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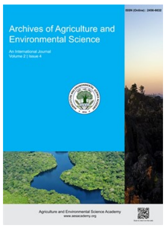

ORIGINAL RESEARCH ARTICLE

CrossMark

Reproductive potential and histological profiling of the wild female anadromous shad (*Tenualosa ilisha*) in lower Meghna Estuary, Bangladesh

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ABSTRACT

Hilsha shad (*Tenualosa ilisha*, Hamilton 1822) is the most commercially important and national fish of Bangladesh, belongs to the family of Clupeidae of the Clupeiformes. The study was conducted to identify the seasonal gonadal cycles through histological study and the peak breeding season combining histological and Gonado Somatic Index (GSI). One hundred and twenty fish samples with an average weight of 756.75±6.25 g was collected from the Tentulia river for the study throughout the year. Monthly mean GSI values of females ranged from 6.36±0.69 to 15.02±1.33. The lowest mean GSI value was found in December and the highest GSI value was in October. From the histological observation of the ovary, early perinucleolar stage, late perinucleolar stage, yolk vesicle stage, yolk granule stage, pre-mature and mature stages were identified. The highest percentage (75%) of mature oocytes and peak breeding season were observed in October and the breeding season continues from October to November. These results will be helpful for fishery managers to impose adequate regulations for sustainable fishery management in Bangladesh.

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INTRODUCTION

Bangladesh is the largest delta in the world, formed by the Ganges, the Brahmaputra, and the Meghna (GBM) river system and their tributaries. This delta's flood plains and coastal mangrove swamps cover nearly a third of the country. Bangladesh has 293 species of freshwater indigenous fishes, 475 species of marine fishes, and 36 species of marine shrimps (Hossain, 2010). Even though Bangladesh has a potential hilsha fishery, this potential has not been fully realized due to a variety of factors, including a lack of effective conservation measures. To implement suitable management strategies for the hilsha fishery, thorough information on population dynamics is required. Anadromous stocks, whose natural home is the lower reaches of estuaries and shoreline areas, ascend rivers during the breeding season and then return to their original habitat after spawning (Panhwar *et al.*, 2011). Any organism's survival on the planet depends on its ability to reproduce. When an organism reproduces within its population, it forms a new individual. Studies in reproductive biology aid in understanding gonadal development, reproductive cycles, and artificial propagation in hatcheries as well as aquaculture. Poor management techniques may stem from a lack of awareness of reproductive physiology. Understanding the biology, population, and management of species, as well as their fisheries, regulation, and determining the environmental suitability of a particular fish in a certain place, requires recording the length and weight of fish (Rahman *et al.*, 2020). The fish stock assessment also requires information on functional length and weight. The level of maturation and the start of the spawning season are determined by the GSI. Because the weight of the ovary grows as it matures, the histology process may be able to detect the stages of maturation and spawning season. The state of the female, her ovaries, and egg size must all be known to calculate the breeding season (Islam and Das, 2006). The calculation of the spawning frequency of a population, which is vital for its management, requires knowledge of gonadal development and the spawning season of a species. Although understanding a fish species' gonadal development is also regarded as a crucial stage for a fish culturist. A species' gonadal maturation and spawning season are determined by histological observations. To understand its breeding biology and season, a histological investigation of gonadal development and measurement of GSI values is required. Its marine range includes Iran and Iraq in the Persian Gulf, as well as the Arabian Sea and the Bay of Bengal on India's west coast (Gomes *et al*., 2004). Reproduction is an ongoing developmental process that necessitates energy, ecological, anatomical, biochemical, and endocrinological adaptations throughout ontogeny (Saha *et al*., 2019). Aquaculture also requires information on fish reproduction. In any commercial species, the availability of good seeds and the ability to manage fish reproduction are limiting considerations (Merson *et al*., 2000). This became a significant component in meeting the need for a year-round supply of table fish and fish seeds (Cakici and Ucuncu, 2007). These prerequisites can be addressed with adequate information on reproductive factors and developmental biology (Kunz, 2004). Fish gonadal histological alterations are a useful tool for determining the reproductive period, which includes developmental phases (Unver and Saraydn, 2012). However, considering these facts the goal of the present study was to learn more about gonadal developments in female *T. ilisha* during the natural reproductive cycle.

