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Research Article

Reproductive biology of *Mugil cephalus* (Linnaeus, 1758) in the Sundarbans estuary, Bangladesh

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Abstract

The reproductive biology of *Mugil cephalus* in the Sundarbans estuary, Bangladesh, is essential for determining its spawning season and informing sustainable fisheries management and conservation strategies. The study aims to investigate the gonadosomatic index, fecundity, ovarian histology, and the spawning season of *M. cephalus* for the first time in this habitat. A total of 120 female specimens were collected through monthly sampling from three major rivers of the Sundarbans estuary between July 2022 and June 2023 for the study. Gonadosomatic index increased from July onward and reached a maximum in December (11.45±2.32%), followed by a marked decline, consistent with the post-spawning phase. The highest mean fecundity was observed in December (2003901.40±516749.64 eggs female^{-1}). Histological observations revealed distinct ovarian developmental stages, a pattern consistent with group-synchronous ovarian development, and the presence of vitellogenic oocytes from October to February. Both histologically and macroscopically mature female individuals were observed from November to January. The length–weight relationship was significant (R^2=0.951, p<0.001), revealing negative allometric growth (b=2.889). Gonad weight and fecundity showed significant relationships with total length and body weight. Estimated total length at which 50% of females were sexually mature (L_{50}) of *M. cephalus* was 45.83 cm. The study provides baseline information on the reproductive characteristics of *M. cephalus* in this estuary and the findings may support future studies on male reproduction, stock assessment, while further research incorporating environmental variables would help clarify their potential influence on the reproductive dynamics of *M. cephalus*.

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Introduction

The Sundarbans estuarine system, located along the southwestern coast of Bangladesh, constitutes one of the region's most important aquatic ecosystems. The system encompasses an extensive network of rivers and tidal channels, including the Baleshwar, Shibsa, and Pasur rivers, which flow through the Sundarbans before entering the Bay of Bengal. These ecologically diverse and highly productive habitats serve as critical feeding, spawning, and nursery grounds for a diverse assemblage of fish species (Elliott and Whitfield, 2011; Whitfield *et al.*, 2012; Day *et al.*, 2013). Among these, the flathead grey mullet, *Mugil cephalus* (Linnaeus, 1758), a member of the family Mugilidae, is widely recognised for its economic importance in warm-temperate to tropical estuarine fisheries (Blaber, 2000; Crosetti and Blaber, 2015; Rao and Babu, 2016). This cosmopolitan species inhabits coastal waters across major seas and oceans between approximately 42°N and 42°S latitudes, spanning tropical, subtropical, and temperate regions (Whitfield *et al.*, 2012; Bhakta *et al.*, 2024). Mullet roe, often referred to as "grey gold," commands high market value globally and is considered a premium culinary product (Whitfield *et al.*, 2012). As a euryhaline fish, *M. cephalus* demonstrates remarkable adaptability to freshwater, estuarine, and marine environments, although its spawning is typically confined to marine waters (Thomson, 1955; Ibáñez and Gutiérrez-Benítez, 2004). The species is characterised by rapid growth and a high sensitivity to environmental fluctuations and anthropogenic stressors, making it both an ecologically informative and economically valuable species (Hixon *et al.*, 2014; Bekova and Raikova-Petrova, 2017; Lowerre-Barbieri *et al.*, 2017). Mullets are already being cultured alongside shrimp in traditional tide-fed systems in Bangladesh and has gained greater prominence as a primary aquaculture species in neighbouring regions (Rekha *et al.*, 2021).

Despite its potential, mullet aquaculture in Bangladesh remains heavily reliant on the collection of wild fries, a practice that concedes the ecological concerns associated with shrimp post-larvae harvesting (Naylor *et al.*, 2000; Young and Potter, 2002; Flores *et al.*, 2026). Coastal aquaculture in Bangladesh is presently dominated by shrimp farming (Primavera, 2006; Ahmed and Glaser, 2016). This situation underscores the need to diversify cultured species and adopt resilient aquaculture practices. In this context, *M. cephalus* emerges as a promising candidate for coastal and brackish water aquaculture due to its herbivorous feeding behaviour, lower trophic level positioning, and potential contribution to sustainable fish protein production (Primavera, 2006; FAO, 2022). These factors are driving to the development of hatchery-based seed production therefore essential to ensure sustainability and reduce pressure on natural stocks (Rosenberg *et al.*, 2000; Welcomme and Petr, 2004). Understanding reproductive parameters will be helpful for the sustainability, conservation and management practices/policies (Afonso-Dias *et al.*, 2005; Dinh, 2018) and planning for artificial seed production under hatchery conditions. The reproductive traits of *M. cephalus* have been extensively investigated across its distribution range. Previous studies have described seasonal reproductive cycles, fluctuations in gonadosomatic index (GSI), fecundity patterns, and ovarian development, demonstrating that reproductive

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characteristics vary with environmental conditions and geographic locations (Assem *et al.*, 2015; Rao and Babu, 2016; Bekova and Raikova-Petrova, 2017; Rekha *et al.*, 2021; El-Aiatt, 2022; Lamraouhi *et al.*, 2022; Bhakta *et al.*, 2024; Flores *et al.*, 2026). Despite these investigations, information on the reproductive biology of *M. cephalus* from the Sundarbans estuary of Bangladesh remains limited. In particular, comprehensive data integrating reproductive indices like gonadosomatic index, fecundity, histological observations, and total length at which 50% female individuals get mature are scarce, which limits the development of management strategies for this commercially important species.

Therefore, the present study aimed to investigate the comprehensive reproductive biology of female *M. cephalus* for the first time in the Sundarbans estuary of Bangladesh by evaluating changes in the gonadosomatic index, estimating fecundity, examining ovarian histology, and identifying the spawning season.

