period

A. coila samples were collected from the fishermen of River Jamuna, River Atrai and Choto Jamuna of Sirajgonj and Naogoan districts of Bangladesh on a monthly basis during the period of January 2022 till December 2022 (Fig. 1). Fishes were caught using seine net and cast net to ensure the different size groups of fish.

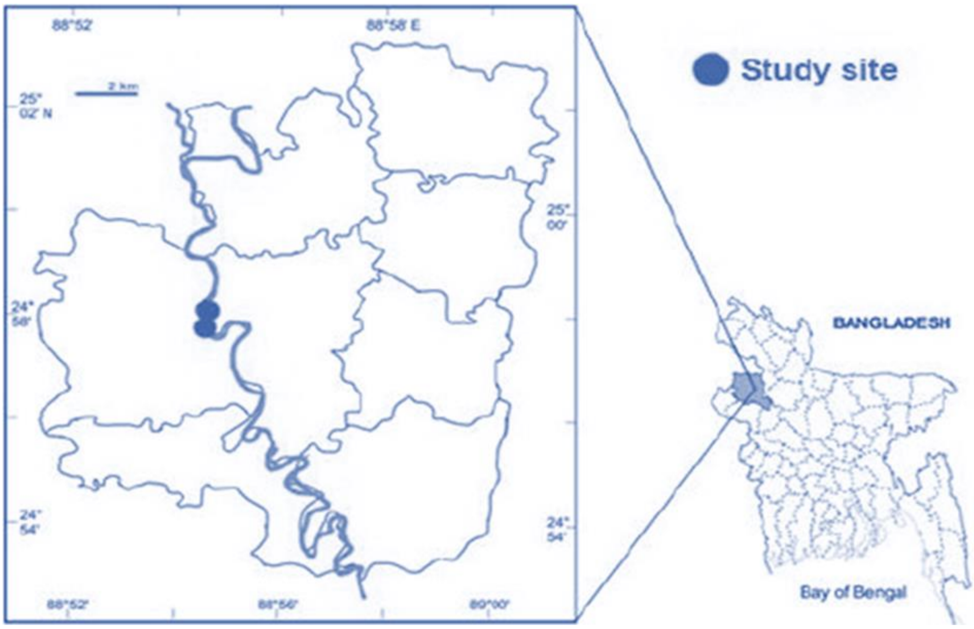

Figure 1. Location of the surveyed area of *Ailia coila*

Sampling and footage of length and weight

A total of one hundred fish samples were collected every month and brought to the laboratory of Floodplain sub-station, Santahar, Bogura, Bangladesh during the study period. The fish were washed with running tap water to remove filth and dirt; the adhered water was dried up and left for 30 minutes for removal of moist completely. The lengths (cm) of the fish were measured in a centimeter scale with an accuracy of 0.1 mm. Body weight (g) and gonad weight (g) were measured by precise digital electronic balance (FX-300i Precision Balance, A&D Company, Ltd.).

Measurement of Gonadosomatic Index (GSI) and Fecundity

For the identification of peak breeding season and the gonadal cycle of *A. coila*, 100 gonad samples of female fish were collected in a monthly basis from January 2022 to December 2022 and the total length and body weight of individual fish were also measured during that time. The value of the gonadosomatic index for each fish was calculated by using the following formula (Afonso-Dias et al., 2005):

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

Fishes collected during the month of April to September were subjected to the calculation of fecundity. Gravid female fish were selected for fecundity estimation and Simpson modified Gilson's fluid (60% alcohol – 100 mL + water – 880 mL + 80% nitric acid – 15 mL + glacial acetic acid – 18 mL + mercuric chloride – 20 g) was used to lessen the oocytes. This fluid hardens the eggs and also liberates them from the ovarian tissues. 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 the sub-sample; "G" is the 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 the '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 an 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 Bio-system, Germany), and the sections were placed in a pre-heated (40 ºC) water bath (Paraffin Bath-Leica Model HI1210, Leica Bio-system, 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, Hamburg), equipped with a camera and photographs were taken for further observation.