MATERIALS AND METHODS

Experimental site

Hilsha-shad samples were collected from the fish landing center in Barisal town. An instantaneous participatory appraisal of oral interview with ten hilsha fishers selling their catch in the landing center concluded that almost all hilsha being landed in the center usually are caught from Tentulia river in the lower Meghna estuary passing between Barisal and Bhola districts.

Sampling of fish

Hilsha is caught in the river during the night using gill nets primarily. Samples were bought once a month over a calendar year from January to December 2015. About 120 fishes covering various size groups were ensured at each event. All specimens were preserved with crushed ice in cool fish boxes and brought to the laboratory as soon as possible.

Gonado-somatic index (GSI)

The reproductive cycle of a species for the year-round fortnightly or monthly intervals may be determined by the gonado-somatic index (GSI). It is a very useful method to indicate the spawning season of a species at the field level. GSI assumes that gonad increases in size with increasing development compared with the weight of the gonad (GW) to the total weight of the animal (BW). The gonado-somatic index of each fish was calculated by the using following formula as described:

$$GSI = (GW/BW) \times 100$$

Histological assay

The histological examination is an important method for predicting the fish's gonadal maturity. Knowing the spawning season and the numerous gonadal phases of fish is also beneficial. A total of 120 gonad samples were subjected to histopathological analysis. The histological profiling was carried out using the Shihab *et al.* (2017) approach. The tissues were cleansed and chopped into smaller pieces after being washed. The tissues were divided into three sub-samples: anterior, posterior, and middle. The sample tissues were then labeled and put into a histology cassette. Tissue cassettes were cleaned in tap water for 6-8 hours before being loaded into an automatic tissue processor machine, where they were processed via several grades of isopropyl alcohol, xylene, and lastly embedded in paraffin wax. For sectioning, the implanted tissues were molded into blocks. Transverse slices of 3-5 m were obtained using a semiautomatic rotary microtome after the blocks were cut. Hematoxylin and eosin were used to stain the sections, which were placed on slides. A compound microscope was used to examine the created slides. Hilsa shad maturity stages were confirmed using histological sections.

Stages of oocytes

The stages of oocytes are described below.

Early perinucleolar stage (EPN): Concomitant with oocyte growth, the nucleus increases in size, and multiple nucleoli become located around the periphery of the nucleus. In the early perinucleolar stage (EPNO) oocytes were the most immature type. Cytoplasm at this stage stained deeply with haematoxylin while nucleus was more or less clear except the nucleoli.

Late perinucleolar stage (LPN): The late perinucleolar stage can be distinguished from the previous stage by the enlargement of the oocyte. During this period (diplotene stage of meiosis), lamp brush chromosomes are formed which disappear immediately before the breakdown of germinal vesicles during oocyte maturation. The cytoplasm tended to lose affinity for haematoxylin.

Yolk vesicle stage (YV): Oocytes became bigger and stained with haematoxylin. White yolk vesicles started to appear initially in the outer part of the cytoplasm, but gradually increased in size and number situated randomly in the cytoplasm. The nucleoli were usually present at the periphery of the nucleus, but in some cases, they also appeared elsewhere in the nucleus.

Yolk granule stage (YG): Small and spherical yolk globules began to appear among yolk vesicles in the peripheral region of the cytoplasm. Yolk globule occupied more than half of the cytoplasm. Yolk globules increased in size and oil droplets appeared within the cytoplasm.

Pre-mature stage (PM): After the nucleus migration, the nuclear membrane broke down. Yolk globules coalesced and no nucleus was observed, although the follicle layer was still visible.

Mature stage (M): Complete vision of yolk Globules was seen together with an overall increase in oocyte translucency.
Besides, GVB (Germinal Vesicle Breakdown) occurred when female hilsha partially spent.

