

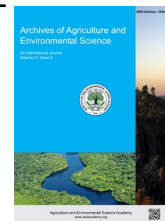


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ORIGINAL RESEARCH ARTICLE



Reproductive biology and gonadal cycle of Indian Potasi, *Neotropius atherinoides* (Bloch 1794) in Bangladesh

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ABSTRACT

The present study was conducted to investigate the reproductive biology of *Neotropius atherinoides* in Bangladesh Fisheries Research Institute, Floodplain Sub-station, Santahar, Bogura, Bangladesh. A total number of 70 fish samples were collected on monthly basis from Atrai River and Jamuna River during the period from September 2021 to August 2022. The highest mean value of the gonado-somatic index (GSI) was recorded in July (12.55 ± 2.50), whereas the lowest was found in January (0.46 ± 0.11). Highest individual fecundity (7000 ± 1900) and ova diameter (0.50 ± 0.07 mm) was also observed in July. From the histological observation of the ovary, early peri-nucleolar stage, late peri-nucleolar stage, yolk vesicle stage, yolk granule stages were identified where the highest percentage (80%) of mature oocytes were observed in July. Based on the GSI, fecundity, and gonadal histology, the breeding season of *N. atherinoides* was observed from May to August and recorded a remarkable peak in July. In case of length weight relationship (LWR), the coefficient of determination value (r^2) was found 0.95 and slope was found $b=1.02$ 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.

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INTRODUCTION

Fisheries sector is considered as one of the most productive and dynamic sectors 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). A total of 55 catfish species are found in inland waters of Bangladesh (Rahman and Akhter, 2019), of which Indian Potasi, *Neotropius atherinoides*, (Bengali name: Batashi) is one of them which belongs to the family Schilbeidae and genus *Neotropius*. The availability of Indian Potashi or Batashi 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 widely distributed in Bangladesh, India, Pakistan, Nepal, and Myanmar. In Bangladesh, it is commonly found in

floodplains, rivers, beels, haors and baors (Rahman, 2005). Like other SIS, this fish species has high nutritional value and rich in protein ($15.84 \pm 1.50\%$), fat ($2.24 \pm 0.40\%$) with other micro and macro nutrients (Bogard et al., 2015; Mazumder et al., 2008). 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 *N. atherinoides* 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 *N. atherinoides*, 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.

MATERIEALS AND METHODS

Study area

The fish samples (n=70) of *N. atherinoides* were collected from

the fishermen of the River Jamuna, River Atrai & Roktodahe Beel of Sirajgonj, Naogoan, and Bogura districts of Bangladesh on a monthly basis during the period of September 2021 till August 2022 (Figure 1). To ensure the different size groups, fishes were caught using seine net and cast net.

Measurement of different parameters

The Length and weight of the collected specimens were measured using measuring scale and digital electronic balance (Model FX- 300), with 0.1g accuracy in the Laboratory of Bangladesh Fisheries Research Institute, Floodplain Sub-station, Santahar, Bogura. The gonads were taken out carefully after dissecting the fish in an intact form. The gonads were preserved in well labeled vials with 10% buffered formalin and then kept in the Bouin's Fixative for histological study.

Gonado-Somatic index (GSI)

Female gonad of *N. atherinoides* was collected in monthly basis from September, 2021 to August, 2022. During the experimental period, 70 fish samples were collected to measure the total length and body weight of individual fish. Then the ovary of each fish was taken out very carefully and preserved in 10% buffered formalin with labeled vials and then fixed in the Bouin's fluid for further histological study. The weight of the ovary was measured very carefully with the help of a sensitive portable electronic balance (Model FX- 300).

GSI of *N. atherinoides* was calculated according to the formula (Afonso-Dias et al., 2005):