Materials and methods

Field sampling and laboratory analyses were carried out following standard protocols. Fish specimens of *Mugil cephalus* were collected monthly, and morphometric, reproductive, and histological analyses were performed to evaluate the study objectives. Gonadal development was assessed macroscopically in both sexes, whereas detailed reproductive analyses, including ovarian histology and fecundity estimation, were conducted for females.

Study area

The study was conducted in Sundarbans estuary (Fig. 1), located on the south-western coast of Bangladesh from July 2022 to June 2023. Fish samples were collected from three estuarine locations inside Sundarbans reserve forest of Bangladesh: the Arpangasia River near Shyamnagar, Satkhira (22.100° N, 89.260° E), the Shibsa River near Koyra, Khulna (22.258° N, 89.475° E), and the Pasur River near Mongla, Bagerhat (22.351° N, 89.630° E).

Fish sampling

A total of 120 female fish specimens were collected throughout the year where 10 female each month using commercial gill nets, seine net and cast nets with the assistance of local fishermen. The sampling location varied among months according to specimen availability, and the specimens collected in a given month were not necessarily distributed equally among the three rivers. Because these three rivers are part of the same Sundarbans estuarine system. Immediately after capture, the fish were placed in ice boxes and transported to the Fisheries Biology Laboratory in Bangladesh Fisheries Research Institute, Paikgacha, Khulna for further analysis.

Morphometric measurements

Total length (TL) was measured from the tip of the snout to the end of the caudal fin to the nearest 0.1 cm using a measuring board. The total body weight (BW) and gonad weight (GW) were recorded using an electronic balance (EK-3200i, A&D Company, Japan) with a precision of 0.01 g.

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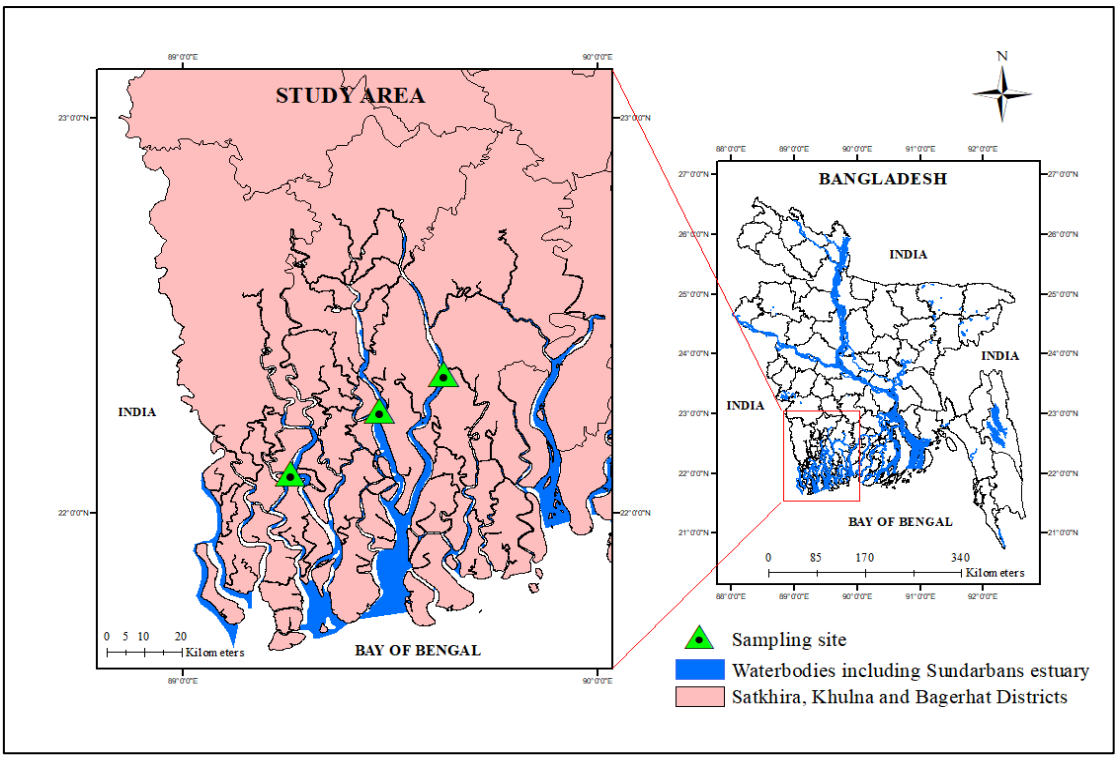

Figure 1: Location of the sampling sites (Arpangasia, Shibsa, and Pasur rivers) in the Sundarbans estuary of Bangladesh.

Reproductive biology

Fish were dissected through a ventral incision, and the gonads were carefully removed, cleaned of adhering tissues, and weighed immediately on an electric balance at 0.01 precisions. Additionally, a total of 36 males were selected for only macroscopic observation round the year and female gonads were documented on using different approaches like macroscopic, microscopic and morphometric measurements.

Gonadosomatic index (GSI)

To assess the reproductive cycle and identify the peak spawning period of *M. cephalus*, female gonad specimens were analysed over the sampling period.

The gonadosomatic index (GSI) was calculated as follows (Akhi *et al.*, 2024):

$$GSI = \frac{GW}{BW} \times 100$$

Where, *BW* is the total body weight of the fish before dissection (g), and *GW* is the wet weight of the ovary (g).

