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ORIGINAL RESEARCH ARTICLE

Spawning season and size at first sexual maturity of freshwater mussel *Lamellidens marginalis* (Lamarck, 1819) in the Brahmaputra River, Bangladesh

Arun Chandra Barman[1,2*], Mohosena Begum Tanu[2], Mohammad Ferdous Siddique[2], Sonia Sku[3], Md. Nazmul Hossen[3], Md. Ayenuddin Haque[2] and Yahia Mahmud[2]

[1]Habiganj Agricultural University, Habiganj 3300, BANGLADESH
[2]Bangladesh Fisheries Research Institute, Headquarters, Mymensingh - 2201, BANGLADESH
[3]Bangladesh Fisheries Research Institute, Freshwater Station, Mymensingh - 2201, BANGLADESH
[*]Corresponding author's E-mail: aruncbt@yahoo.com

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ABSTRACT

Spawning season and size at first sexual maturity of Freshwater mussel *Lamellidens marginalis* was studied from the specimens collected from Brahmaputra River, Mymensingh district, Bangladesh from July 2015 to June 2016. The present study has investigated sex ratios, gamogenetic cycle, condition index, and size at first sexual maturity by means of standard histological procedures. The results indicated no significant difference in the overall sex ratio (M: F = 1:1.3). The qualitative analysis of gonad developmental stages has provided confirmation of the presence of a yearly reproductive cycle characterized by prolonged gonadal activity. The highest percentages of ripe gonads were observed in July for males (77.78%) and August for females (53.85%). The spawning activity was highest in October for males (50%) and November for females (83.33%). Furthermore, ripening and spawning stages in different shell lengths ranged from 58 –63 to 88–93 mm for both sexes. The findings from the qualitative observation of gonad developmental stages, a single annual spawning peak observed between October and November. A statistically significant correlation was detected between the average condition index of male and female. Males reached sexual maturity at smaller standard length (SL_{50} = 63.25 mm) compared to females (SL_{50} = 72.10 mm SL). Acquired knowledge regarding the present state and distinctive gonad developmental characteristics of *L. marginalis* will aid fisheries management professionals and conservation biologist in the effective management of this particular species of mussels in the waters of Bangladesh.

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INTRODUCTION

Lamellidens marginalis (Lamarck, 1819), commonly known as the pearl mussel, is a widely distributed species of freshwater bivalves that can be observed in diverse river systems, ponds, and derelict aquatic habitats across Bangladesh (Mishra *et al*., 2008; Hussain *et al*., 2022). This species plays a significant role in providing a basis of protein for animals and generating income for indigenous communities. This particular species has garnered significant attention as a subject of research due to its potential to support small-scale fisheries and its suitability as a aspirant species for pearl production (Thippeswamy *et al*., 2014; Siddique *et al*., 2020; Tanu *et al*., 2019; 2021; 2022). *L. marginalis* demonstrates a strong ability to tolerate stress and a greater vulnerability to pollutants, rendering them a dependable bio-indicator and sentinel species for assessing water body pollution levels (Nobles and Zhang, 2015; Yasmeen and Pathan, 2021). Since the local populations do not eat *L. marginalis*, their economic significance is still low in Bangladesh. However, recent developments in the growth of aquaculture, ecosystem

protection, and pearl production have emphasized their significance (Chowdhury *et al*., 2016; Niogee *et al*., 2019).

Freshwater mussel species in Bangladesh exhibit high sensitivity to various factors that pose significant risks to their populations. Moreover, there has been a worldwide decrease in the biomass of mussels in natural aquatic environments in recent times. This decline can be attributed to various factors, including the extensive use of pesticides, the discharge of metals into water bodies, industrial runoff, and the unintended capture of mussels during fishing activities, and a decreased water depth during the winter season (Haag, 2019; Sun *et al*., 2019). Hence, the primary recognized hazards that endanger these organisms and their corresponding populations include selective exploitation, habitat destruction, and pollution. In spite of being classified as "least concern" by the International Union for Conservation of Nature (IUCN) (Madhyastha *et al*., 2010), there has been a notable decrease in the populations of *L. marginalis*, in recent years. This decline has resulted in substantial losses in the ecosystem services provided this mussel species (Haag and Williams, 2014; Vaughn, 2018). Understanding mussels' reproductive processes and morphometrics would thus help future conservation initiatives and pollution related studies (Hossain *et al*., 2023). A comprehensive understanding of the reproductive cycle is crucial to formulate an effective exploitation strategy for this fishery resource.