$$\text{GSI} = (\text{Gonad Weight} / \text{Total weight}) \times 100$$

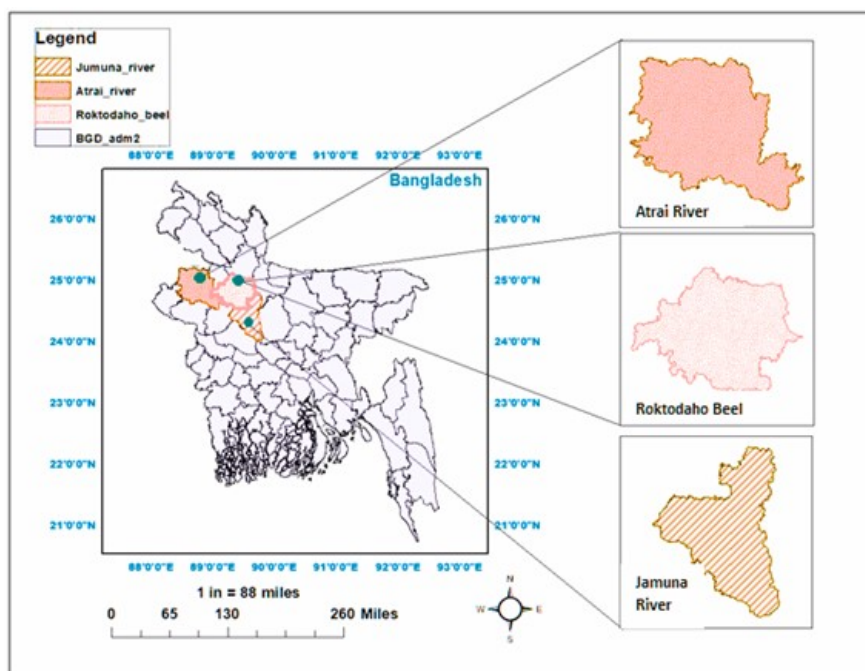


Figure 1. Location of the capture area of *N. atherinoides*.

Fecundity

Von Vayer method was applied to estimate the fecundity of relatively large size eggs of *N. atherinoides* where the ovaries were dissected out by a pair of scissors. The external connective tissues were removed from the surface of each pair of ovaries and moisture was removed with the help of a blotting paper. The ovary weight of each fish was measured with an electronic balance (Model FX- 300) and 10 mg from each ovary was taken separately from anterior, middle and posterior portions of each lobe accurately. The number of mature and maturing eggs from each portion was estimated separately by actual counting. Then the mean number of eggs in 10 mg was multiplied by the total weight of the ovary, which gave the total number of eggs.

Histology of gonad

The histological examination is an important tool for predicting the fish gonadal maturity and the spawning season. The histological study was conducted in the Laboratory of Shrimp Health Management, Bangladesh Fisheries Research Institute, Bagerhat, Bangladesh. The tissues were divided into three subsamples: anterior, posterior, and middle. The sample tissues were then labeled and put into a histology cassette. The 'animal tissue technique' method (Humason, 1972) was used where tissue dehydration was performed 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 μ m thick) were cut with a rotating microtome (Leica RM2255, Leica Bio-system, Germany), and the sections were placed in a pre-heated (40°C) water bath (Paraffin Bath-Leica Model HI1210, Leica Biosystem, Heidelberg, 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 (Achakzai et al., 2013) 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:

$$Y = a + bX$$

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 (Froese, 2006) as follows:

$$W = a \times TL^b$$

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 the fish tends to increase as the fish reach maturity and after spawning, it declines and become lowest during the resting phase. The GSI values of females of *N. atherinoides* were changed from 0.46 ± 0.11 to $12.55 \pm 2.50\%$ with the change of seasons (Figure 2). The highest mean GSI value of female *N. atherinoides* was found 12.55% in July, and the lowest mean value was found 0.46% in January. GSI values progressively increased starting from March and reach to its maximum in July before witnessing a sharp fall in September. Higher values of GSI were found during the month of June, July, and August. Highest values of GSI ($12.55 \pm 2.50\%$) in the month of July (Figure 2) indicated that July is the peak breeding season of *N. atherinoides*. The GSI values began to fall abruptly from August to September and gently fall down from October to January. However, previously some reproductive aspects of *N. atherinoides* were studied by Hossian et al. (2020), where the highest gonadosomatic index was observed in the months of April, May, and June. But in the present experiment the higher GSI value were found in the month of June, July, and August. The increasing GSI value of *N. atherinoides* suggested that the percentage of yolk laden ripe eggs in ovary was found higher in June, July, and August.