Fecundity estimation

Fecundity was estimated from 50 female specimens selected based on the presence of maturing and mature ovaries during the study period. These specimens were selected to obtain representative fecundity estimates across the reproductive development stages. After estimating gonad weight, gonads were cut into three subsamples of known weight at 0.01 precision from different regions of each ovary. Then, the oocytes were separated gently in physiological saline, count each subsample, and use the mean oocyte density to estimate absolute fecundity. Absolute fecundity (F) was estimated using the gravimetric method (Mishra *et al.*, 2018):

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$$Absolute\ Fecundity = \frac{No.of\ eggs\ in\ the\ ovary\ sample}{Ovary\ Sample\ Weight} \times\ Ovary\ Weight$$

Relative Fecundity was determined by the following formula (Bagenal, 1978):

$$Relative\ fecundity = \frac{Absolute\ fecundity}{Body\ weight}$$

Histological analysis of ovary

A total 6of 0 (sixty) female gonads were used for the histological study. Where, dissected fish gonads were cut into three regions (anterior, middle, and posterior) and placed in Bouin's solution for 6 hours and then immersed in 70% ethanol for 24 hours. Histological analysis was performed at the Anatomy and Histology Laboratory, Bangladesh Agricultural University, Mymensingh. Then, labelled histological cassettes were used for tissue processing and cutting into thin, smaller pieces. Tissue processing was carried out using an automated tissue processor (Leica ASP300S, Leica Biosystems, Germany), including dehydration in a graded ethanol series (70-100%), clearing in xylene, and embedding in paraffin wax. Sections of 4-5 µm thickness were prepared using a rotary microtome (Leica RM2255, Leica Biosystems, Germany). Sections were floated on a water bath at 40 °C, mounted on glass slides, and dried overnight. Subsequently, slides were deparaffinized in xylene, rehydrated through a descending ethanol series, and stained with haematoxylin and eosin (H&E) following standard histological procedures (Humason, 1972). Finally, sections were mounted with Canada balsam and covered with cover slips. Prepared slides were examined under a light microscope (Olympus BX53, Olympus Corporation, Japan) equipped with a digital imaging system. Photomicrographs were obtained to document and analysed the different stages of ovarian development.

Morphometric relationships

The relationships among total length (TL), body weight (BW), gonad weight (GW), and absolute fecundity (F) were analyzed using power-function and logarithmic regression models. The length–weight relationship (LWR) was described by the power function: $BW = aTL^b$. Similarly, the relationships between GW and TL and between F and TL and BW were described using power functions. For logarithmic regression, both the dependent and independent variables were transformed using the natural logarithm (ln) before analysis. The transformed relationships were fitted using ordinary least-squares regression as: $\ln(Y) = \ln(a) + b\ln(X)$, where Y is the dependent variable and X is the independent variable. Regression coefficients were estimated using the ordinary least-squares method, and the coefficient of determination (R^2) was used to assess model fit. Statistical significance was evaluated at $p<0.05$. These regression approaches are widely used in fisheries biology (Murua and Saborido-Rey, 2003; Froese, 2006; King, 2007; Wootton and Smith, 2015). Size at which 50% females are sexually mature (L_{50}) was estimated by finding the value of L_{50} by plotting the cumulative percentage of gonadal mature fish with the size of the fish total length, this yielded a standard logistic curve, and the intersection point between the curve and the 50% cumulative frequency represents the

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total length at which 50% of the fish get mature gonadally (Tamsil and Hasnidar, 2024).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Data are expressed as mean±standard deviation (SD). Sampling locations were mapped using ArcGIS version 10.8 (Esri, Redlands, CA, USA). Monthly differences in biological parameters were assessed using one-way analysis of variance (ANOVA). The assumptions of normality and homogeneity of variances were assessed before analysis. Welch's one-way ANOVA was used for TL, BW, and GW when the assumption of homogeneity of variances was violated, followed by Games–Howell post-hoc comparisons. For relative fecundity, when the assumptions of conventional ANOVA were satisfied, one-way ANOVA followed by Tukey's honestly significant difference (HSD) test was used for pairwise comparisons. Relationships among TL, BW, GW, and F were evaluated using regression analysis, and the coefficient of determination (R^2) was used to assess model fit. Statistical significance was set at $p<0.05$.

Results

Monthly variation was assessed using Welch's ANOVA due to heterogeneity of variances across months. Total length (TL) did not differ significantly among months ($F(11,42.41)=0.673$, $p=0.755$), nor did body weight (BW) ($F(11,42.41)=0.720$, $p=0.713$). In contrast, gonad weight (GW) differed significantly among months ($F(11,42.13)=35.376$, $p<0.001$), indicating pronounced seasonal variation in gonadal development. Games-Howell post-hoc comparisons showed that GW in December was significantly higher than in July, August, September, October, February, March, April, May and June ($p<0.05$), but did not differ significantly from November ($p=1.000$) or January ($p=0.878$). GW exhibited pronounced seasonal fluctuations, with the lowest value recorded in March (19.18±7.23) and the highest in December (139.40±33.51). The progressive increase in GW from May onward indicates seasonal gonadal development, with December representing the period of maximum gonadal development (Table 1).

The gonadosomatic index (GSI) showed clear seasonal variation, increasing progressively with gonadal maturation and declining sharply after spawning (Fig. 2a). In female *Mugil cephalus*, GSI values gradually increased from July and rose markedly from October (7.06±1.83%), reaching a peak in December. Thereafter, GSI values declined steadily from February to March, indicating the post-spawning and resting phases. Lower GSI values were recorded between March to July (1.65±0.78% to 4.05±1.10%), corresponding to immature or resting stages. Moderate values were observed during August and September (5.75±1.65% and 6.23±2.00%), reflecting transitional phases of gonadal development. The highest GSI values, recorded between November, December and January, confirm active maturation and spawning activity during this period, as also indicated by the presence of gravid females (Fig. 2b). GSI varied significantly among months

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(Welch's ANOVA, F(11,42.26)=187.043, p<0.001). The highest mean GSI was recorded in December (11.45±2.32%), although it did not differ significantly from November (p=0.655) or January (p=0.239).