Statistical analysis

All data are presented as (means ± SE). Data were checked for normality by Kolmogorov– Smirnoff test and analyzed for statistical differences within months by one-way ANOVA. The graphical presentation was done with the help of 'Microsoft Office 2010' computer-based software.

RESULTS AND DISCUSSION

Length and weight

The total length of female hilsha was measured from January to December 2015. Different sizes of females were considered for the present study. The highest total length was found in July and the lowest in January (Figure 1). In the present experiment, different size group female hilsha were sampled and their weight was ranged from 420.00g to 1472.00 g (Figure 2). The highest body weight was observed in September and the lowest in January.

Determination of spawning season

Gonado-somatic index: To detect the size of maturity, subsequent spawning, and the period of peak spawning activity, one of the approaches is to calculate the Gonado-Somatic Index (GSI) and observe throughout the year. In the present study, the GSI value for female hilsha was found to vary from 6.36±0.69 to 15.02±1.33. The specimen that was collected in December showed the lowest mean GSI (6.36±0.69) and the highest mean GSI value (15.02±1.33) was found in October. It is important that higher GSI was also found in September (11.27±2.32) and November (13.14±2.39). Therefore, we can primarily consider October as the peak spawning season of *T. ilisha* (Table 1 and Figure 3) in the Tentulia river in the lower Meghna estuary. Monthly variations in the Gonadosomatic index (GSI) were quite apparent (Roomiani *et al.*, 2014). Gonadosomatic index (GSI) values of *T. ilisha* from River Indus were recorded for six months during summer from April to September and in winter from October to March (Narejo *et al.*, 2008). The determination of spawning and frequency of reproduction within the season and the life-cycle of the fish are prerequisites in assessing the reproductive potential of a population (Jhingran and Verma, 1972). The gonadosomatic index increases with the maturation of fish, being maximum during the period of peak maturity and declining abruptly thereafter, when fish become spent (Le Cren, 1951). The monthly changes in GSI reflect the ovarian activity of fish and it must be noticed that GSI is a reliable tool to determine peak spawning season (Vladimir, 2000). The mean GSI values of female *T. ilisha* ranged from (6.36±0.69 to15.02±1.33) with the highest 15.02 in October. The highest GSI value in October illustrated that the peak breeding season of hilsha shad in the Tentulia rivers in October, and this result agreed with a few previous studies. Hossain (1985) studied the GSI of female hilsha and showed the highest peak in October in the case of hilsha in the Meghna river and August in the case of Marine hilsha. Ahmed *et al.* (2008) also observed the peak GSI value of females in riverine and estuarine environments in October. Halder (2004) determined the peak breeding period of hilsha is placed during the full moon in October, which agrees with the present findings. Females having ovaries at mature stages were considered those spawning or near to spawning. The occurrence of mature ovaries examined by external characteristics began in January and continued up to December. Percent (%) occurrence of mature ovaries started to increase in February, peaked in October (75%), and decreased in December, January, and April. Examination of external characteristics of ovaries suggested

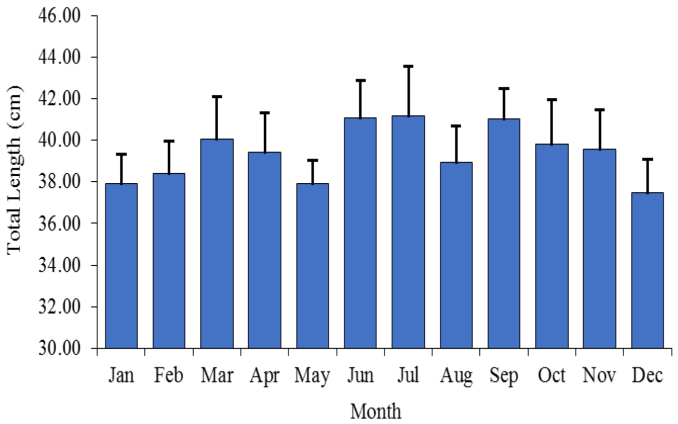


Figure 1. *Month-wise observed total length of female hilsha collected from Tentulia river in the lower Meghna estuary during January 2015 to December 2015.*