Body morphometrics is a commonly employed methodology in the domains of protection, biological evaluation, and resource organization pertaining to aquatic organisms (Mozsár *et al*., 2015, Okuthe and Bhomela, 2020). Reproductive studies often utilize qualitative indicators obtained from cytological characterizations of the gonads and histological assessments. Additionally, quantitative indicators such as condition index, and maturation size are commonly utilized (Arago´n-Noriega *et al*. 2007; Zaidman *et al*. 2012). Knowing the reproductive strategies of *L. marginalis* is essential for efficiently gathering young individuals for commercial cultivation or developing a hatchery to guarantee a steady provision of juveniles. Freshwater mussels exhibit a distinctive life cycle that distinguishes them apart from other freshwater bivalves. In order to support a clutch of larvae during this unusual life cycle, a female must have a modified reproductive system. These larvae have evolved to undergo parasitic development on fish (Dillon, 2004). The male organism expels spermatozoa into the water column, and the female organism captures them using the incurrent syphon. Internal fertilization occurs within specialized suprabranchial chambers known as marsupia, wherein the embryos undergo development and transform into fully formed larvae referred to as glochidia (Mackie, 1984). The process of transforming larvae into juvenile mussels requires the encystment and attachment of glochidia in the host fish. The juveniles are ultimately liberated from fish and subsequently settle at the benthic region of the aquatic environment. Once settled in the sediment, they undergo rapid growth (Dillon, 2004).

This entails knowledge of the spawning and larval brooding seasons, as well as specific details regarding the favourable ecological conditions, therefore, required for successful reproduction. Numerous methodologies were utilized to evaluate the reproductive form of bivalve molluscs (Park and Choi, 2004; Uddin *et al*. 2012). Histological methods are extensively utilized and commonly employed due to their ability to provide visual evidence regarding gonadal materials (Lango-Reynoso *et al*. 2000). The condition index demonstrates a high level of sensitivity to fluctuations in reproductive growth and generally displays a robust association with the average gonad index (Ojea *et al*., 2004; Peharda *et al*., 2006). Gribben *et al*. (2004) suggested the condition index computation as a quick assessment technique for broods' reproductive status. The length at first maturity of a species is also essential parameters to know for the sustainable management of that species within an ecosystem. There exist numerous techniques for approximating the length at maturity, commonly denoted as the SL_{50}, which represents the length at which 50% of the individuals have got maturity. The ogives of SL_{50} as well as the maturity state are mutually exclusive; specifically, the SL_{50} may initiate the onset of the maturity condition. Hence, the precision of SL_{50} is significant as it would serve to identify the female individuals that are selected for inclusion in reproductive population.

The present study was to acquire in-depth understanding of the gamogenetic cycle and gonadal development of *L. marginalis* originating from the Brahmaputra River in Bangladesh. We employed a combination of qualitative gonadal stage analysis and quantitative evaluations to ascertain the temporal aspects of reproductive growth and spawning. The data acquired serves as a foundation for the implementation of sustainable strategies in the management of naturally occurring populations.

MATERIALS AND METHODS

Study area and duration

Specimen of *L. marginalis* was collected from three sites of Brahmaputra River, Bangladesh (Figure 1) with the help of professional fishers. A total of 240 individuals were sampled for one year (20 individuals per month from July 2015 to June 2016). In the laboratory, mussels were subjected to measurements of shell length (SL, mm), shell height (SH, mm) using a digital caliper with a precision of 0.01 mm, and total weight (TW, g) using a digital balance with a precision of 0.01 g. Subsequently, the mussels underwent dissection, and the mass of their soft body was properly documented. The shells were dried and then their weights were measured.

Histology of the gonads

Every month, a total of ten individuals were chosen for the purpose of conducting histological analysis on their gonads, with the aim of determining the gametogenic cycle and periods of spawning. In *L. marginalis*, the gonad is closely connected to the underlying digestive gland, posing challenges when attempting to separate without causing damage or loss of gonadal tissue. The tissue assemblage, comprising the digestive gland and gonad, underwent fixation in Davidson's solution for duration of 48

