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Seasonal Variation of Reproductive Indices *Tenualosa Ilisha* (Hamilton, 1822) In Relation to Environmental Factors in The Hooghly Estuary (Bay of Bengal), West Bengal, India

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ABSTRACT

This study intended to examine the reproductive indices of Hilsa shad, *Tenualosa ilisha*, a commercially important anadromous fish, using reproductive parameters and the macroscopic structure of gonads. Fish samples were collected from the Hooghly River since March, 2021 to February, 2022 from the local sites. Seasonal variations of GSI, Dobriyal index and ova diameter indicated that Hilsa shad had prolonged spawning season with two peaks, major one in August–September (Monsoon) and a subsidiary peak in February-March (Pre-monsoon). Regression analysis indicated that mean absolute fecundity was strongly associated with ovary weight ($r2 = 0.99$), ovary length ($r2 = 0.79$) and total body weight ($r2 = 0.45$). Data revealed that spawning season was strongly associated with mean seasonal air temperature (°C) and rainfall (mm) ($p < 0.05$). The finding of this result may become very helpful for artificial breeding, conservation and fishing management of *T. ilisha*.

Introduction

Tenualosa ilisha, an anadromous fish, is mainly found in coastal and estuarine water and has been reported from the Arabian Gulf, Bangladesh, Burma, China, Iran, South Vietnam, and India. It is one of the most economically important and demanded edible fish due to its flavor, taste, and culinary properties in West Bengal and Bangladesh (Alam et al. 2025; De et al. 2019; Ganguly et al. 2018; Mohanty et al. 2011) and represents an important component of the inland resources of the country. Approximately 14% of the total fish catching, hilsa is a major component of the West Bengal marine fishery section (DoF 2019). A significant number of fishermen are dependent on the hilsa fishery in West Bengal. Fluctuation of high yield of hilsa was found in the nineties onwards (Gupta 1993; Mitra et al. 1997). The riverine catch of hilsa has declined due to closure of migration routes by dams/barrages, degradation of habitats, indiscriminate harvesting of broods and juveniles and climatic variability (Dutton et al. 2018; Miah 2015; Sinha et al. 1996). Hilsa ascends in the up-shore areas mainly for reproduction, and the juveniles after attaining about 10 cm start their downward migration. Hilsa spawn throughout the year with a major peak in September–October, followed by a minor peak in February–March (Haroon 1998). On the other hand, Pillay (1958) opined that the same fish does not breed twice in a year. Most of the time, external morphological features make it difficult to distinguish male and female hilsa. Reproductive studies on teleost fishes necessitate an understanding of the stage of gonad development. One of the most important aspects in the

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field of fishery biology is to identify the annual reproductive cycle of fishes (Unver and Saraydin 2004). The methods used for evaluating gonad development stages in fish include measurements of determination of gonadal indices, fecundity, oocyte size, histological examination, and external appearance of testis, ovaries, and oocytes. Histology is regarded as the most precise technique and is a powerful tool for the detection of fish maturity stages though it is time-consuming and expensive. Gonadal weight and related indices give the idea of the fish reproductive biology. The Gonado somatic index (GSI) is an accurate predictor of hilsa that have spawned or are about to spawn (Devlaming et al. 1982). The overall general suitability of macroscopic staging for adult versus immature fish was validated by the histological investigation. Macroscopic observation and GSI in combination may be useful to determine maturity conditions for some maturity stages; however, histological examination of the gonad is the most accurate method for all stages (Assem 2000; Honji et al. 2006).

Due to the high importance of hilsa in fisheries, many studies have been conducted regarding various aspects of biology but unfortunately, information regarding the reproductive biology of hilsa is very scared particularly in the present study area. Many authors have studied the biology and reproduction of hilsa shad in Bangladesh (Majid et al. 2007; Amin et al. 2008; Hossain 1985). In light of the foregoing, the current work has been undertaken with the following goals, macroscopic identification of the gonadal development in male and female hilsa, and histological assessment during the pre-monsoon, monsoon, and post-monsoon periods.

Materials and methods

Sampling

The entire work of the present study was conducted for one year, i.e., March 2021 to February 2022. Hooghly River is known to be one of the most important offshoots of the river Ganga in India. Three sampling sites along the Hooghly River were chosen for the study- Barrackpore (latitude 220 76' 74" N and longitude 880 22' 84" E), Bagbazar (latitude 220 12' 00" N and longitude 880 48' 00" E), and Raichak (latitude 220 12' 41" N and longitude 880 07' 39" E) (Fig. 1). Samples were collected from March 2021 to February 2022 from the local fishermen in pre-monsoon (February-May), monsoon (June-September), and post-monsoon (October–January) season. Pooled data from the three sampling stations were used for statistical analyses.