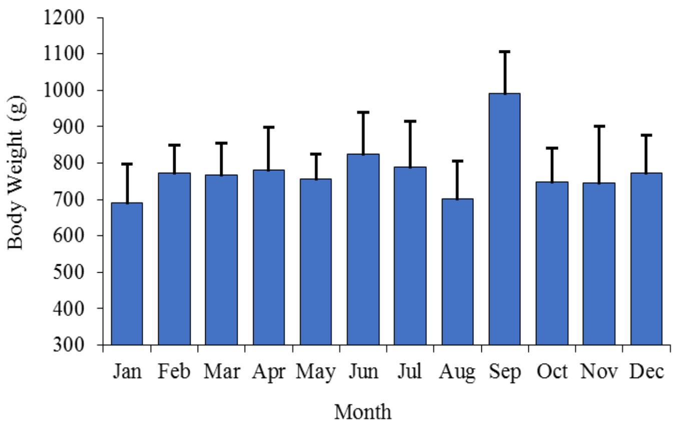


Figure 2. *Month-wise body weight of female hilsha collected from Tentulia river in the Meghna estuary from January 2015 to December 2015.*

Table 1. Month-wise gonadosomatic index of female *T. ilisha.*

Month	No. of sampled fish	GSI range	Mean GSI (± SE)
January	10	4.44-12.04	7.52±1.68
February	10	4.27-12.20	8.27±1.61
March	10	4.22-15.05	8.52±1.91
April	10	5.68-12.78	6.92±1.29
May	10	4.96-12.17	8.44±1.43
June	10	4.23-11.32	8.47±1.32
July	10	6.43-20.66	11.27±2.32
August	10	6.55-11.32	8.88±0.98
September	10	4.66-15.98	9.73±2.03
October	10	9.98-17.64	15.02±1.33
November	10	6.74-18.01	13.14±2.39
December	10	5.25-8.30	6.36±0.69

Table 2. Percent (%) occurrence of the oocyte stages of ovarian development found in the histological study of hilsha ovary during the study period January to December, 2015.

Month	EPNO	LPNO	YV	YG	PM	M	GVB
January	5	5	20	20	15	35	-
February	10	10	25	-	10	45	-
March	10	15	-	20	15	40	seen
April	20	20	20	10	15	15	-
May	-	-	15	30	20	35	-
June	15	15	10	10	20	30	-
July	5	5	10	-	20	60	seen
August	10	15	15	-	20	40	-
September	-	10	-	20	20	50	Seen
October	5	5	-	10	5	75	-
November	-	5	-	10	25	60	-
December	30	40	-	-	20	10	Seen

Here, M = Mature, PM = Pre-mature, YG = Yolk granule, YV = Yolk vesicle, LPNO = Late perinuclear oocyte, EPNO = Early perinuclear oocyte, GVB = Germinal Vesicle Breakdown.

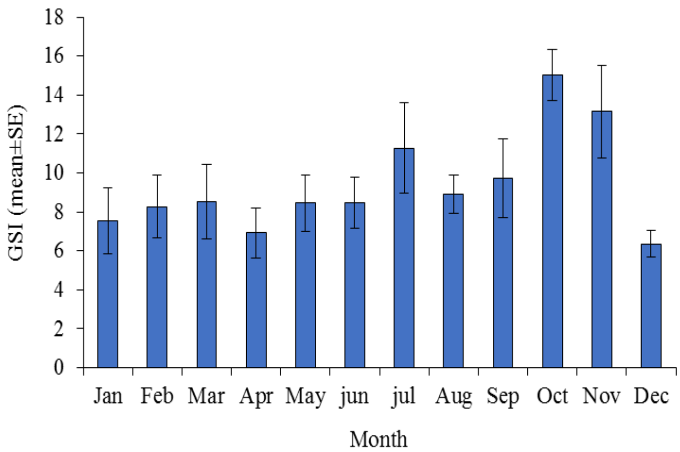


Figure 3. *Month-wise observed Gonado-somatic Index (GSI) of female hilsha collected from Tentulia river in the Meghna estuary during January 2015 to December 2015.*

that *T. ilisha* spawn throughout the year but major spawning takes place in October –November and peaked in October. Pillay and Rosa (1963) studied the maturation and spawning of the hilsha, *Hilsa ilisha* (Hamilton) of the Saurashtra coast and have observed five maturity stages (maturing, mature, partially spent, and spent).