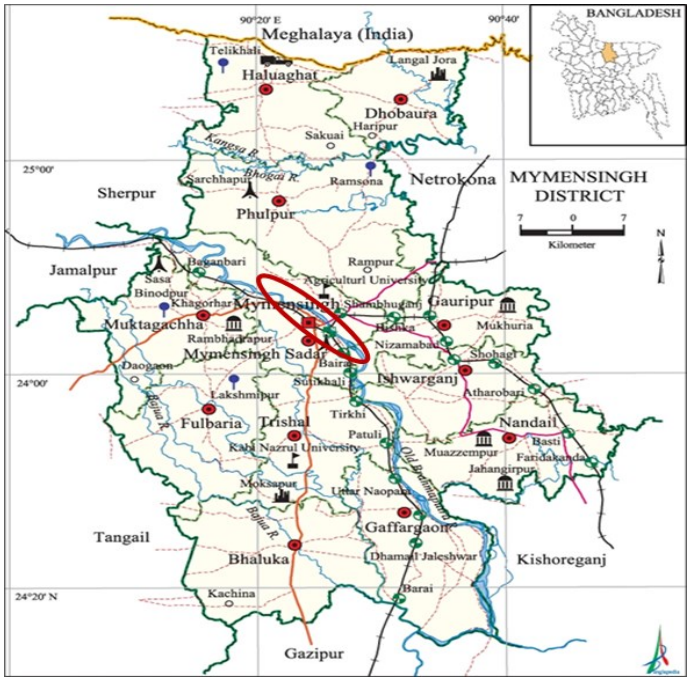

Figure 1. *Sampling sites in the Brahmaputra River, Mymensingh district, Bangladesh.*

hours and was subsequently preserved in a solution of 70% ethanol. Following that, a transverse incision measuring 2 to 3 mm in thickness was performed. The tissue samples underwent dehydration by a series of graded ethanol solutions, followed by infiltration and embedding in paraffin wax. The blocks were trimmed with upper and lower edges straight and parallel and kept in freezer for 30 minutes. The blocks were cut with 5 µm blade in microtome machine (KD-2258). The ribbon was immersed in an electrothermal mounting bath, specifically a water bath, and maintained at a temperature of 40°C. The blocks underwent xylene clearance, followed by rehydration with alcohol. Subsequently, they were stained, rehydrated once more using Haematoxylene, counterstained with Eosin, and ultimately dehydrated using alcohol and clearing xylene. The slides were prepared by mounting them with Canada balsam and subsequently observed for duration of 12 hours. Examination was conducted using a light microscope, specifically the OPTIKA B-150 model, with varying magnification levels ranging from X4 to X40, depending on the dimensions of the germinal cell. The histological slides were captured using a securely mounted digital camera (TOUPCAMTM- UCMOS-3.1MP ½ ", Model No. 3.2). Gonadal maturity stages in both sexes were recognized using a microscopic to assess the development of the gonads (briefly described in Figure 3). Maturity stages were classified into five categories: I: Early developing; II: Late developing; III: Ripe; IV: Spawning; and V: spent (Siddique *et al*., 2019).

Condition index

The condition index of the mussels being studied was calculated by dividing the weight of the dried tissue by the weight of the dried shell for each individual mussel, using the methodology outlined in the study conducted by Uddin *et al.* (2010).

$$CI = \text{Dry tissue weight (g)} / \text{Dry shell weight (g)} \times 100$$

Size at first sexual maturity

The size at first maturity (SL_{50}) is a quantitative measure that represents the length at which exactly 50% of the population reaches sexual maturity. The specimens used for the microscopic analysis of gonadal components were utilized to determine the SL_{50}. The developmental Stages 3 (ripe) and 4 (spawning) were designated as mature individuals for the calculation of SL_{50}.

The determination of sexual maturity size was performed by creating a graph that plotted the proportion of individuals with mature reproductive organs against their shell length. The shell length was divided into eight categories, each measuring 8 mm in size. The data was used to fit a logistic regression model of the following equation:

$$\%\text{Maturity} = 1 / [1 + e^{-r(SL - SL_{50})}]$$

where %Maturity is the proportion of mature individuals in the sample, r is the regression slope and SL_{50} is the size at first sexual maturity.