Table 1: Monthly variation in total length (TL), body weight (BW), gonad weight (GW) of female *M. cephalus* in Sundarbans estuary, Bangladesh (n=120).

Month	TL (cm) (Mean±SD)	BW (g) (Mean±SD)	GW (g) (Mean±SD)
January	44.52±4.07	1219.93±290.6	105.21±57.92[a]
February	42.94±3.18	1140.91±265.6	53.39±50.94[b]
March	44.15±3.73	1230.47±284.99	19.18±7.23[b]
April	42.47±3.18	1120.89±280.48	24.21±7.33[b]
May	41.61±2.5	1039.91±214.23	36.38±6.96[b]
June	42.13±2.8	1065.9±211.0	42.49±9.38[b]
July	42.46±3.94	1110.43±288.67	45.03±12.62[b]
August	42.0±2.72	1046.41±183.96	59.63±8.75[b]
September	43.36±2.67	1174.97±216.47	73.72±18.07[b]
October	43.08±3.46	1148.42±273.69	80.93±19.71[b]
November	43.64±3.15	1207.93±264.43	129.77±30.08[a]
December	43.68±2.85	1209.48±237.66	139.4±33.51[a]

Note: Different superscript letters within the GW column indicate there is no significant differences among months according to the Games-Howell post-hoc test following Welch's ANOVA (p<0.05).

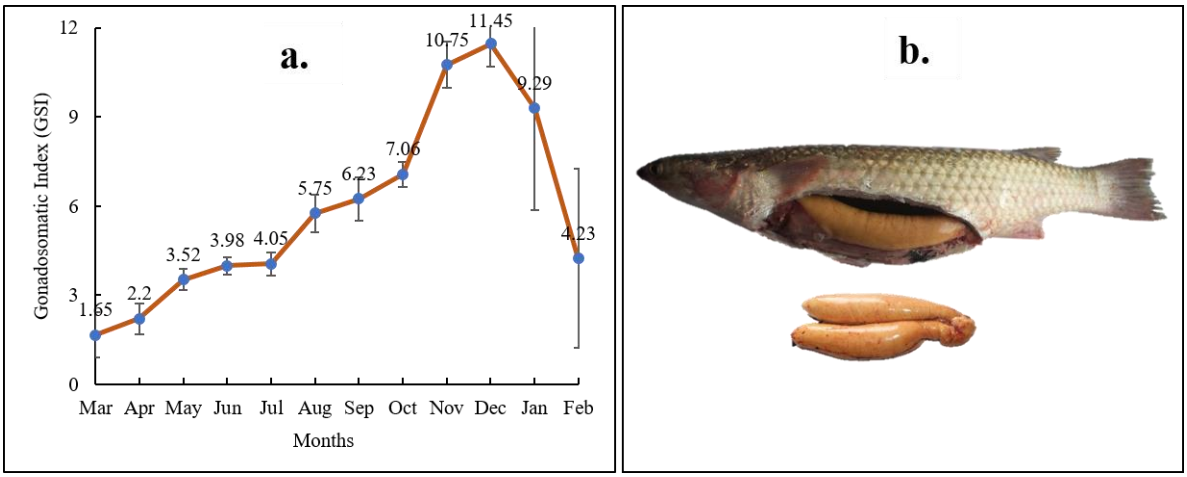

Figure 2: a. Monthly variation in female gonadosomatic index (GSI) and b. gravid female *M. cephalus* in Sundarbans estuary, Bangladesh.

Fecundity exhibited significant monthly variation during the reproductive period. Absolute fecundity differed significantly among months (Welch's ANOVA, F(5,7.778)=177.00, p<0.001). Games-Howell post-hoc comparisons showed that absolute fecundity was significantly higher during November-February than in September and October, whereas no significant differences were observed among November, December, January and February. Absolute fecundity reached its highest mean value in December (2,003,901.40±516,749.64 eggs female^{-1}) and remained high through January and February. Relative fecundity also varied significantly among months (one-way ANOVA, p<0.001). Tukey's HSD test indicated that December had significantly higher relative fecundity than all other months, while September and October had the lowest values and did not differ

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significantly from each other. Relative fecundity in November, January and February did not differ significantly from one another (Table 2).

Table 2: Monthly variation (mean±SD) in fecundity of *Mugil cephalus*, including absolute fecundity and relative fecundity at Sundarbans estuary, Bangladesh (n=50).

Months	No. of fish examined	Body Weight (g) (Mean±SD)	Gonad Weight (g) (Mean±SD)	Fecundity Ranges (no. of ova)	No. of ova/g of gonad weight (Mean±SD)	Absolute Fecundity (nos.) (Mean±SD)	Relative Fecundity (Mean±SD)
September	7	1199.75 ±186.63	79.15 ±16.55	711330-1331640	13300.25 ±2898.27	1054744.14 ±232344.28^{a}	872.4 ±56.13^{a}
October	10	1148.421 ±273.69	80.93 ±19.71	762420-1779260	13530.48 ±3622.46	1100836.10 ±300128.82^{a}	955.52 ±64.97^{a}
November	10	1207.93 ±264.43	129.77 ±30.08	327820-2630266	13925.08 ±3521.76	1812451.25 ±448237.32^{b}	1497.08 ±118.43^{b}
December	10	1209.48 ±237.66	139.40 ±33.51	1412587-2941352	14325.82 ±3138.08	2003901.40 ±516749.64^{b}	1641.79 ±131.49^{c}
January	8	1330.47 ±278.05	136.81 ±33.87	1241506-2477905	14442.86 ±2588.81	1977522.57 ±495058.72^{b}	1475.59 ±108.75^{b}
February	5	1508.13 ±224.76	148.72 ±29.18	187743-2033251	13150.64 ±707.11	1955343.50 ±110177.84^{b}	1296.11 ±51.78^{b}

Note: Different superscript letters within each column indicate significant differences among months (p<0.05), based on Games–Howell post-hoc test for absolute fecundity and Tukey's HSD test for relative fecundity.