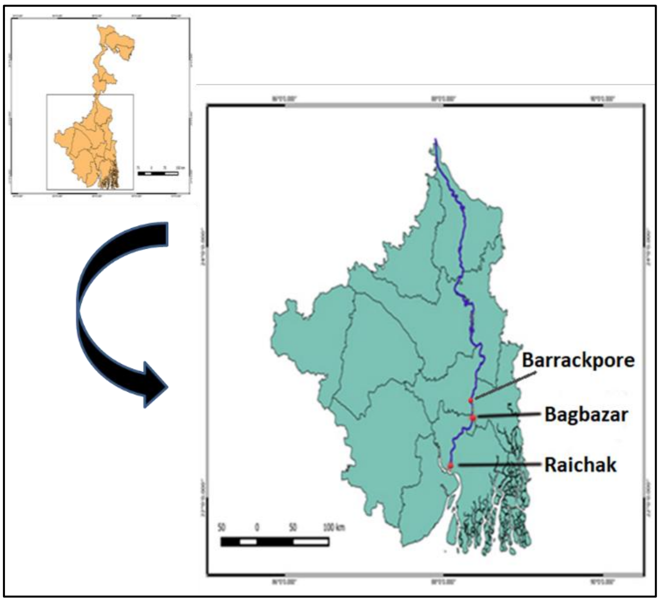

Fig. 1. The sampling Sites (Barrackpore, Bagbazar and Raichak) of *T. ilisha* along the Hooghly River during the study period.

Morphological studies

Standard morphological measurements of the fish, including total length (TL) and gonad length, were recorded to the nearest 0.1 mm using a digital Vernier caliper. In addition, total body weight and gonad weights were recorded to the nearest 0.01 g with using an electronic balance. Each fish was sampled for fork length (nearest cm), body weight (g), gonad weight (nearest g), and maturity stage based on macroscopic visual observation with criteria of degree of opacity, consistency and vascularisation, oocytes or sperm visibility and overall coloration of the gonads (Lagler 1978; Shinkafi et al. 2011).

Reproductive biology

Gonado somatic index was calculated as follows (Roff 1983)-

$$GSI=\frac{GW(g).100}{BW.(g)}$$

Here, GW- gonad weight, BW- total body weight.

Dobriyal index was calculated by using the following formula (Dobriyal et al. 1999)-

$$DI=\sqrt[3]{GW}$$

Fecundity was estimated by Gravimetric method using the formula (Islam and Talbot 1968)-

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

Where, F - fecundity, n -number of ova in subsamples, G - total weight of ovary, and g - weight of sub-sample.

Gonadal histology

After sacrificing the fish, both ovaries and testes were dissected out, and the mid part of the gonad was fixed in alcoholic Bouin's solution, dehydrated in graded ethanol solution, and embedded in paraffin. Embedded tissue samples were sectioned to a thickness of 0.5 µm with the help of a Rotary microtome (Weswox). Sections were stretched on albumenized glass slides and stained with Haematoxylin and Eosin and observed with a compound microscope (Leica, DM 2500, 11888139). Different stages of the gonad were identified according to the standard literature (Gentek et al. 2009).

Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test has been done to compare among and between the acceptable difference dobriyal index, and ova diameter. The relationship of fecundity and reproductive morphometries (total length, body weight, ovary length, and ovary weight) were calculated using the linear regression analysis. Spearmen's correlation was used to understand the association of environmental parameters (rainfall and temperature) and spawning season. Statistical analyses were completed by using GraphPad, OriginPro and a *P* value of 0.05 or less was considered statistically significant.

Results

Length-weight and gonadosomatic index

In the present study females of *T. ilisha* had the mean total length (TL) 32.28 ± 1.45 cm and mean body weight (BW) 528.66±47.14 g, and were larger than males (28.96 ± 2.24cm TL and 331 ± 92.15 g), with $p<0.05$. There was evidence of predominance of males over females throughout the sampling season. The seasonal changes in the mean GSI values for female and male hilsa are shown in Fig. 2 (a) and (b). The result of ANOVA

showed that mean GSI values of female and male hilsa were significantly different among the season (F-76.23, $p<0.0001$, r^2- 0.96 for female hilsa and F-28.46, $p<0.0009$, r^2- 0.9 for male hilsa). Turkey's multiple comparison test revealed a significant difference in means of gonado somatic index, female GSI between monsoon and post-monsoon season (adjusted $p<0.001$) and between pre-monsoon and monsoon (adjusted $p<0.0007$) in the case of male GSI. The present study