Statistical analysis

The data was subjected to testing in order to evaluate its normality and determine if the variances are equal. A t-test was utilized to compare the sizes of specimens (specifically SL, SH, and TW) between different sexes. The chi-square test was employed to assess the association between the sex ratio of the population and parity (1:1) using SPSS (Statistical Package for Social Sciences, version 25.0, IBM Corporation, Armonk, NY, USA), with a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Morphometric measurements

Descriptive statistics of morphometric measurements of *L. marginalis* is shown in Table 1. A total of 240 individuals were analyzed during the study period. Shell length was ranged between 49.32 to 103.45 mm with the mean value of 79.73±12.70 mm. Minimum and maximum values of shell height was 10.28 and 53.24 mm, respectively with the mean value of 36.19±12.38 mm. The mean value of total weight was 17.00±4.89 g, with the minimum and maximum values of 6.45 and 31.35 g, respectively. No significant differences ($P < 0.05$) were observed in the morphometric measurements of *L. marginalis* between male and female individuals over the study period. Mondol *et al.* (2016) reported a mean shell length, shell height, and total body weight of 59.90 mm, 29.80 mm, and 25.49 g, respectively. These findings suggest the presence of a relatively smaller yet robust population of *L. marginalis* in the current study. Nahar *et al.* (2019) documented the presence of mussels with increased body weight in the Northwest region of Bangladesh, while Natarajan and Susithira (2016) observed a similar phenomenon in culture ponds located in Tamil Nadu, India. The observed variations in morphological traits among species may be attributed to the distinct hydrological and sedimentological characteristics found in different geographical regions (Gaspar *et al*., 2002).

Table 1. Descriptive statistics of body measurement of *L. marginalis* in Brahmaputra river.

Measurements	Sex	N	Min	Max	Mean±SD	95% CL of mean	P-value
SL	Male	111	51.25	103.45	80.29±12.67	77.91-82.68	0.404
	Female	126	49.32	101.59	78.91±12.69	76.68-81.15	
	Unidentified	3	89.60	99.20	93.33±5.14	80.56-106.11	
	Combined	240	49.32	103.45	79.73±12.70	78.12-81.35	
SH	Male	111	12.61	53.24	36.45±12.38	34.12-38.78	0.631
	Female	126	10.28	53.19	35.68±12.43	33.49-37.87	
	Unidentified	3	46.10	49.30	47.77±1.60	43.78-51.75	
	Combined	240	10.28	53.24	36.19±12.38	34.61-37.76	
TW	Male	111	6.45	31.35	17.35±5.35	16.34-18.36	0.303
	Female	126	8.40	27.59	16.69±4.50	15.90-17.49	
	Unidentified	3	15.85	17.30	16.67±0.74	14.82-18.51	
	Combined	240	6.45	31.35	17.00±4.89	16.37-17.62	

Table 2. Variations in the percentage of male and female *L. marginalis* and sex ratio.

Months	Male	Female	Sex ratio	χ^2 value	P-value
Jul	45.00	55.00	1:1.22	1.23	>0.05
Aug	31.58	68.42	1:2.17	1.36	>0.05
Sep	55.56	44.44	1:0.80	2.52	>0.05
Oct	40.00	60.00	1:1.50	1.75	>0.05
Nov	70.00	30.00	1:0.43	5.26	<0.05
Dec	45.00	55.00	1:1.22	2.23	>0.05
Jan	40.00	60.00	1:1.50	1.63	>0.05
Feb	45.00	55.00	1:1.22	0.96	>0.05
Mar	55.00	45.00	1:0.82	1.55	>0.05
Apr	20.00	80.00	1:4.00	4.59	<0.05
May	60.00	40.00	1:0.67	1.64	>0.05
Jun	55.00	45.00	1:0.82	2.44	>0.05

χ2 -test, p < 0.05 = Significant difference, p > 0.05 = Insignificant difference.

Sex ratio

The outcomes of 240 studied individuals of *L. marginalis* presented that 111 (46.25%) mussels were males, 126 (52.5%) were females and 3 (1.25%) were sexually undifferentiated individuals (Table 2). However, no hermaphrodite individuals were noticed during the study period. The sex ratio calculated was 1:1.13 which was insignificant (x^2 = 15.71, P = 0.152). The sex ratio varied from month to month, with females predominating in 8 (July, August, September, October, December, January, February, and April) of the 12 months of the research. However, males were dominated during November, March, May, and June). Monthly variation in sex ratio was only found during November and April. A previously documented observation also indicated statistically insignificant but a prevalence of female dominance (male-female sex ratio = 1.00:1.08) within *L. marginalis* populations inhabiting pond ecosystems in Bangladesh (Niogee *et al*., 2019). A study conducted by Hossain *et al*. (2023) in the freshwater Swamp Forest of Bangladesh also reported statistically indifferent but female dominated population of *L. marginalis*. However, male's dominance in the mussel population of a lentic environment of Bangladesh was also reported by Siddique *et al*. (2019). Gaikwad and Kamble (2013) also reported insignificant male to female sex ratio of the similar species from Indian waters. The hydrological and temporal factors in the study area may contribute to the observed higher prevalence of females compared to males in the current study.