Relationship between different parameters

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

$$Y = a + bX \text{ (Achakzai et al., 2013)}$$

Where: Y = Fecundity 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 the power equation as follows:

$$W = a \times TL^b \text{ (Froese, 2006)}$$

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

The mean GSI value of fishes was tended to increase as the fish reach maturity and after spawning, it declined and became lowest during the resting phase. The GSI values of females of *A. coila* were changed from 0.15 ± 0.09 to 8.78 ± 1.95% with the change of seasons (Fig. 2). The highest mean GSI value of female *A. coila* was found 8.78% in July and the lowest mean value was found 0.15% in December. GSI values progressively increased from March and reached their maximum in July before witnessing a sharp fall in August. The single highest values of GSI (8.78 ± 1.95%) in the month of July in a year indicated that July is the peak breeding season of *A. coila* and they breed only one time in a year (Fig. 2). The GSI values began to fall abruptly from August and then gently fall down till January. The highest GSI value of *A. coila* in the month of July suggested that during this month the percentage of yolk laden ripe eggs in the ovary were higher.

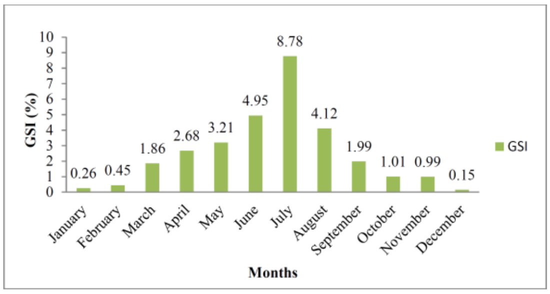

Figure 2. Monthly mean values of gonado-somatic index (GSI) of female *Ailia coila*

Fecundity

The lowest individual value of fecundity (715) was found at a total length of 7.36 cm in April, with a body weight of 4.99 g; whereas, the highest individual value (3,020) was recorded at 13.11 cm total length and 7.33 g body weight (Table 1). This suggests that big sized fish have more energy and a 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 other 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 *Ailia coila*

Month	Fecundity range	Mean Fecundity (nos.) (Mean ± SD)	Ova diameter (mm) (Mean ± SD)
April	715–985	850 ± 135	0.12 ± 0.03
May	1,070–1,800	1,435 ± 365	0.15 ± 0.05
June	1,640–2,620	2,130 ± 490	0.25 ± 0.06
July	1,880–3,020	2,450 ± 570	0.38 ± 0.07
August	1,236–2,194	1,715 ± 479	0.27 ± 0.05
September	785–1,015	900 ± 115	0.15 ± 0.02

Relationship among different parameters

Length-weight relationship (LWR)

The logarithmic linear regression relationship of pooled data between the total length and body weight of *A. coila* over the study period was estimated as Log BW = 1.504(Log TL) + 0.045 (Fig. 3). The intercept "Log a" was 0.045 and slope "b" was 1.504 for this species, indicating the pattern of negative allometric growth of this species as b < 3. It means that the body length of *A. coila* increases faster than the body weight although variation in the LWR may depend on the population, season and environmental conditions (Froese, 1998). Paiboon & Mengumphan (2015) studied the length-weight relationship of *Pangasianodon gigas* and showed a negative growth pattern where *b* values were 2.63 and 2.03. Chakraborty et al. (2019) estimated the length weight relationship between *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 *Mystus cavasius* from natural catch (Latif et al., 2018). Mithun et al. (2020) also found a negative allometric growth pattern on length weight relationship of *M. cavasius* in cage culture in closed environmental conditions. The coefficient of determination 'R^2' was found 0.94, showing a good linear regression between the length and weight of the species.

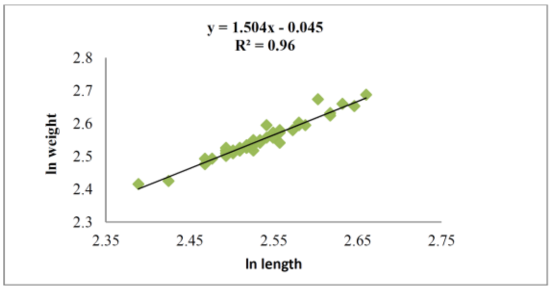

Figure 3. Relationship between total length and body weight of *Ailia coila*

Relationship of fecundity with other parameters

Linear and positive co-relationships were obtained between fecundity with total length, body weight and gonad weight. The co-efficient of determination (R^2), equation of these relationships and values of 'a', 'b' are given in (Table 2). A positive, linear and significant relationship (R^2 = 0.95) 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 0.51 and 0.83, respectively. The relationship of gonad weight with fecundity (R^2 = 0.95) showed high positive co-relationships; whereas, a moderate positive co-relationship was observed between Fecundity and body weight (R^2 = 0.83) and a low positive co-relationship was observed between fecundity with total length (R^2 = 0.51).