Histological study of oocytes: Ovarian development is subdivided into distinct developmental stages according to physiological, biochemical, morphological, and histological criteria. Maturity stages were determined following the key outline as described by Matsuyama and Matsuyama (1982). Different stages found in the present ovarian histological study are presented in Table 2 and Figure 4.

From table 2, In January, 5% of the specimens had early perinuclear oocyte, 5% in the late perinuclear oocyte, 20% in yolk vesicle, 20% in yolk granule, 15% in pre-mature stage, and 35% in the mature stage. In February, no yolk granule stages were found and 10% in the early perinuclear oocyte, 10% in the late perinuclear oocyte, 25% yolk vesicle stage, and 10% in pre-mature, and 45% in mature stages were observed. In March, 10% of female oocytes were in the early perinuclear oocyte, 15% in the late perinuclear oocyte, 20% in yolk granule stages, 15% in the premature stage, 40% in mature stages, and no yolk vesicle stages were found. In April, no late perinuclear stages were found, 10% in the early perinuclear oocyte, 20% yolk vesicle stage, 10% in the yolk granule, 15% pre-mature stage, and 35% in mature stages were found. In May, 15% of the specimens had a yolk vesicle stage, 30% in yolk granule, 20% in pre-mature, and the 35% in the mature stage, early perinuclear oocytes, and late perinuclear oocytes were not found in this month. In June, 30% of female oocytes were in the mature stage, 20% in pre-mature, 10% in yolk granule, and 10% in yolk vesicle, 15% in early perinuclear oocyte and15% in the late perinuclear oocyte. In July, 5% in the early perinuclear oocyte, 5% in the late perinuclear oocyte, 10% in yolk vesicle stage, 20% in pre-mature, and 60% in mature stages were found and no yolk granule stages were found. In August, 10% early perinuclear

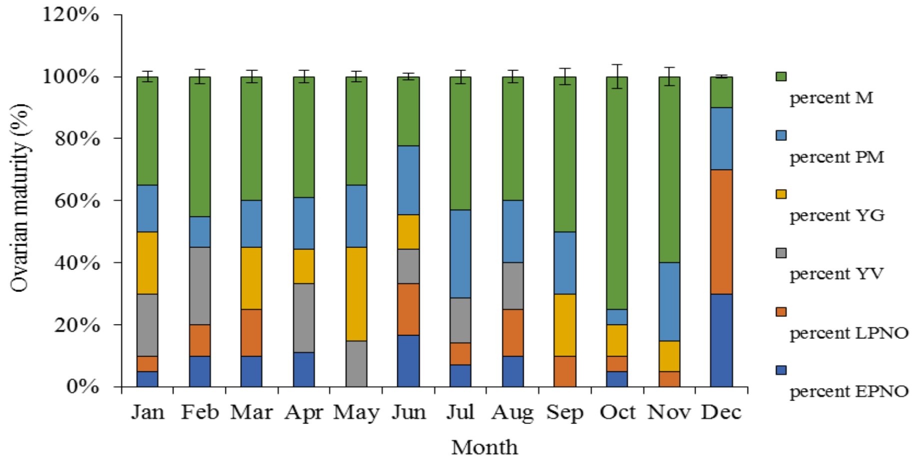
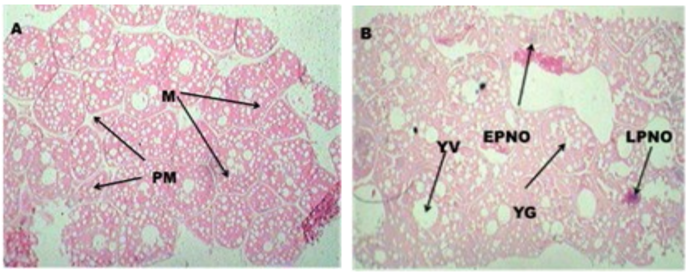