Gametogenic cycle and spawning activity

Based on the examination of the gonadal section, it was observed that individuals of *L. marginalis*, regardless of their sex, exhibited five distinct maturation phases that were determined by the developmental state of each gonad. The reproductive cycle consists of five distinct maturity stages: early developing, late developing, ripe, spawning, and spent stages. Each stage was noticeable with several different microscopic characters (Figure 2).

Early developing stage: Spermatogenesis occurs inside the acini of the testes in males. The lobes of the testis have a packed germinal epithelium and do not contain spermatids or spermatozoa. Additionally, the testis volume is relatively smaller compared to the visceral mass. (Figure 2A). In females, plentiful oogonia along with the pre-vitellogenic oocytes are observed along the periphery of the acini, no oocytes are found freely within the lumen (Figure 2B).

Late developing stage: In males spermatocytes undergo differentiation into spermatids, which subsequently migrate towards the central region of the lumen. A tiny proportion of spermatozoa are observed within the lumina of the acini. The germinal epithelium is situated in the outermost region (Figure 2C). In females, free oocytes are freely located within the lumen, while the left-over oocytes are merged to the surface. The acini are enclosed by connective tissue and have very few interstitial gaps (Figure 2D).

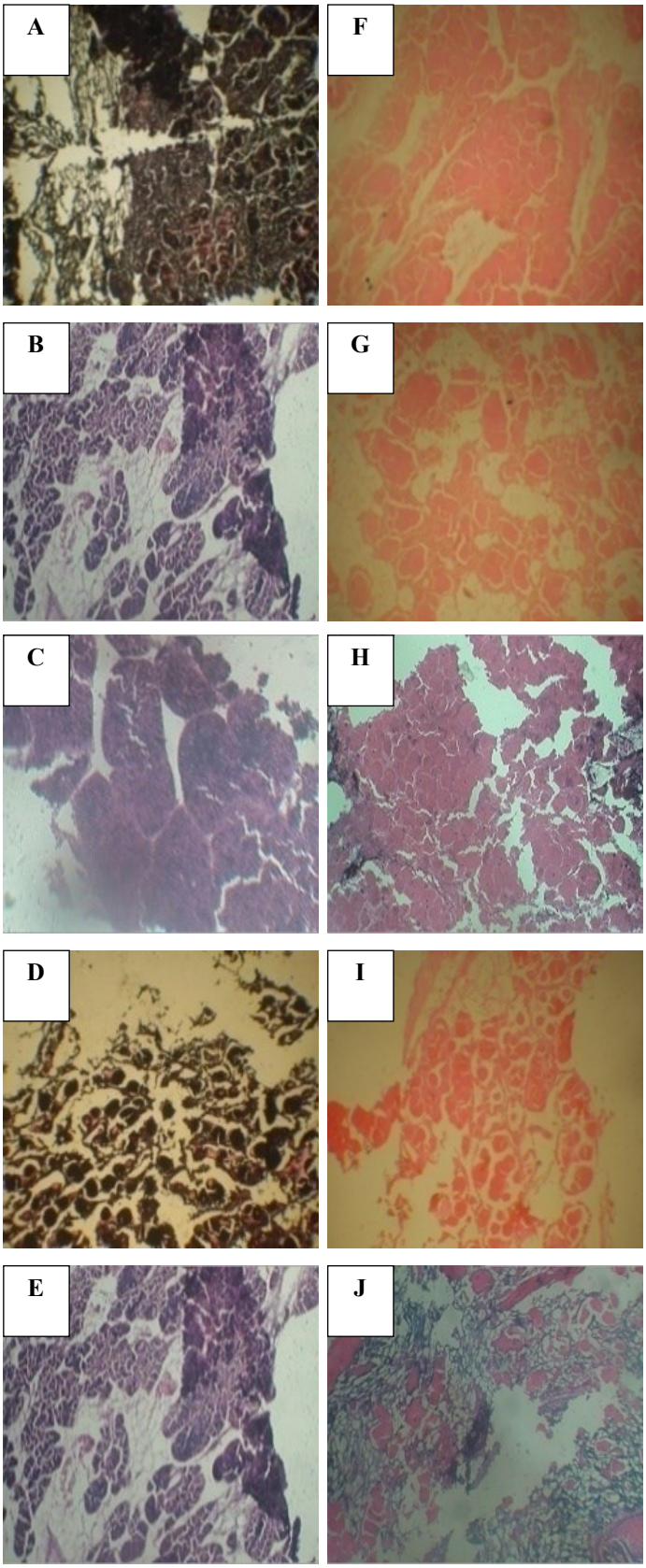

Figure 2. *Gonad developmental stages of L. marginalis at Haor. (A-E) - males; (F-J) - females; (A, F = Early developing stage, B, G = Late developing stage, C, H = Ripe stage, D, I = Spawning stage, E, J = Spent stage.*

Ripe stage: In males, the acini contain fully developed spermatozoa, oriented with their tails directed towards the central region of the lumen. In highly mature specimens, bands of spermatozoa are observed near the follicle wall (Figure 2E). In females, the ovary exhibits a consistent coloration. Acini contain free oocytes within the lumen. Oocytes become polygonal due to their abundance, and the follicular wall thins (Figure 2F).

Spawning stage: In males, there is a reduction in the quantity of spermatozoa located in the central region of the acini, where their flagella are oriented to the lumen. There are observable areas that indicate the continuous release of gametes (Figure 2G). In females, the walls of the follicles undergo degradation, resulting in partially empty acini. The acini contain varied post-vitellogenic oocytes reliant on the stage of spawning. Some follicles are blank as they have released their gametes (Figure 2H).