Histological observations revealed successive stages of oocyte development, ranging from chromatin-nucleolus and peri nucleolar oocytes to advanced vitellogenic stages (Fig. 3). Early development included CNO, EPNO, LPNO and primary oocytes. These were identified in the sample from the month February to October. Vitellogenesis was characterized by the progressive appearance and accumulation of yolk granules, from EYGO and YG to LYGO and LVt (Fig. 3a-c). The presence of atretic oocytes was consistent with oocyte degeneration and post-spawning ovarian regression (Fig. 3c). These stages were most prominent between November to January, confirming the peak reproductive period. The coexistence of different oocyte stages within the ovary consistent with a group-synchronous pattern of development.

Immature ovaries were mainly observed from March to June, while maturing ovaries were found from June to October. Fully mature ovaries were large, yellow, and occupied most of the abdominal cavity, with clearly visible oocytes, especially from November to January. Regression ovaries appeared flaccid and reduced in size, with residual oocytes and empty follicles, primarily observed February-March (Table 3).

The length-weight relationship of *Mugil cephalus* was highly significant (R^2=0.951; p<0.001), with a growth exponent (b=2.889) indicating negative allometric growth with Pauly test (Table 4).

The gonad weight was significantly correlated with both total length (R^2=0.529;

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p<0.001) and body weight (R^2=0.528; p<0.001), demonstrating size-dependent reproductive investment. Absolute fecundity showed moderate but significant relationships with gonad weight (R^2=0.639; p<0.001), total length (R^2=0.517; p<0.001), and body weight (R^2=0.508; p<0.001) (Fig. 4).