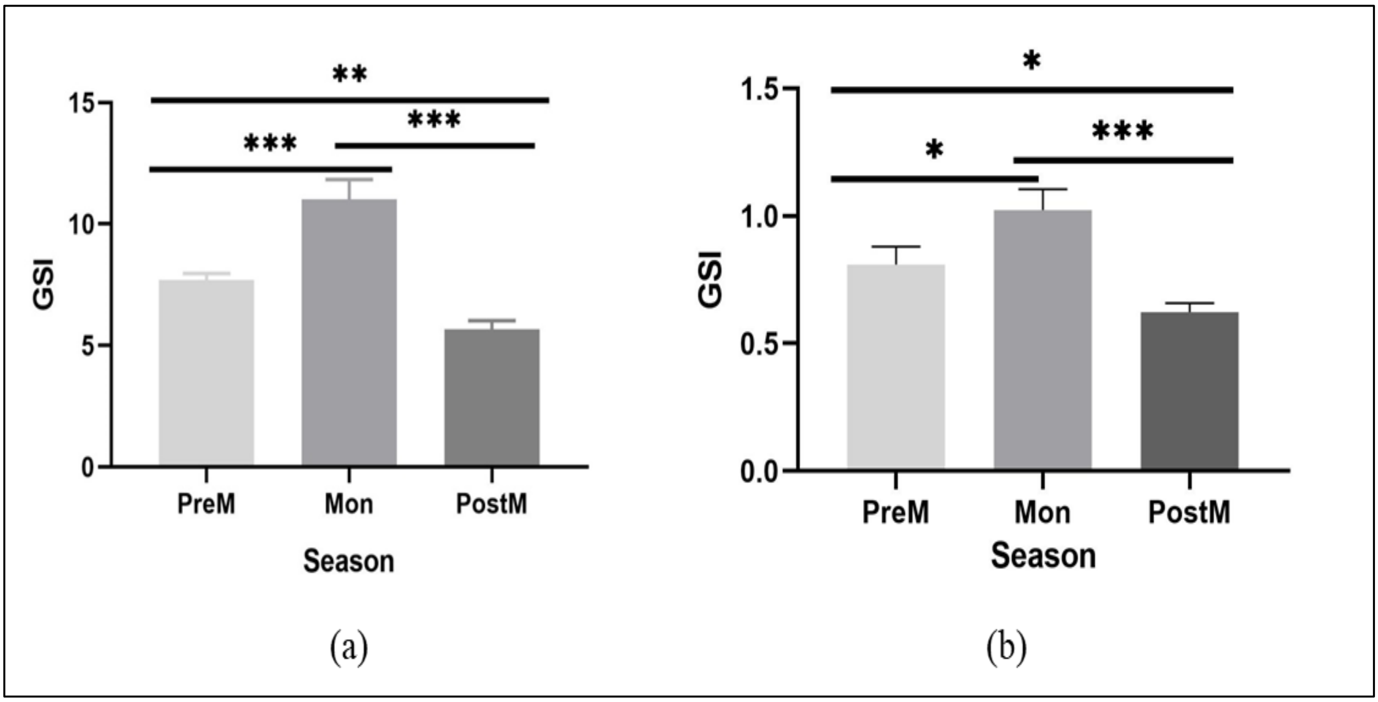

Fig. 2. Seasonal changes of gonadosomatic index (GSI) of *Tenualosa ilisha*. (a) female gonado somatic index and (b) male gonadosomatic index. (Abbreviation: PreM- Premonsoon, Mon- Monsoon and PstM- Postmonsoon).

depicted two distinct peaks of GSI for both the sexes. The first peak was during the pre-monsoon season (February-May) and the second peak was in monsoon (June-September). This result revealed that the spawning season was prolonged from February to September, with distinct spawning peaks at the beginning and the end of the spawning migration.

Dobriyal index

Mean dobriyal index for both male and female hilsa in different seasons are shown in Fig. 3. ANOVA result showed a significant difference in dobriyal index

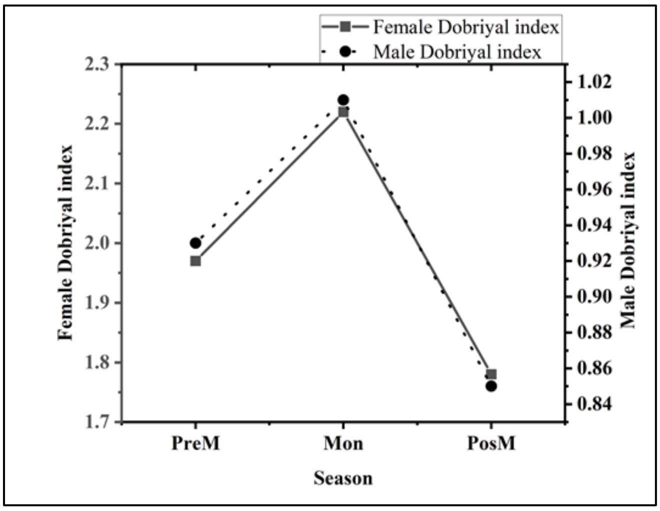

Fig. 3. Mean dobriyal index for both male and female hilsa in different season (Abbreviation- PreM: Premonsoon, Mon: Monsoon, PostM: Postmonsoon)

values among the seasons for both male (F-35.72, p<0.001, r^2-0.92) and female (F-382.9, p<0.001, r^2-0.99) hilsa. According to the result of Turkey's multiple comparison test, there were prominent differences in dobriyal index between monsoon and post-monsoon in the case of male hilsa (adjusted p<0.0004), on the other hand, significant differences were recorded between pre-monsoon and monsoon as well as monsoon and post-monsoon in case of female hilsa (adjusted p<0.0001 and p<0.0001 respectively). Seasonal variation in dobriyal index coincided with the seasonal variations in GSI which pretend prolonged spawning season with two distinct peaks.