Spent stage: In males, there is the presence of degenerated connective tissue, along with a limited quantity of undischarged spermatozoa and a significant number of hemoglobin-containing cells in the tubules (Figure 2I). In females, follicles are fragmented, dispersed, and depleted in number; resorbing follicles contain just a few remaining oocytes, and phagocytes have been seen within and between the follicles (Figure 2J). Gonadal presence was observed consistently throughout the year, albeit in relatively low proportions (Figure 3). The gonadal development stages were monitored over the course of the year, and varying percentages were observed. However, the highest values for early and late developing stages were recorded in January and March, respectively. The presence of ripe gonads was observed in July for male (77.78%) and August for female (53.85%). The activity of the spawning *L. marginalis* for male was highest during October (50%) and for female during November (83.33%). The distribution of the maturity stages based on shell length is depicted in Figure 4. The maturity stages were classified according to the shell length, ranging from 49 mm to 108 mm. No apparent pattern in the arrangement of maturity stages was noticed throughout the duration of the study. The smallest ripe male was observed in the shell length 49-53, while in females from 58-63 mm. Spawning stage was appeared in the length of 58-63 mm both in male and female. However, ripening, and spawning stages were in different shell lengths ranging from 58-63 to 88-93 mm for both sexes. Hence, it is feasible to evaluate the maturation and spawning of *L. marginalis* at shell lengths falling within the aforementioned ranges. Several spawning events have formerly been confirmed for populations of *L. marginalis* (Mishra *et al.*, 2008; Verma *et al.*, 2017). However, the present findings contradict with Siddique *et al.* (2020) and Diadhiou *et al.* (2019) who reported spawning peak of this species during rainy season based on the histological observation of gonads. The potential reasons for this phenomenon could be attributed to the increased accessibility of food resources, favourable hydrological conditions, and enhanced opportunities for mating in the studied riverine water (Morriconi *et al.*, 2002; Saeedi *et al.*, 2009; Silva-Cavalcanti *et al.*, 2018). Furthermore, the frequency and duration of spawning events can exhibit significant variation depending on factors such as species, geographical location, and environmental conditions (Gosling, 2003).

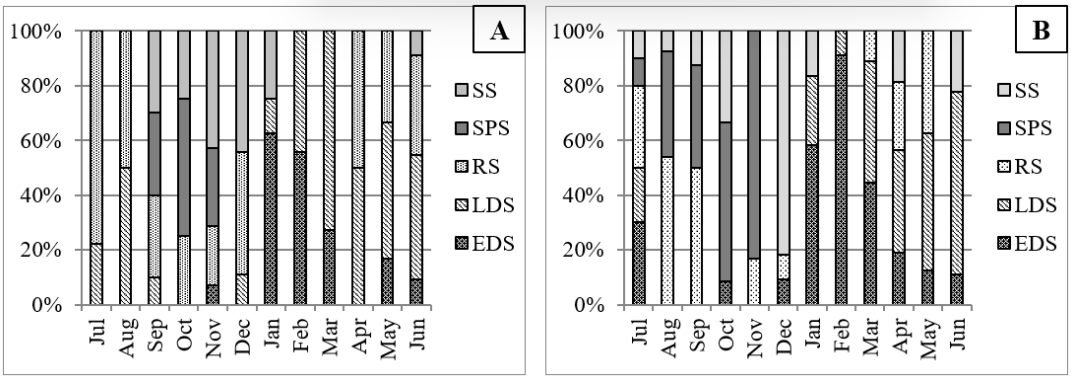

Figure 3. *Monthly percentage composition of gonad developmental stages, A = Male, B = Female.*