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 = 389.8TL + 499.47	499.47	389.8	0.51
Fecundity (F) vs. Body Weight (BW)	F = 1,017.5BW – 1,356.70	1,356.70	1,017.50	0.83
Fecundity (F) vs. Gonad Weight (GW)	F = 4,877.4GW + 1,250.62	4,877.4	5,002.10	0.95

In the present study, the range of fecundity of *A. coila* varied from 715–3,020 for a corresponding length and weight 7.36–13.11 cm and 4.99–7.33 g. The average number of eggs of *A. coila* 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. It is familiar that the gonadosomatic index (GSI) increases with the maturation of fish, becomes maximum during the period of peak maturity and declines abruptly thereafter (Parween et al., 1993). In *A. coila*, the gonadosomatic index was maximum during July when the 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 = 389.8TL + 499.47) representing the relationship between fecundity and total length was found as linear and the value of R^2 = 0.51, which is less significant (Table 2). 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 & Hye (1970) in *Hilsa ilisha* species. The relationship between fecundity and body weight was significant (R^2 = 0.83) and found to be linear (F = 1,017.5BW – 1,356.70) (Table 2). 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 = 4,877.4GW + 1,250.62 (Table 2). Fecundity increased with increasing gonad weight, which also agrees with Sultana (2010). The fecundity and gonad weight relationship was highly positive as the fecundity increased with the increasing of gonad weight and this was happened till the maturity of the gonad.

Histological observation

Histological observation of the ovaries revealed different maturity stages of the fish. The oocyte development observed throughout this study period revealed that eggs from the ovary showed group synchronous oocyte development but went through discrete developmental stages, before reaching full maturity. The ovarian histology of *A. coila* showed group synchronous oocyte development. Five different oocyte stages were observed in ovaries: (A) Chromatin nucleolar stage (Fig. 4 A), (B) Perinucleolar stage (Fig. 4 B), (C) Yolk vesicle stage (Fig. 4 C), (D) Yolk granule stage (Fig. 4 D) and (E) Spent stage (Fig. 4 E).