Oocyte diameter

Oocyte diameter is a measurement to determine oocyte growth. ANOVA results depict the oocyte diameter was significantly different (F- 25.53, p< 0.001, r^2-0.89) in different sampling seasons (Fig. 4). In the present study, it was noticed that the ova diameter increases from 0.4 to 0.6 mm following the progression of maturity stages. The lowest value (0.3 ± 0.02 mm) was recorded in the post-monsoon season and the highest value (0.06 ± 0.03 mm) was recorded in the monsoon season. Seasonal variation in ova diameter was according to the seasonal variations in

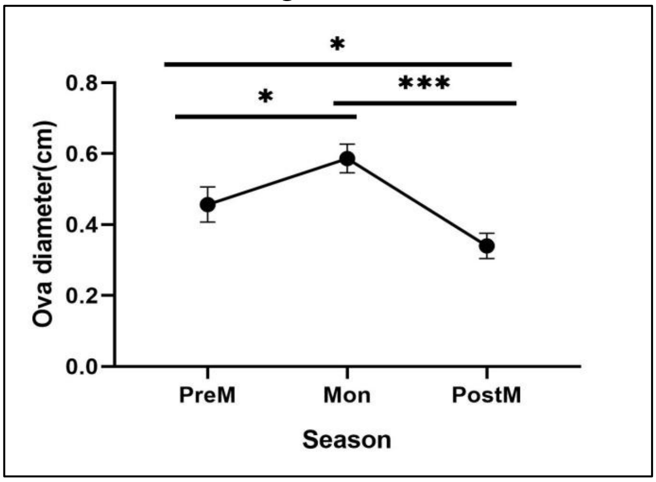

Fig. 4. Seasonal changes of ova diameter (mm) of female Tenualosa ilisha. (PreM- Premonsoon, Mon- Monsoon and PstM- Postmonsoon)

GSI and dobriyal index which indicate prolonged spawning season with two distinct peaks.

Fecundity

Mean absolute fecundity value of hilsa was found to be varied among the different season, maximum 721,512.00 was found in monsoon season, 209723.00 was recorded in pre-monsoon season and 139473.00 the lowest value was found in post-monsoon season. Regression analysis showed that absolute fecundity and body length relationship of *T. ilisha* showed low correlation [r^2 = 0.15, F = 5838 (TL) - 2E + 06] (Fig. 5a), which indicate body length was not correlated to

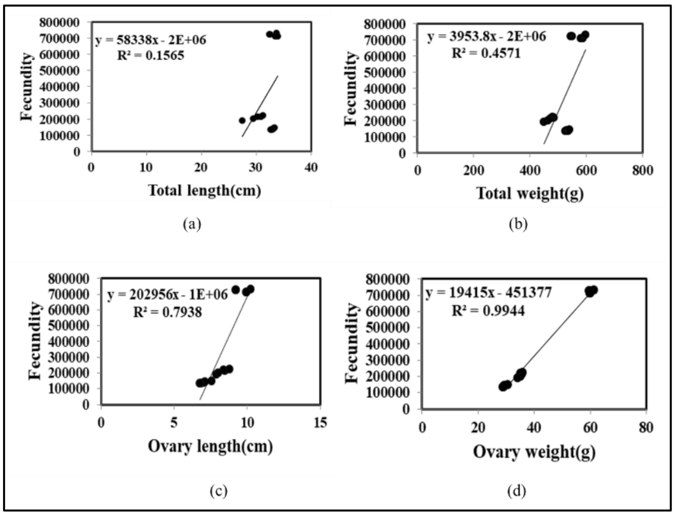

Fig. 5. Relationship of ovary length (cm) and ovary weight (g) (A) and total length(cm) and body weight (g) (B) throughout the season of *Tenualosa ilisha.*

the fecundity. The absolute fecundity was moderately correlated with body weight [r^2 = 0.46, F = 3953.8 (TW) - 2E + 06] (Fig. 5b) while strongly correlated with ovary length [r^2 = 0.79, F = 202956 (OL) - 1E + 06] (Fig. 5c) and ovary weight [r^2 = 0.99, F = 19415 (OW) - 451377] (Fig. 5d).