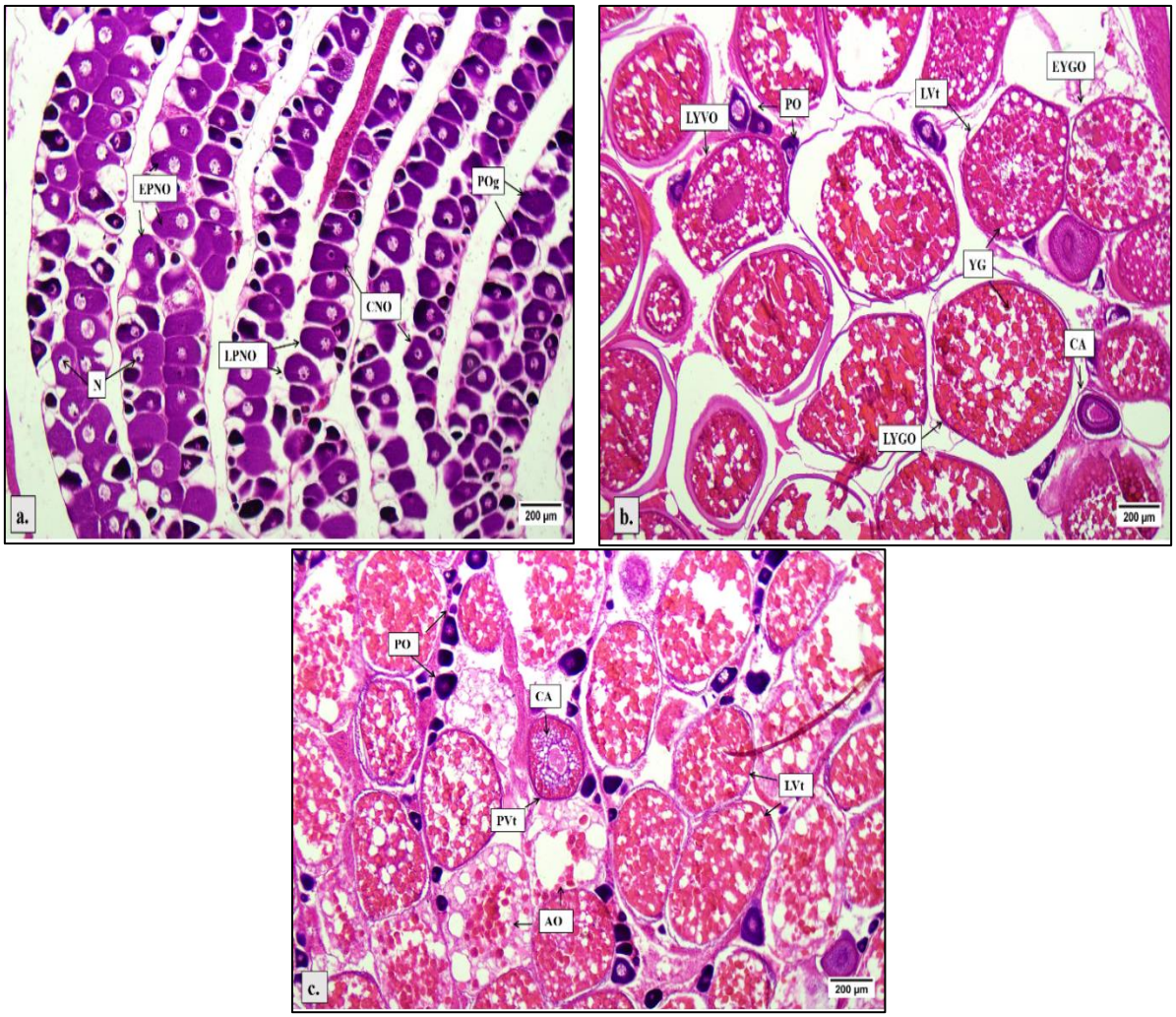

Figure 3: Histological characteristics of ovarian development microscopically at 10X magnification in *Mugil cephalus*. (a) Early oocyte developmental stages, including chromatin-nucleolus and perinucleolar oocytes; (b) vitellogenic development showing early yolk-granule, yolk-granule and late vitellogenic oocytes; and (c) advanced vitellogenic oocytes with previtellogenic/cortical-alveolar stages and atretic oocytes. CNO, chromatin-nucleolus oocyte; EPNO, early perinucleolar oocyte; LPNO, late perinucleolar oocyte; POg, primary oocyte; EYGO, early yolk-granule oocyte; YG, yolk granules; LYGO, late yolk-granule oocyte; PVt, previtellogenic oocyte; LVt, late vitellogenic oocyte; CA, cortical alveoli; AO, atretic oocyte; N, nucleus; PO, primary oocyte.

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Table 3: Macroscopic classification of gonadal maturity stages in male and female *Mugil cephalus*, describing morphological and structural characteristics of ovaries and testes across developmental phases at Sundarbans estuary, Bangladesh.

Stages	Female	Male
Immature (Stage 1)	Ovaries are smaller, transparent, pale-pink, and gelatinous, connected at the posterior tip. Occupying one-quarter of the abdominal area. Blood vessels are not noticeable.	Filiform testes connected at the posterior extremity, semi-translucent with slight red color; occupy between one-third and one-half of the abdominal space.
Developing (Stage 2)	Ovaries are larger, cylindrical and pinkish or pale yellow in color. Granular surface as a result of the appearance of vitellated oocytes to the unaided eye. Occupying between one-third and one-half of the abdominal cavity. Lobules of varying lengths. The visibility of blood vessels increases.	Testes whitish, broader than in the last stage, exhibit a flattened shape with dull and smooth surface. Occupying between one-half and one-third of the abdominal cavity. A groove from the vas deferens is apparent on the inner lateral side of the testis. Semen is not expelled when pressure is exerted on the abdomen.
Mature (Stage 3)	Ovaries enlarged, distended, and golden yellow to a vivid orange color. The oocytes are noticeable with the naked eye, and certain hydrated oocytes are expelled with gentle pressure to the abdominal cavity. They occupy three-quarters of the abdominal cavity. Blood vessels are more apparent.	Testes are creamy white, turgid, and fill the whole abdominal cavity. The semen is expelled with slight abdominal pressure.
Regressing (Stage 4)	Reduced ovarian size. Lobules resembling stage 1, although exhibiting a pinkish-brown colour. Blood vessels decreased but are still visible.	Testes are flaccid, with no semen release upon pressing. Lobes exhibiting dark, brownish borders and covering one-half to two-thirds of the abdominal cavity.

Table 4: Regression equations, coefficients of determination (R^2), regression parameters (a, b), significance levels (p-values), and sample sizes (n) of different relationship in M. cephalus in Sundarbans estuary, Bangladesh.

Relationship Parameters	Regression Equation	R^2	a	b	*P*-value	n
Total Length (TL) - Body Weight (BW)	$BW=0.0215\ TL^{2.889}$	0.951	0.0215	2.889	<0.001	120
Gonad Weight (GW) - Total Length (TL)	$GW=0.0003\ TL^{3.422}$	0.529	0.0003	3.422	<0.001	120
Gonad Weight (GW) - Body Weight (BW)	$\ln(GW)=1.193\ \ln(BW) - 3.770$	0.528	0.0231	1.193	<0.001	120
Absolute fecundity (F) - Gonad Weight (GW)	$\ln(F)=0.557\ \ln(GW)-0.408$	0.639	0.6649	0.557	<0.001	50
Absolute fecundity (F) - Total Length (TL)	$F=1.5244\ TL^{3.651}$	0.517	1.5244	3.651	<0.001	50
Absolute fecundity (F) - Body Weight (BW)	$F=187.91\ BW^{1.268}$	0.508	187.91	1.268	<0.001	50

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The estimated total length at which 50% females were sexually mature (L_{50}) of *M. cephalus* at 45.83 cm (95% Cl: 43.87-47.93 cm) TL (Fig. 5a). Individuals below 45.83 cm TL were predominantly immature, whereas the proportion of mature fish increased markedly with increasing body length. The distribution of gonadal maturity stages round the year showed a distinct ontogenetic shift in reproductive status (Fig. 5b).