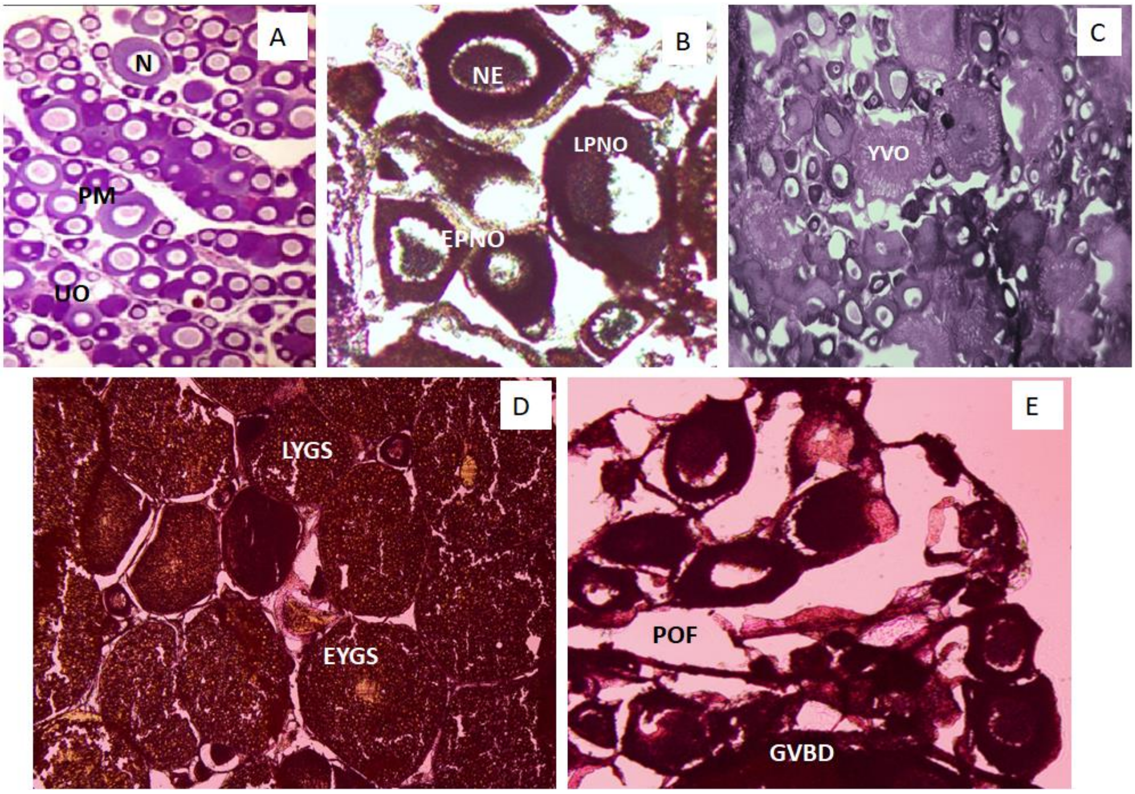

Figure 4. (A) Chromatin nuclear stage (N = Nucleoli; UO = Undeveloped oocyte, PM = Premature oocyte); (B) Perinucleolar stage (NE = Nucleolus, EPNO = Early perinucleolar oocyte, LPNO = Late perinucleolar oocyte); (C) Yolk vesicle stage (YVO = Yolk vesicle oocyte; (D) Yolk granular stage (EYVO = Early yolk vesicle stage oocyte, LYGO = Late yolk granule stage oocyte; (E) Spent (GVBD = Germinal vesicle breakdown, POF = Post ovulatory folicle)

Developmental stages of *Ailia coila* oocyte with histological characteristics

A. Chromatin Nucleolar Stage: Immature stage observed in the youngest oocytes characterized by the chromatin threads, undeveloped oocyte (UO) and often oogonia. This stage was found in the months of January and February (Fig. 4A).

B. Perinucleolar Stage: Oocyte's nucleus begins to expand, nuclear and cytoplasmic volumes are increase, a large number of nucleoli with varying sizes are found around the periphery of the nucleus. Cytoplasmic volume increased and vitteline envelope formation began at this stage. This stage was observed between March and April (Fig. 4B).

C. Yolk vesicle stage: Oocyte contained small yolk vesicles and these yolk vesicles appeared firstly around the cytoplasm and then gradually spread more into the middle of the cell, nucleolus appeared around the nucleus or inside the nucleus. This stage was predominant from mid-April to May (Fig. 4C).

D. Yolk granule stage: Oocyte contained a number of yolk granules stained in light pink color, yolk granules appear firstly around the cytoplasm and then gradually spread more into the middle of the cell. At this stage, the diameter of the oocytes and the quantity of yolk granules increased sharply and oil droplets inside the cytoplasm were evident. This stage was found predominantly in late

June, July and early August when the oocytes were fully matured (Fig. 4D).

E. Spent phase: At this stage the follicle became empty and post ovulatory follicles are predominant although a few mature oocytes were observed. This spent and resting phase of the ovary was observed from September to November (Fig. 4E).

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 of *A. coila* (phase after spawning). In the present study, the gonadal maturity stages of *A. coila* were identified in females based on the description mentioned by different authors with slight modifications (Coward & 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.

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 and March. The rapid development of oocytes with the shifting towards maturing stage was observed between April and May where the average oocyte diameter was found to be 0.12 mm and 0.15 mm, respectively (Table 1). There was a significant mature stage oocyte observed in June, July and August with average oocyte diameter of 0.25 mm, 0.38 mm and 0.27 mm, respectively (Table 1). The immature stage oocytes was found to be minimal from May to August while the maximum number of maturing stage and mature stage oocytes were present in May and July, respectively. The highest percentage of the spent stage of the ovary was observed in September. In the current study, the macroscopic and histological observations of the gonads, GSI, fecundity, and oocyte diameter showed a good agreement that the spawning season of *A. coila* extends from June to August with the major peak in July.

Table 3. Developmental stages of gonad of *Ailia coila*

Stage	Maturity level	Description	
		Male	Female
Stage I	Immature	Testis transparent and thread-like	Ovary strip-like and transparent
Stage II	Maturing	Testis pinkish white, strip- like and about 1/2 of body cavity	Ovary dull greyish, granular and about 1/2 of the body cavity
Stage III	Mature	Testis whitish and band-like	Ovary greyish and about the size of the entire body cavity. Transparent ova visible
Stage IV	Spent	Testis shrink	Ovary pinkish brown and sunken to about 1/2 of the body cavity

CONCLUSION

This study revealed that *A. coila* has a single reproductive cycle in a year and the peak breeding season is July. The detection and characterization of various gonadal development stages, GSI, fecundity, egg diameter, and the 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. coila* 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 the open water bodies of Bangladesh.

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