Macroscopic and microscopic structure of gonads

Gonads of female hilsa were found to be paired, elongated, and located in the dorsal portion of the body cavity as in another teleost. Ovaries were observed a bi-lobed structure and the left ovary was connected with the right one. The right ovary is slightly larger in length than the left. In the present study, the ovary of *T. hilsa* has been classified into four stages of maturity depending on macroscopic observation as shown in Fig. 6 and Table 1.

Table 1. Maturity stages of female and male gonads of *Tenualosa ilisha* of Hooghly River

Gonadal stage	**Macroscopic Examination**
Female gonad	
Immature and Developing (I and II)	Ovary translucent, reddish orange in colour; ovary small, no eggs visible, blood vessels less.
Pre-spawning (II and III)	Right ovary is slightly larger than left ovary; ovary orange coloured; occupies ½ of the ventral cavity; numerous blood vessels; eggs are not yet to be hydrated likely to spawn later the year.
Spawning (IV)	Ovary occupies whole body cavity. Most eggs are translucent from hydration. Ova run with pressure. Thin stretched ovarian wall. Huge blood vessels and capillaries found.
Spent (V)	Ovary empty and flaccid. Thick ovarian wall.
Male gonad	
Immature (I)	Slightly pinkish in colour, faint appearance of blood vessels
Maturing (II) to ripe running (III)	Pinkish in colour, blood vessels found; milt flowing with gentle pressure.
Spent (IV)	Dark brown in colour. Blood vessels obscure; milt was not released/absent with gentle pressure.

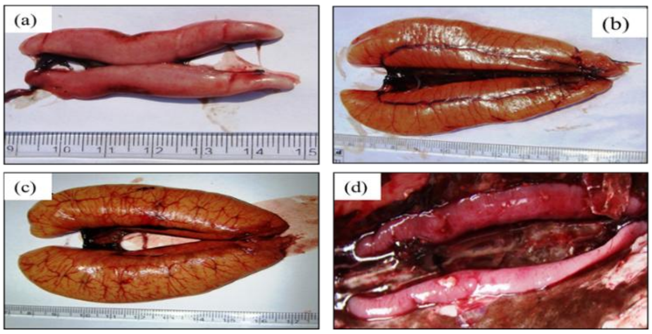

Fig. 6. Macroscopic ovary structure of female hilsa (a) Immature Ovary, (b) Pre-Spawning, (c) Spawning, (d) Spent

The slide of the histological section of the ovary has shown in Fig. 7a-c. The testes were bilobed and the right testis is slightly larger than the left testis lobe. The males which were observed mainly containing immature, maturing, ripe running, and spent stages (Table 1 and Fig. 8). The histological structure of the testis is depicted in Fig. 9a-c.

GSI and environmental parameters

The relationship between male and female GSI and environmental parameters are given in Table 2 and Fig. 10. During the study, from Barrackpore to Raichak average temperature was ranged from 20.50 to 29.18 0C. The highest temperature was recorded from the monsoon season (29.18 ± 0.04 ^{0}C), the lowest was in the post-monsoon season (20.50 ± 0.63 ^{0}C) and 28.5 ± 0.05 ^{0}C was in pre-monsoon season. In monsoon and pre-monsoon GSI of male and female hilsa was also high, which indicates the optimal water temperature was present for spawning. The results indicated that the highest rainfall was observed in

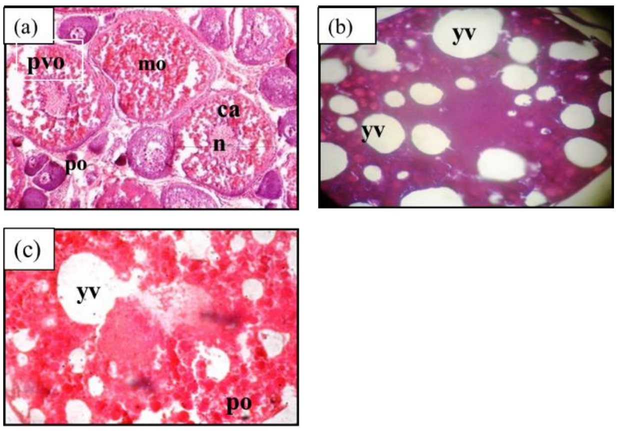

Fig. 7. Transverse sections through ovary of *Tenualosa ilisha* in different maturation stages: (a) Developing stage, (b) Late yolk globule stage, (c) Ripening stage. Abbreviation: po- primary oocyte; pvo- primary vitellogenic oocyte; mo- mature oocyte; ca- cortical alveoli; n- nucleus; yv- yolk vesicle.

monsoon (401.14 ± 16.23 mm), but the lowest was in pre-monsoon (56.05 ± 1.63 mm). To verify the effects of temperature and rainfall on GSI, a non-parametric relationship, Spearman rank correlation test, were used. The results indicated that temperature and rainfall were significantly correlated with the GSI (Table 2).

significance of this type of sex difference might be the cause of minimize the intraspecific competition as well as maximizing egg-production biomass (Clarke 1983).