Figure 4. *Month-wise observed frequency occurrence of ovarian maturity of female hilsha collected from Tentulia river in the Meghna estuary during January 2015 to December 2015 [Here, M = Mature, PM = Pre-mature, YG = Yolk granule, YV = Yolk vesicle, LPNO = Late perinuclear oocyte, EPNO = Early perinuclear oocyte].*

oocyte, 15% in the late perinuclear oocyte, yolk vesicle stages were 15%, 20% pre-mature stage, 40% in mature stages were observed and no yolk granular stages were found. In September, no yolk vesicle and early perinuclear oocyte, 10% in the late perinuclear oocyte, 20% in the yolk granular stage, 20% in pre-mature stages, and 50% in mature stages were found. In October, the highest percentage (75%) comprised of mature stages and 5% in pre-mature, 10% in yolk granule, 5% in the late perinuclear oocyte, and 5% in the early perinuclear oocyte. In November, on early perinuclear oocyte and yolk vesicle stage, 5%late perinuclear oocyte, 25% pre-mature, and 60% mature stage. In December, of the specimens with no yolk granule and yolk vesicle stage, 30% in the early perinuclear oocyte, 40% in the late perinuclear oocyte, 20% pre-mature, and 10% mature stage were observed. Yolk vesicle stages were found throughout the year except for March, September, October, and November, December. Yolk granule stages were also found throughout the year except for February, July, August, December. Early perinuclear oocytes were also observed throughout the year except, May, September, November. Late perinuclear oocytes were observed throughout the year except for April and May. Pre-mature and mature oocytes appeared throughout the year during the study period. The advanced oocyte i.e., the mature oocyte was found in the very month of the study period. The occurrence of the mature oocyte in every month of the study period confirmed that *T. ilisha* spawns precisely throughout the year, but major spawning takes place in October (Figure 5).

In this study, oocytes developmental stages were classified into the early perinuclear oocyte, late perinuclear oocyte, yolk vesicle, yolk granule, pre-mature, and Mature stages. The mature stage was observed as the advanced stage in the study. The advanced mature stage indicated that spawning or near to spawn time. Advanced mature stages of oocytes were found in each month during the study period, January to December 2015. Panhwar *et al.* (2011) studied the reproductive pattern and some biological features of anadromous fish *T. ilisha* from Pakistan. They identified five stages of oogenesis, nearly ripe, fully developed, running ripe, partially spent, and spent. Flura *et al.* (2015) stated that large GSI values were observed in the months of October and November in almost all cases. Cheng *et al.* (2005) studied with *Leiognathus equulus* and they found eight maturity stages of oocytes as chromatin nucleus, perinucleus yolk vesicle, primary yolk, secondary yolk, tertiary yolk, migratory nucleus, and ripe stage by histological examination of ovaries. Akter (2011) studied on *Pangasius pangasius*, found that the highest GSI of female *P. pangasius* was 9.52 in July and the lowest was 0.59 in September. GSI in females increases from May to July then decreased gradually. The highest female GSI value in July is indicative of the peak breeding season of *P. pangasius*. Again, the lowest GSI value in September indicates the spent status of the female ovary. High GSI value during June to August indicates the spawning season of this fish in the Chandpur region around the Meghna River. In the study of Maya (2011), it was found that the highest mean GSI for male *Mystus cavasius* was 3.41 in early July and the lowest was 0.05 in late September and for females, it was 24.54 in late July and the lowest was 0.19 in October. The highest GSI value of testis in early July and that of the ovary in late July indicates that male matures earlier than female. Narejo (2003) studied on *Monopterus cuchia*, observed that the highest value of GSI was observed in May which was 6.002±1.672 and the lowest value was observed in September which was 0.232±0.015. The highest GSI value in May is indicative of the fact that mud eel may, have peak breeding season in May. Again, in the study on *Ompok pabo* from the Sylhet basin, Alam (2009) recorded that the highest GSI for males was 1.006±0.326 in June and the lowest was 0.614±0.053 in April and for females, it was highest in June 14.45±2.37 and lowest in April 10.51±0.82. These findings are partially different from the findings of the present study. In the fish ovary, the mature stages are generally observed at the most advanced stage of the ovary when the fishes are ready for spawning and when mean GSI values are comparatively high. In this study, advanced mature oocytes were found every month during the study period. The histological study of oocyte development, examination of external characteristics of ovaries, and GSI values suggested that *T. ilisha* spawn throughout the year but major spawning takes place in October–November and peaked in October.