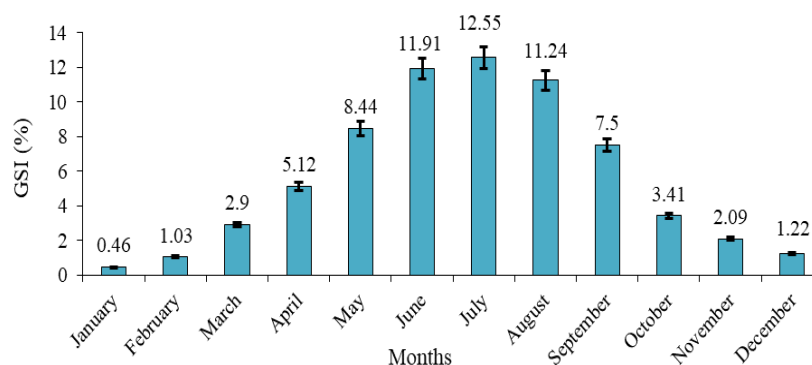


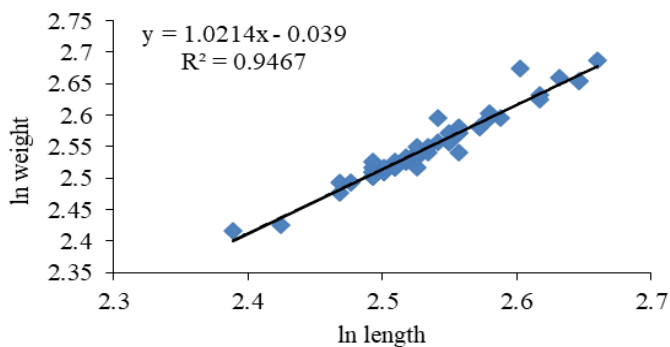
Figure 2. Monthly mean values of gonado-somatic index (GSI) of female *N. atherinoides*.

Table 1. Fecundity of *N. atherinoides*.

Month	No. of fish examined	Fecundity range	Mean Fecundity (nos.) (Mean±SD)
April	10	1100-1800	1450±350
May	10	2670-4200	3435±765
June	10	3160-6500	4830±1670
July	10	5100-8900	7000±1900
August	10	2636-5794	4215±1579
September	10	1300-1900	1600±300

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)	$F = 449.71TL + 2041.30$	2041.30	447.70	0.67
Fecundity (F) vs. Body Weight (BW)	$F = 1038.1BW - 1577.60$	1577.60	1038.10	0.86
Fecundity (F) vs. Gonad Weight (GW)	$F = 5002.1GW + 1740.20$	1740.20	5002.10	0.94

**Figure 3.** Relationship between total length and body weight of *N. atherinoides*.

Fecundity

The lowest individual value of fecundity (1,100) was found at the total length of 6.5 cm in April, with body weight of 5.11 g; whereas, the highest individual value (8,900) was recorded at 12.4 cm total length and 11.21 g body weight (Table 1). This 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). Previously, Hossian *et al.* (2020) determined the highest fecundity of 10,043 oocytes in *N. atherinoides* collected from the Kangsha River flowing through Netrokona district, which is higher than that of the present study which was collected from Jamuna River and Atrai River. The variation in fecundity is common in fish (Reddy and 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).

Relationship among different parameters

Length-weight relationship (LWR)

Logarithmic linear regression relationship of pooled data between total length and body weight of *N. atherinoides* over the study period was estimated as $\text{Log BW} = 1.02(\text{Log TL}) + 0.039$ (Figure 3). The intercept "Ln a" was 0.039 and slope "b" was 1.02 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 0.94 indicating a good linear regression between length and weight of the species.

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 ($R^2=0.94$) 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 0.67 and 0.86, respectively. The relationship of gonad weight with fecundity ($R^2=0.94$) showed high positive co-relationships; whereas, moderate positive co-relationship was observed between Fecundity and body weight ($R^2=0.86$) and fecundity with total length ($R^2=0.67$).

In the present study the range of fecundity of *N. atherinoides* varied from 1100-8900 for a corresponding length and weight 6.5-15.5 cm and 5-12g. The average number of eggs of *N. atherinoides* 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).