Gonadosomatic index

Biologists frequently use GSI to indicate the maturity and periodicity of spawning as well as the periodicity of a fish's breeding season. Stages of maturity of

Table 2. Relationship between environmental parameters with GSI of *Tenualosa ilisha* in the Hooghly River

Relationship	r_s value	95% CL of r_s	*p* values	Significance
Female GSI vs rainfall	0.78	0.35 to 0.94	0.004	**
Male GSI vs rainfall	0.77	0.34 to 0.93	0.004	**
Female GSI vs temperature	0.71	0.22 to 0.92	0.011	**
Male GSI vs temperature	0.71	0.21 to 0.91	0.011	**

*GSI gonadosomatic index, rs, Spearman rank correlation values, CL confidence limit, p the level of significance, **Highly significant ($p \leq 0.01$)*

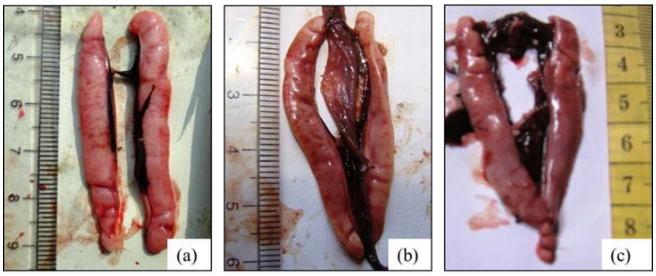

Fig. 8. Macroscopic testis structure of male hilsa. (a) Immature, (b) Maturing, (c) Spent

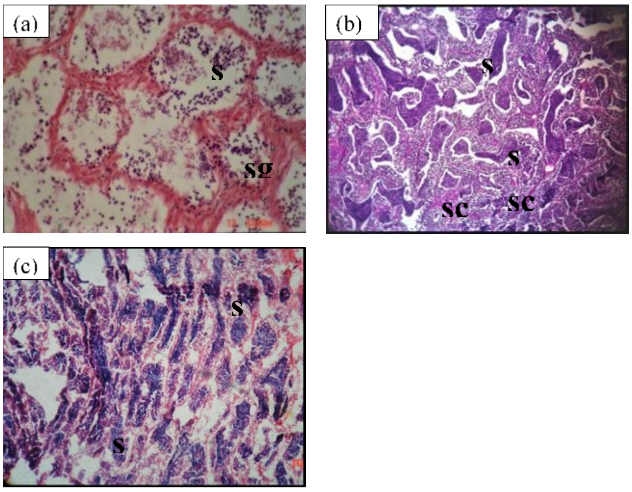

Fig. 9. Transverse sections through testes of *Tenualosa ilisha* in different maturation stages: (a) immature (Stage I), (b) Maturing (Stage II) to Ripe running (Stage III), (c) Spent (Stage IV). Abbreviations: sg - spermatogonia; sc - spermatocytes; sz -spermatazoa. All sections were stained with haematoxylin and eosin.

Discussion

The female hilsa fish were generally with higher body length and weight compared to males. The adaptive

gonads are directly proportional to the GSI values (Nautiyal 2011). Gonado somatic index is an important indicator for determining the ripeness of the gonad as well as development (Nandikeswari et al. 2014). The seasonal variation is used to know the peak of the spawning period (Vladimir 2000). The maturation of the gonad gradually increases with the progressive increase of GSI and reaches its maximum value at the time of the highest degree of maturity. In the current study, male and female hilsa had higher GSI (female-11.02, male-1.02) in August-September (monsoon) followed by female -7.7, male - 0.81in Feb-March (pre-monsoon), whereas, it exhibited lower values in October–January (post-monsoon). The present study observed a prolonged spawning season from February to September, with two peaks at the starting and the end of spawning migration. The spawning activity of hilsa in the Shatt Al Arab River extended from March to November, with two distinct peaks during March–June and August–October (Almukhtar et al. 2016). Spawning of hilsa occurs almost throughout the year, peak of spawning period is September and October with a minor peak in January-February in Bangladesh (Rahman et al. 2012). In the Khuzestan Province Rivers of Iran, the spawning season of *T. ilisha* was reported to occur from May to August (Rahman et al. 2012). Observed differences indifferent reports about the spawning periods of hilsa populations in different areas may be due to genetics and environmental factors.

Dobriyal index

Dobriyal index is used as a consideration factor of spawning season, sexual maturity, and frequency of spawning (Dobriyal et al. 1999). In the calculation, DI does not involve the body weight, rather than it depends on food availability, environmental and physiological stress, and feeding intensity.