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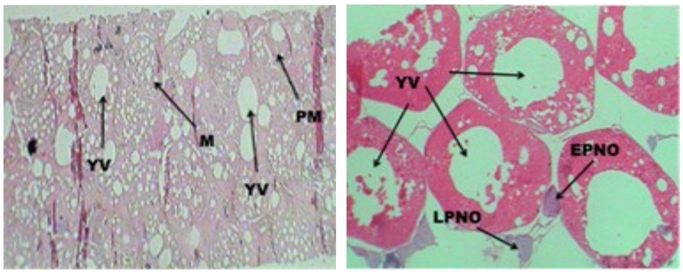

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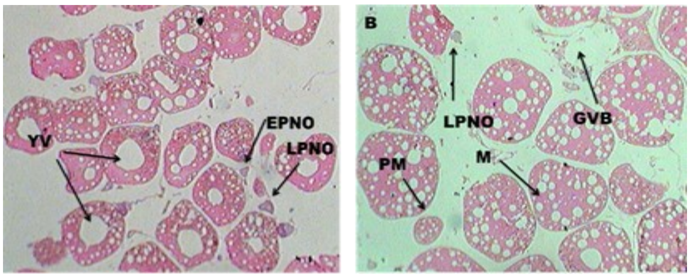

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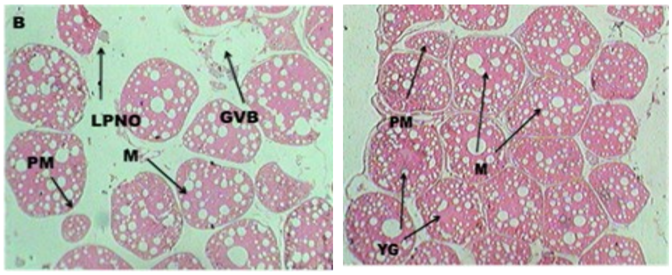

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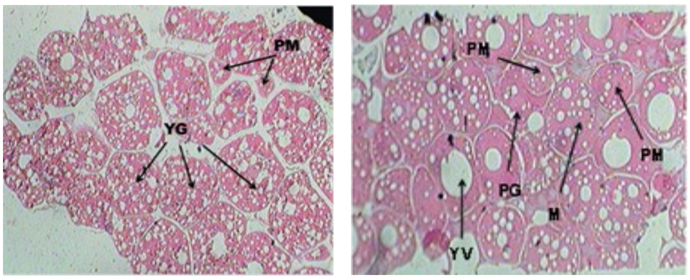

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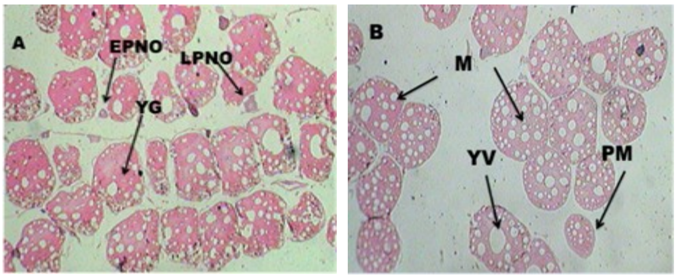