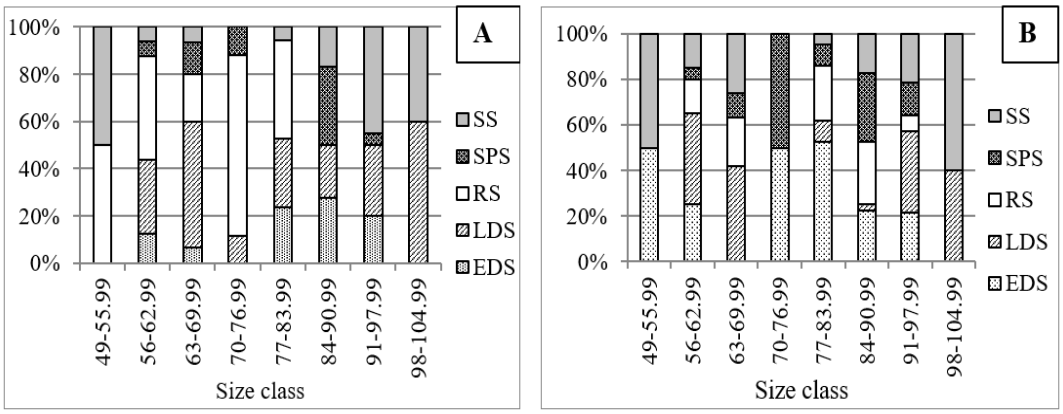

Figure 4. *Percentage composition of gonad development stages according to specimen size, A = Male, B = Female.*

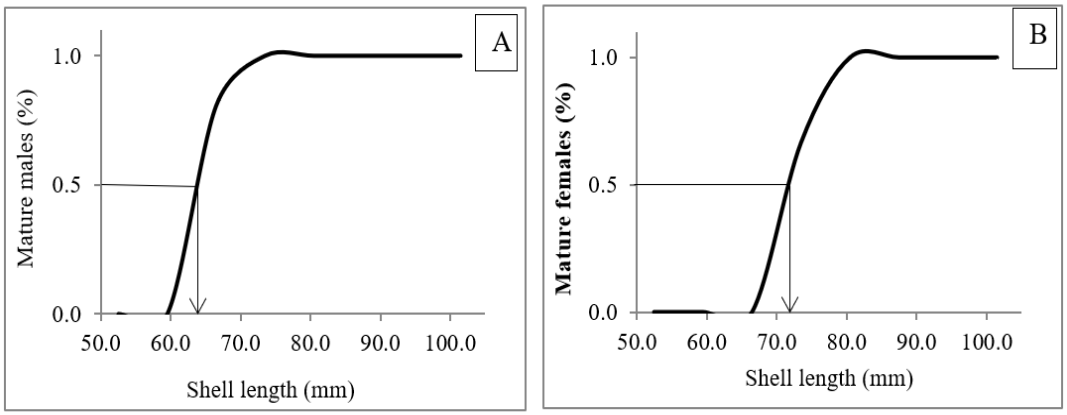

Figure 5. *Logistic curves for estimation of the size at first sexual maturity (L_{50}) of L. marginalis (A) female and (B) male in the Brahmaputra River.*