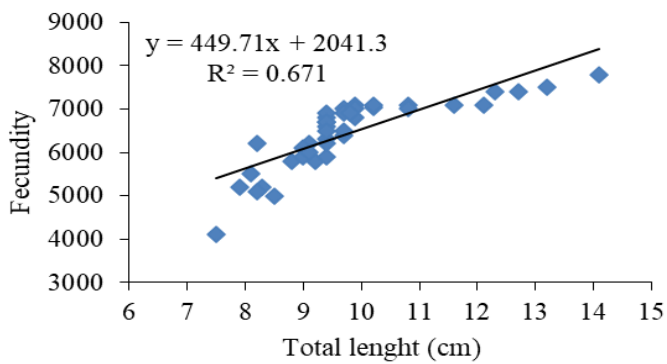


Figure 4. Relationship between fecundity and total length of *N. atherinoides*.

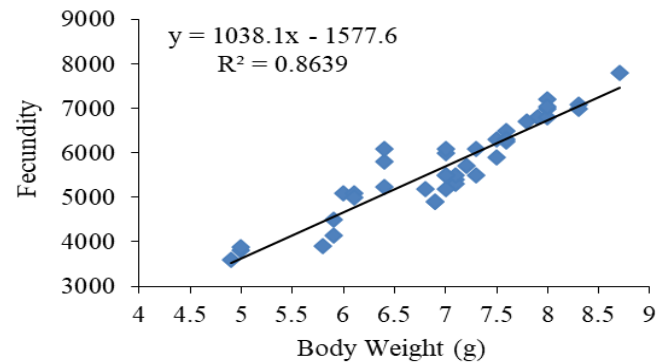


Figure 5. Relationship between fecundity and body weight of *N. atherinoides*.

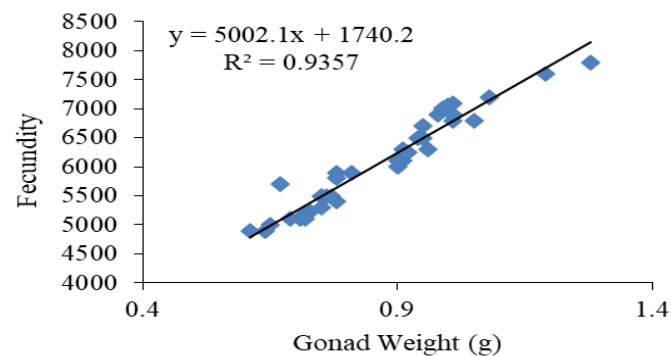


Figure 6. Relationship between fecundity and Gonad weight of *N. atherinoides*.

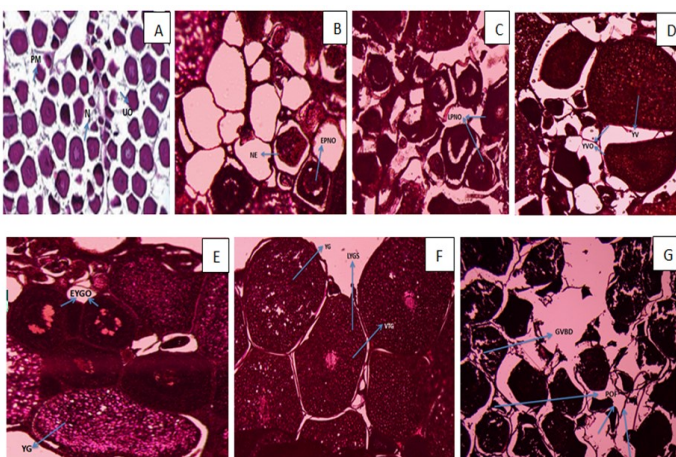


Figure 7. (A) Chromatin nucleolar stage (N= Nucleoli; UO= Undeveloped oocyte; PM= Premature oocyte), (B) Early Perinucleolar stage (NE= Nucleolus; EPNO= Early perinucleolar oocyte), (C) Late perinucleolar stage (LPNO= Late perinucleolar oocyte), (D) Yolk vesicle stage (YVO= Yolk vesicle oocyte; YV= Yolk vesicle), (E) Early yolk granular stage (EYGO= Early yolk granule stage oocyte; YG= Yolk granule), (F) Late yolk granular stage (LYGO= Late yolk granule stage oocyte; VTG= Vitellogenin stage), (G) SPENT (GVB= Germinal vesicle breakdown; POF= Post ovulatory follicle).

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 *N. atherinoides*, the gonadosomatic index was maximum during July 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 ($F = 449.71TL + 2041.30$) representing the relationship between fecundity and total length was found as linear and the value of $R^2 = 0.67$, which is moderately significant (Figure 4). 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 ($R^2 = 0.86$) and found to be linear ($F = 1038.1BW - 1577.60$) (Figure 5). 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 ($R^2 = 0.94$) and the equation was $F = 5002.1GW + 1740.20$ (Figure 6). 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.