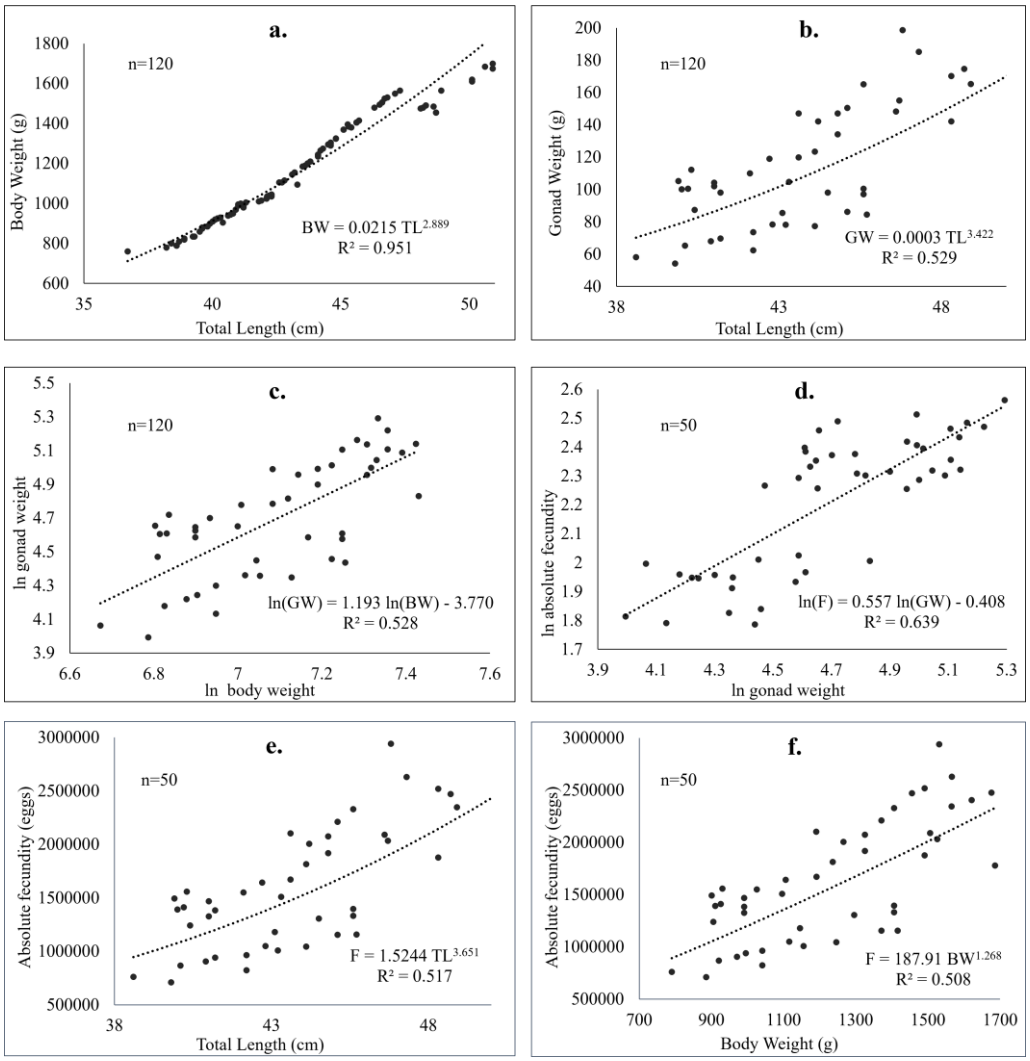

Figure 4: Relationships between total length (TL), body weight (BW), gonad weight (GW), and absolute fecundity (F) of *Mugil cephalus* in the Sundarbans estuary of Bangladesh: (a) TL-BW, (b) TL-GW, (c) BW-GW, (d) GW-F, (e) TL-F, and (f) BW-F relationships. Lines represent fitted regression curves.

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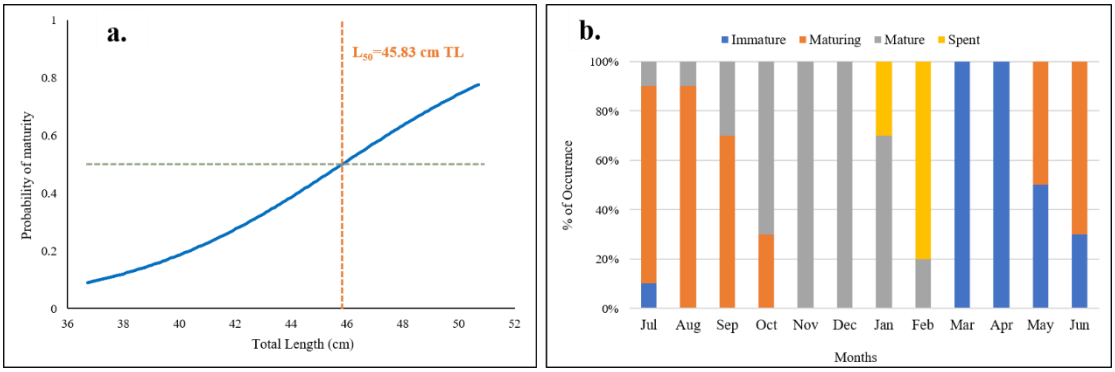

Figure 5: a. Logistic maturity showing the relationship between the proportion of mature female *Mugil cephalus* and total length (TL), with the estimated total length at which 50% females were sexually mature (L_{50} = 45.83 cm TL) indicated by the vertical dashed line. The horizontal dashed line represents the 50% maturity level; b. Monthly distribution of gonadal maturity stages in sampled female M. cephalus, expressed as percentage occurrence of immature, maturing, mature, and spent individuals at Sundarbans estuary, Bangladesh.

Discussion

In a whole, current study assessed the reproductive parameters of female *Mugil cephalus* at the Sundarbans estuary of Bangladesh. Narrower variation ranges in total lengths and body weights of sampled individuals were observed throughout the year due to selected sample was narrow size range individuals. In the middle of the year, gonadal maturing stage energy flow to reproductive activity. Similarly, this kind of activity has been reported in mullet species inhabiting estuarine conditions (Whitfield *et al.*, 2012; Crosetti and Blaber, 2015).

Reproductive parameters showed clear seasonal variation. This well-defined reproductive cycle of *M. cephalus* indicates the spawning season exists from November to January and reaches its peak in December. Likely seasonal patterns have been widely documented in another teleost (McDonough *et al.*, 2003; Assem *et al.*, 2015) but there are some variations due to environmental cues like temperature, food availability and geographical differences (Wootton and Smith, 2015; Rao and Babu, 2016; Bekova and Raikova-Petrova, 2017; Rekha *et al.*, 2021; El-Aiatt, 2022).