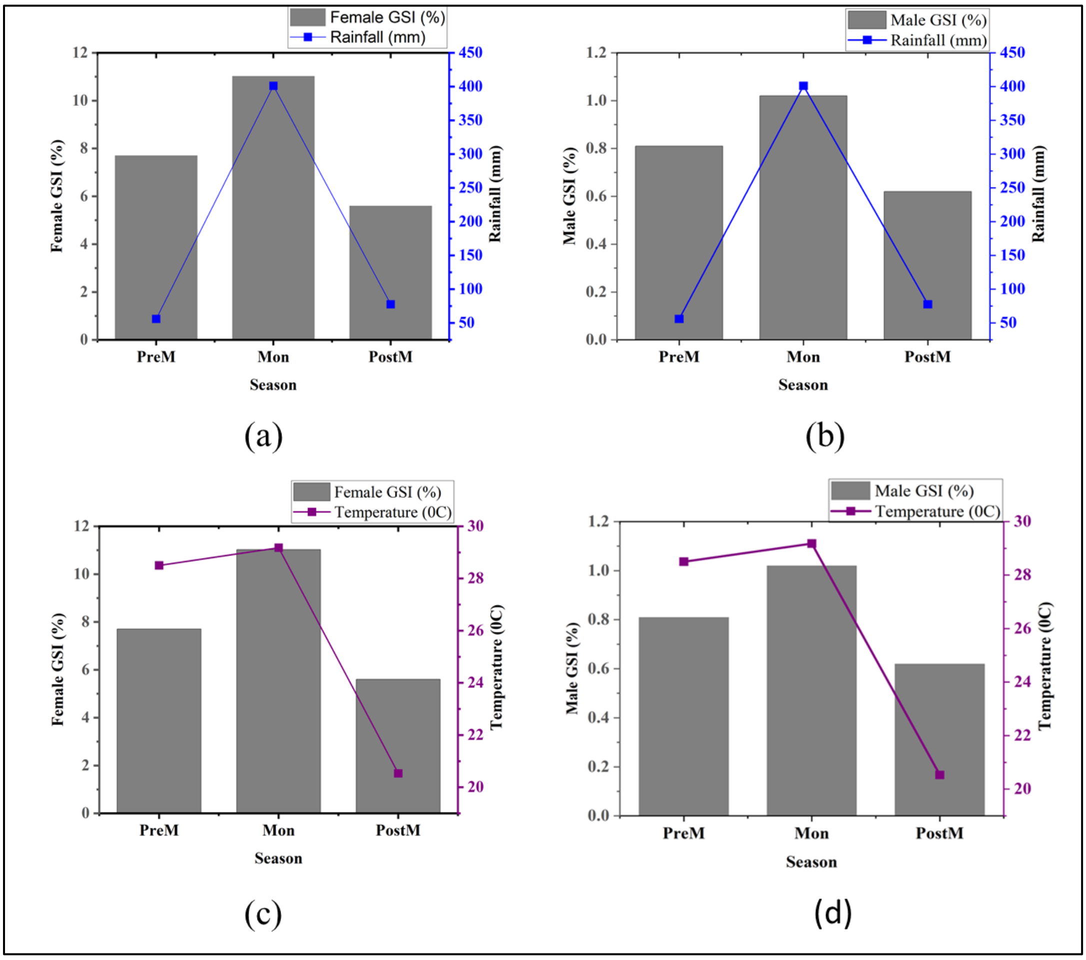

Fig. 10. Relationship of female and male GSI of T. Ilisha and environmental parameters (Rainfall and temperature) in different season. (a) female GSI and Rainfall, (b) male GSI and Rainfall, (c) female GSI and temperature, (d) male GSI and temperature. (Abbreviation: PreM- premonsoon, Mon- monsoon and PostM- postmonsoon.

Therefore, it may consider a more reliable index of spawning and gonad maturity. Mean Dobriyal index of male and female hilsa depicted two peaks one in Feb-March (Pre-monsoon) (female-1.97±0.02, male-0.93±0.03) and the other in August-September (Monsoon) (female-2.22±0.05, male-1.01±0.03). This finding was similar to Almukhtar et al. 2016, based on the same species from Iraq. Seasonal variation in dobriyal index coincided with the seasonal variations in GSI which showed prolonged spawning season with two distinct peaks.

Oocyte diameter

Oocyte diameter was measured to detect the spawning period of *T. ilisha*. The mean oocyte diameter was increased in accordance with the maturational stage of the ovary. The oocyte diameter progressively increased from February to September with two peaks, i.e., one in February–March (0.46 ± 0.05 mm) and another peak was in August–September (0.59 ± 0.04 mm) (Roomiani et al. 2014). The lowest ova diameter was found in post-monsoon season. Oocytes were considered mature when their diameter reached to 0.5 mm (Wu et al. 2008). The diameter of the ripe oocyte by different previous studies was from 0.70 to 0.75 mm (Jones and Menon 1951) and 0.76 to 0.87 mm (De 1986). This difference in oocyte diameter may be due to the size range of *T. ilisha*. Different sizes of oocyte were found at the same time, which conclude that *T. ilisha* is an asynchronous spawner as well as has a prolonged breeding season.