Condition index

The annual fluctuations in condition index (CIs) are depicted in Table 3. The condition index of *L. marginalis* appeared to be influenced by gametogenic development, as indicated by our findings. The mean value of the condition indices reached its peak in July in males (0.96±0.32) and September in females (0.96±0.24), coinciding with the period of gonad ripening. Nevertheless, it is likely to attribute the abrupt decrease in CIs observed in males between July and September, and in females between September and October might be due to the physiological effects of weight loss caused by the discharge of gametes and the subsequent energy expenditure during spawning. While the condition index of *L. marginalis* generally aligned with its reproductive stages, specifically indicating the initiation of spawning, it did not exhibit a strong correlation with other stages of gonadal development. This phenomenon can be accredited to the occurrence of individuals in varying gonadal stages among both males and females within a given month. In November, a majority of males exhibited partial spawning, while a subset displayed early development, maturation, and subsequent expenditure of reproductive resources. This observation may provide an explanation for the absence of a correlation between the condition index and reproductive stages. The CIs and their peak months were varied from the results of Hossain *et al.* (2023), Siddique *et al.* (2020) and Niogee *et al.* (2019). The contrasts between the current results and those of the prior research imply that the morphometry and maturation of the mussel are more closely related to its environmental characteristics (Lagos *et al.*, 2016).

Table 3. Monthly variations in the condition index (mean ± SD) of *L. marginalis*.

Months	Male	Female
Jul	0.96 ± 0.32	0.62 ± 0.13
Aug	0.92 ± 0.12	0.90 ± 0.32
Sep	0.62 ± 0.10	0.96 ± 0.24
Oct	0.60 ± 0.11	0.32 ± 0.09
Nov	0.55 ± 0.21	0.56 ± 0.16
Dec	0.88 ± 0.28	0.55 ± 0.10
Jan	0.56 ± 0.18	0.46 ± 0.09
Feb	0.44 ± 0.11	0.48 ± 0.10
Mar	0.40 ± 0.18	0.56 ± 0.12
Apr	0.72 ± 0.23	0.59 ± 0.08
May	0.68 ± 0.14	0.77 ± 0.19
Jun	0.65 ± 0.16	0.42 ± 0.12

Table 4. Shell length of first sexual maturity of *L. marginalis* from Brahmaputra River.

Size class	Male		Female	
	Number	Maturity (%)	Number	Maturity (%)
49-55.99	2	100.00	2	0.00
56-62.99	11	9.09	20	20.00
63-69.99	18	44.44	19	31.58
70-76.99	7	71.43	4	50.00
77-83.99	23	56.52	21	33.33
84-90.99	27	48.15	40	57.50
91-97.99	21	4.76	14	21.43
98-104.99	6	0.00	5	0.00
Total	111		126	

Size at first sexual maturity

Based on gonad microscopic inspection, the sexual maturity was estimated of 111 males and 126 females sampled in July 2019 to June 2020 (Table 4). Histological investigation was done to certify the shell length of the individuals that reached gonadal maturation. In males, smallest length for sexually mature individuals was 49-55.99. Furthermore, 100% of the individuals were matured with the SL 49-55.99 mm and >50% were sexually mature with the SL of 70-76.99 and 77-83.99. In females, smallest individuals with sexually mature gonads were SL, 56-62.99 and the collected 20% of the individuals were sexually mature. Furthermore, >50% mature females were observed at SL, 70-76.99, and 84-90.99 length groups, respectively.

The utilization of sigmoid functions enables the examination of the association between the percentage of mature individuals and the overall length of mussels. This approach facilitates the tracking of sexual maturity levels based on size and provides a precise estimation of the length at which 50% of the individual's exhibits sexual maturity. The estimated SL_{50} for male and female mussels were 63.25 and 72.10 mm, respectively with high correlation coefficients R^2 of 0.95 and 0.94, respectively (Figure 5A, B). The variation in the size at which males and females reach sexual maturity could be attributed to disparities in growth rates amid the gender (Power and Keegan, 2001) or a potential interruption in the onset of sexual maturation in females (Cledón *et al.*, 2008). Furthermore, the variations in sexual maturity size between males and females can also be attributed to several additional factors, as identified by Ilano *et al.* (2003). These factors include males' competition for mates, the conflict between growth and reproduction, parasitic infections, and various environmental and fishing pressures.

Conclusion

The current study reports on the sex ratio, spawning season, condition index and lengths at first maturity of *L. marginalis*. From the present data we conclude that freshwater mussel *L. marginalis* collected from the Brahmaputra River had female dominated insignificant sex ratio (M:F = 1:1.3) and a continuous annual gonad developmental stage consisted early developing, late developing, ripe, spawning and spent. By comparing the calculated histological analysis and CI it was observed that the males achieve gonadal maturation before the female. The estimated SL_{50} for male and female mussels were 63.25 and 72.10 mm, respectively. Further studies should investigate to correlate the gonadal developmental stages and size at sexual maturity with environmental factors to establish a breeding protocol for propagation in captivity for aquaculture purpose.

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