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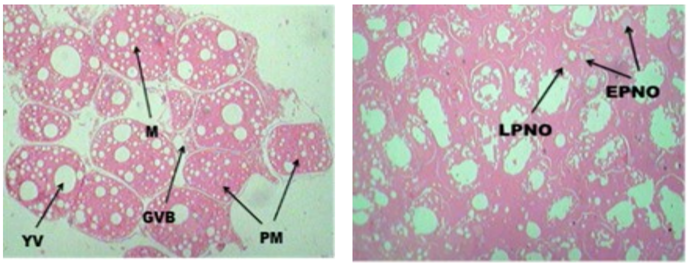

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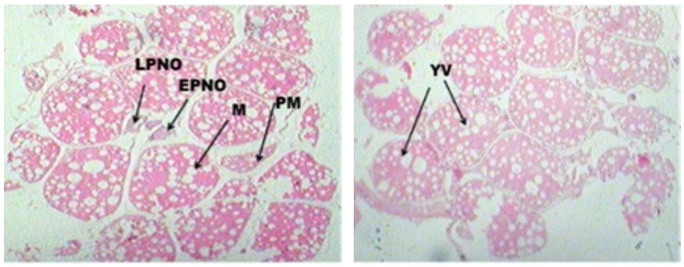

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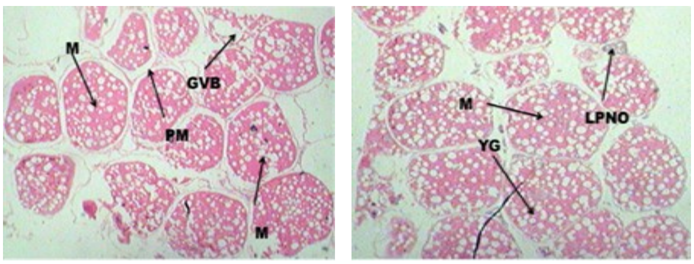

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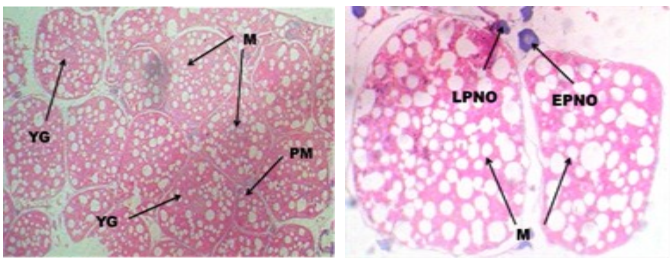

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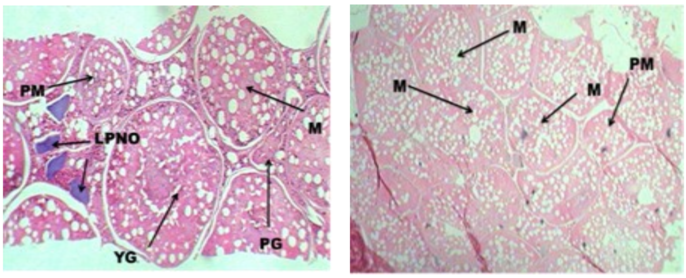

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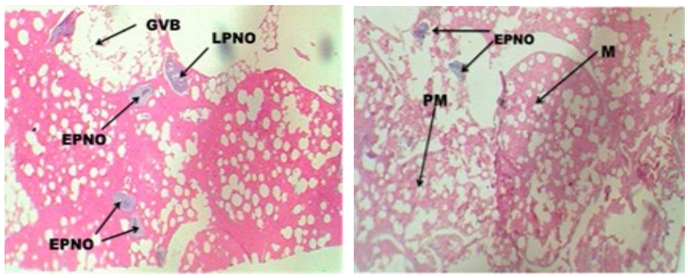

December

Conclusion

It can be concluded that data on reproductive physiology can provide important and basic information on the gonadal maturity, breeding potential and breeding season of a species which are important for fisheries management. This study will be helpful for fishery managers to impose adequate regulations for sustainable fishery management in the Meghna river as well as other rivers of Bangladesh.

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Competing interests

Authors have declared that no competing interests exist.

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