The peaks of gonado somatic index (GSI), dobriyal index (DI), and ova diameter (mm) indicated that the major spawning event, were taken place in August-

September, and a subsidiary spawning occurs in February-March in the Hooghly River.

Fecundity

Fecundity is the estimation of the number of ova in a fish. It is considered an important parameter of reproductive biology in fishes related to reproductive success. The fecundity of hilsa was found to 213560.3 ± 11174.53 in pre-monsoon, 723534 ± 11200.93 in monsoon, and 140705 ± 6192.87 in post-monsoon. In our study, the mean absolute fecundity observe of *T. ilisha* was similar to (Panwar et al. 2017) but less compared to the (Sarkar et al. 2018; Almukhtar et al. 2016). This comparatively low fecundity was due to small size range of the fish. Absolute fecundity was strongly correlated with ovary weight (r^2 = 0.99), Ovary length (r^2 = 0.79), and total weight (r^2 = 0.45), whereas weakly related with total length (r^2 = 0.15). These observations were consistent with the results obtained by (Almukhtar et al. 2016). Thus, the present study suggested that the ovary weight and ovary length are the better indices related to fecundity rather than total weight and total length. Though, in a previous study, Panwar et al. 2017 reported that fecundity was strongly correlated with total length and total weight, which did not coincide with the present findings. Such variation might be due to some factors. Therefore, further research related to other parameters like food availability and geographical region need to be conducted for further clarification about the reproduction potential of this species.

Histological analysis of gonadal maturation

The present study depicted the abundance of hilsa with three stages of the oocyte (Fig. 7a-c).

Primary vitellogenesis stage: It is a developing stage with numerous cortical alveoli (nucleus found in the middle of oocyte). As the oocytes grew, the cortical alveoli proliferated, and the follicle increase in size.

Secondary vitellogenesis stage: Nucleus found in the oocyte. It is the late yolk globule stage, where the number and size of the yolk vesicle increased.

Tertiary vitellogenesis stage (mature oocyte): It is the ripening stage with dispersed yolk in the oocyte. The nucleus could not be observed.

In the present study, the following conditions in the case of male hilsa were substantiated with histological structure (Fig. 9a - c).

Immature stage (Stage I): The lumen of the testicular cells was filled with a spermatogenic cells which were indistinguishable.

Maturing (II) to Ripe running (III): Gonad lumens were packed with smaller-sized spermatozoa whereas spermatocytes being of larger size could be visible in surrounding regions.

Spent (Stage IV): In this case, lumen had a very limited number of spermatozoa as mostly were released but some spermatozoa remain.

Relationship of GSI and

The sampling sites for *T. ilisha* in this study are in a tropical zone with well-defined seasonal variations. Temperature and seasonal rainfall are important for the regulation of teleostean fish reproductive cycles, which may lead to changing the breeding phenotypes in a region-specific way. For both males and females of *T. ilisha*, the spawning season predictor, the mean GSI were associated with seasonal average air temperature and rainfall. Present study revealed a strong association between temperature fluctuations and GSI (r_s = 0.71, p <0.05 for female and r_s = 0.71, p <0.05 for male), and between rainfall and GSI of *T. ilisha* (r_s = 0.77, p <0.05 for male and r_s = 0.78, p <0.05 for female). Present findings are similar to many notable researches, such as (Sarkar et al. 2019; Karnatak et al. 2018). Karnatak et al. 2020 have established a link between GSI and environmental variables, particularly temperature and rainfall. This result suggested that the combined effects of high temperatures and high rainfall enhance the gonad maturation and declined with low temperature and low rainfall. Rainfall also adds to the endocrine function, which leads to hydration of fish gonads that facilitate the normal spawning. There are no prior records about the relation of environmental parameters and GSI of this species to equate with our results.

Conclusion

Presently, it is a target fish for the inland aquaculture sector to meet the consumer demand in West Bengal as well as India. But for seminatural breeding require sufficient knowledge regarding reproductive biology especially fecundity, ova diameter, gonadal change, and spawning period. In male hilsa, sperm cell size decreases with maturation. The increase in GSI values in monsoon is indicative of the stage of ovarian maturation. Study on the gonad condition of hilsa is found to be essential in the sense that it may provide information on the stages of ovary and testis of the available fish groups that will play an important role in proper management and conservation of this species.

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Competing Interests

Authors declare that there is no competing of interest.

Authors' Contributions

Bhuban Mohan Majhi: Conceptualization, Writing–original draft, Software, Methodology, Interpretation of data. Bidita Banerjee and Bhuban Mohan Majhi: Histological analysis. Ashim Kumar Nath: Supervision, Validation and Visualization, Conceptualization, Writing – review & editing.

Ethical Approval

No ethical approval permission was required from the Animal Ethics Committee because no live specimen was used in this study. The specimens were dead but fresh in condition.

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