Histological observation

Histological observation of the ovaries revealed different maturity stages of the fish. The oocyte development observed throughout this study period and 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 *N. atherinoides* showed group synchronous oocyte development. Seven different oocyte stages were observed in ovaries: (A) Chromatin nucleolar stage (Figure 7A), (B) Early perinucleolar stage (Figure 7B), (C) Late perinucleolar stage (Figure 7C), (D) Yolk vesicle stage (Figure 7D), (E) Early yolk granular stage (Figure 7E), (F) Late yolk granular stage (Figure 7F) and (G) Spent stage (Figure 7G). 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 *N. atherinoides* (phase after spawning).

Developmental stages of *N. atherinoides* oocyte with histological characteristics

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 (Figure 7A).

Early peri nucleolar stage: Oocyte's nucleus begins to expand, nuclear and cytoplasmic volume are increase, nucleoli of varying sizes are found around the periphery of the nucleus. This stage was observed between February and mid-March (Figure 7B).

Late peri nucleolar stage: Oocyte is surrounded by follicular layer and large number of nucleoli appeared throughout the nucleus. Cytoplasmic volume increased and vitelline envelope formation began at this stage. This stage was observed in late-March and partially in April (Figure 7C).

Yolk vesicle stage: Oocyte contained small yolk vesicles and these yolk vesicles appeared firstly around the cytoplasm and then gradually spread more into middle of cell, nucleolus appeared around nucleus or inside nucleus. This stage was predominant from mid-April to May (Figure 7D).

Early yolk granule stage: Oocyte contained a numbers of yolk granules stained in light pink color, yolk granules appear firstly around the cytoplasm and then gradually spread more into middle of cell. This stage signaled the end of oocyte maturation. Some of these oocytes were observed in May, while the others were detected in June (Figure 7E).

Late yolk granule stage: 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 the late June, July and August when the oocytes were fully matured (Figure 7F).

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 observed in September to November (Figure 7G).

In the present study, the gonadal maturity stages of *N. atherinoides* 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.

Based on the oocyte prevalence percentage, ovarian developmental phases were classified as immature (Stage 1), maturing (Stage 2), mature (Stage 3), and spent (Stage 4). The immature stage oocytes were found in February and March where the average oocyte diameter was found to be 0.08 mm (Table 3). A rapid development of oocytes (oocyte diameter 0.21 ± 0.03 mm) with the shifting towards maturing stage was observed between April and May where the average oocyte diameter was found to be 0.21 mm (Table 3). There was a significant mature stage oocyte observed in June, July and August with average oocyte diameter of 0.50 mm (Table 3). The immature stage oocytes were 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 spent stage of 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, indicating that the spawning season of *N. atherinoides* extends from May to August with the major peak in July.

Table 3. Developmental stages of ovary with ova diameter of *N. atherinoides* with histological characteristics.

Stages of ovaries	Histological characters	Ova diameter (mm) (Mean±SD)	Months
Immature	Only young females possess this stage. Oogonia and PG follicles are well-organized in the ovigerous lamellae. This ovarian stage contains chromatin threads with no visible eggs, undeveloped oocyte, oogonia and chromatin nucleolar oocytes This stage does not present follicles in vitellogenesis.	0.08±0.02	February & March
Maturing	At this stage, the females are considered entering into the reproductive cycle. Oogonia, primary growth oocytes, cortical alveoli in the oocytes are predominant. This stage also contains early perinucleolar oocytes, late perinucleolar oocytes and oocytes with yolk vesicles in some part of cytoplasm. As maturation progresses, the quantity of vitellogenic oocytes increases.	0.21±0.03	April & May
Mature	This ovarian stage contains oocytes that were filled with yolk granules. Number of vitellogenic oocytes was sharply increasing. Mature and hydrated oocytes were numerous	0.50±0.07	June, July & August
Spent	Abundant post ovulatory follicle (POFs) and germinal vesicle breakdown (GVBD) follicles were observed with a large number of immature oocytes	0.15±0.02	September

Conclusion

This study revealed that *N. atherinoides* 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 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 *N. atherinoides* 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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Conflict of interest

The authors declare no conflict of interest.

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