The high fecundity observed in *M. cephalus* may represent a reproductive strategy adapted to potentially high larval mortality in estuarine environments. Variations in absolute fecundity compared with previous studies (Assem *et al.*, 2015; Rekha *et al.*, 2021) may be associated with differences in fish size and environmental conditions. In particular, seasonal reductions in estuarine salinity may influence reproductive investment and fecundity; however, the present study did not quantify the direct contribution of salinity to the observed variation in absolute fecundity. Therefore, the influence of salinity, together with temperature and other environmental factors, should be investigated in future studies (Blaber, 2000; Whitfield *et al.*, 2012).

Histological observations of ovarian development were generally aligned with the findings from GSI. Early-stage oocytes were associated with the regression phase, while the progression through perinucleolar and cortical alveoli oocytes reflected active

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ovarian maturation. The onset of vitellogenesis coincided with increasing GSI values, while the predominance of yolk globule oocytes corresponded to the period of highest reproductive activity. The occurrence of atretic oocytes further indicated post-spawning or resorption activity. The agreement among histological, morphometric, and GSI-based indicators supports the reproductive cycle described in this study (West, 1990; Lowerre-Barbieri *et al.*, 2017). Although seasonal changes in estuarine salinity may influence gonadal development, GSI, and ovarian investment in euryhaline fishes, the present study did not quantify the direct effect of salinity on these reproductive parameters. Therefore, the extent to which reduced salinity contributed to the observed changes in GSI and ovary weight cannot be determined from the present data. Similar ovarian developmental patterns have been reported in *M. curema* (Oliveira *et al.*, 2011) and other estuarine fishes, including *Polynemus paradiseus*, *Scatophagus argus*, and *Pomadasys hasta* (Mondal *et al.*, 2022a; Mondal *et al.*, 2022b; Naser *et al.*, 2023).

However, multiple spawning events have been reported in *M. cephalus* (Stenger, 1959), while two peak spawning periods within a year were identified by Bhakta *et al.* (2024), indicating possible regional and temporal variation in spawning activity. In contrast, the present study identified a single main spawning season from November to January in the Sundarbans estuary. This pattern was supported by the highest GSI value observed in December (11.45±2.32%) and the predominance of advanced yolk globule oocytes during the peak reproductive period. The observed single annual spawning peak suggests that the spawning pattern of *M. cephalus* in the studied estuary may differ from populations showing multiple spawning peaks. The coexistence of multiple oocyte developmental stages leads to batch spawning throughout the identified reproductive season. This strategy is advantageous in fluctuating environments, enhancing reproductive success under variable conditions (Murua and Saborido-Rey, 2003; Crosetti and Blaber, 2015). Macroscopic observations aligned these findings, showing clear transitions between immature, mature, and spent stages.

The length–weight relationship demonstrated a strong correlation, while the growth exponent indicated negative allometric growth (b=2.88), suggesting that length increased proportionally faster than body weight. The growth pattern observed in the present study was comparable to that reported for *M. cephalus* from South Carolina estuaries (b=2.92) and the Lagos Lagoon, Nigeria (b=2.92), but differed from that reported in the Narmada Estuary, India (b=3.03) (McDonough *et al*., 2003; Lawson and Jimoh, 2010; Bhakta *et al*., 2024). Such regional variation in growth patterns may reflect differences in environmental conditions, including food availability, habitat characteristics, and energy allocation (Froese, 2006; King, 2007).

Reproductive activity was size-dependent as larger individuals exhibited higher gonadal development and fecundity. However, the moderate strength of relationships among parameters suggests that additional factors, including age and

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environmental variability, influence reproductive output (Stearns, 1992; Roff, 2002; Bhakta *et al.*, 2024).

The estimated total length at which 50% of female *M. cephalus* get sexual maturity (L_{50}) is 45.83 cm, which is identical (45.95cm) with those observed in Moroccan South Atlantic population (Lamraouhi *et al.*, 2022) and L_{50} (43.76 cm) values reported for Indian west- and east-coast stocks (Rekha *et al.*, 2021) but higher than those reported (17.1 cm) for populations from the Narmada estuary, India (Bhakta *et al.*, 2024). This may reflect substantial regional and sampling variation in maturation schedules. Such differences in total length at which 50% female get maturity are commonly associated with geographic variation in environmental conditions, food availability, growth rate, population structure, exploitation pressure, and of deliberate selection of sampled individuals.

Protecting larger fish is important because they contribute more to maintaining fish populations (Hixon *et al.*, 2014). The findings of this study provide valuable information for the sustainable management and conservation of *M. cephalus* in the Sundarbans estuary. Implementing seasonal fishing restrictions during the spawning period (November–January) may protect breeding populations, enhance recruitment.

Conclusion

This study provided baseline information on the reproductive biology of *Mugil cephalus* in the Sundarbans estuary, Bangladesh. The observed patterns of gonadosomatic index, fecundity, and ovarian histology suggested that reproductive activity was concentrated during November-January, with the highest reproductive activity observed in December. Histological observations were consistent with a group-synchronous pattern of ovarian development, while absolute fecundity increased with body size. The findings highlight the potential reproductive importance of the Sundarbans estuary for *M. cephalus* and provide useful information for future studies on its reproductive ecology, conservation, and artificial propagation. Also, this study showed the importance of this estuary in supplying fry for rearing in other aquaculture areas, thereby ensuring sustainable aquaculture and reducing pressure on natural stocks. However, because the present study covered only one annual cycle, multi-year monitoring is needed to confirm the consistency of the observed spawning period and to evaluate appropriate fisheries management and broodstock protection measures.

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Conflicts of interest

We declare that this research was conducted without any conflict of interest with any party.

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