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Original Article

Temporal changes in glochidia larval occurrence and condition index in freshwater mussel, *Lamellidens marginalis* (Lamarck, 1819) collected from a wetland of Bangladesh

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Abstract
Information on glochidia larval occurrence and release time from the brooding mussels are crucial for the development of larval rearing system or commercial collection of juveniles from the wilds. We report monthly changes in glochidia larval occurrence and condition index (CI) in freshwater mussel, *Lamellidens marginalis*, collected from a wetland in Bangladesh. Specimens were drawn monthly over a year from October 2021 to September 2022. *Lamellidens marginalis* females displayed glochidia larvae in the supra-branchial chamber over ten months from March to December. No glochidia larva was reported in females during January and February when the water temperature remained below 18°C. All females carried glochidia larvae during March and April, indicating no larval shedding at this time. The percentage of females containing glochidia larvae dropped suddenly in May (22.22%), suggesting that glochidial release from the gill chamber to the environment was initiated by May. Larval presence in only some females (22% to 86%) at each sampling date from May to September implied an extended glochidial discharge period till September when the water temperature was above 24°C. The CIs varied from 0.36 to 0.79, with two distinct peaks in December and March when all females carried glochidia larvae. In conclusion, monthly changes in larval occurrence and discharge could be primarily related to temperature and the existence of larvae in mussels could affect the CIs in *L. marginalis*.

Keywords: condition index; freshwater bivalve; glochidia larvae; lentic habitat; seasonality; temperature; Unionidae

1 | INTRODUCTION

Molluscs are an important fishery today, and bivalves are the most important commercial group of molluscs (Uddin *et al.* 2024). Molluscs, primarily bivalves, contributed over 13.8% of the global edible aquatic animal production in 2020 (FAO 2022). Freshwater mussels play an important role in aquatic ecosystem. Freshwater mussel, *L. marginalis*, is abundant in inland lentic and lotic habitats of south and southeast Asia (Ghosh and Ghose 1972; Dan *et al.* 2001). This species is of high commercial importance in Bangladesh and widely utilised to produce lime and used as fish and poultry feed (Siddique *et al.* 2020). In addition, it is the most suitable freshwater pearl-producing bivalve species in Bangladesh and is widely used for the production of pearls (Tanu *et al.* 2021). However, the wild populations of *L. marginalis* are decreasing, as their supply is solely dependent on wild stocks. Little attempt has so far been undertaken to develop a farming system due to inadequate information on the species' life cycle and glochidia larval release time for collecting juveniles to supply required seeds to the fish farmers (Siddique *et al.* 2020). Mishra *et al.* (2008) evaluated the growth performance of *L. marginalis* under farming system when they studied survival, shell increments and weight gain by using certain drugs to prevent bacterial growth. Sultana *et al.* (2005) reported better growth performance of *L. marginalis* in pond bottom rather than hanging culture method.

Unlike other bivalves, freshwater unionid mussels have a unique life cycle exhibiting brooding larvae called glochidia in the supra-branchial chamber of females, and the drifting glochidia expel from the female mussel to lead a parasitic mode of life in fish before becoming a free-living form (Kat 1984; Helfrich *et al*. 2019). During spawning, male unionids discharge gametes to the water through the excurrent siphon. The female mussels capture the spermatozoa accidentally, along with food particles, through the incurrent siphon. Fertilisation occurs internally in the female's modified gill chamber, where the embryos advance into glochidia larvae (Mackie 1984). Once discharged from the brood, the glochidia larvae search for host fish to lead a parasitic mode of life in it. However, these larvae cannot survive for long outside of the female mussel without a host fish. Having suitable host fish species, the larva attaches and encysts onto the host's body for a couple of weeks until it becomes a juvenile mussel and drops off the fish to lead a free-living mode of life. The main advantages of having fish as hosts are thought to be related to nutrition (Dillon 2004) and dispersal, including upstream colonisation and the promotion of connectivity among populations (Barnhart *et al.* 2008; Schwalb *et al.* 2011; Horký *et al.* 2014). Glochidia larvae are commonly released from females when the surrounding environment is favourable such as suitable temperature, available host fish species and so on (Helfrich *et al.* 2019; Sangsawang *et al.* 2019; Vikhrev *et al.* 2019). The timing of glochidial expulsion may indicate the probable time of juvenile collection from the natural habitat for commercial farming. The environmental parameters during glochidial discharge might be useful for the development of hatchery and nursery systems of the species to supply an adequate quantity of seeds in due time.

There has been no attempt at freshwater mussel culture in Bangladesh due to inadequate knowledge of the timing of glochidial release in natural stock or the lack of mussel hatcheries. Therefore, it is essential to understand the complete scenario of glochidial occurrence and discharge from the female *L. marginalis* of a specific habitat. Considering the aforementioned context, the current study was conducted to investigate the temporal changes in the occurrence of glochidia larvae in the gill chambers of females and the condition index (CI) of *L. marginalis* collected from a wetland at Trishal, Mymensingh, Bangladesh.

2 | METHODOLOGY

2.1 Sampling framework

Freshwater mussel, *L. marginalis*, specimens were collected from Darikathal Beel, Trishal, Mymensingh (24.5827°N 90.3945°E) (Figure 1). Specimens were collected monthly from October 2021 to September 2022 by hand-picking. Following collection, specimens were kept in a bucket and conveyed quickly to the Aquatic Ecology Laboratory of Bangladesh Agricultural University, Mymensingh. Twenty adult mussels (>56 mm shell length; SL) were randomly selected from the monthly specimens. Overall, 240 mussel individuals were analysed over the 12-month study period. Morphometric measurements of each specimen, such as, shell length (SL), shell height (SH), and shell width (SW), were taken using digital callipers (Model: Heng Liang, China). Next, the specimens were dissected chronologically using a keen knife. Then, the soft tissue was taken out from the shell and kept on tissue paper with proper numbering to remove excess water. Wet tissue weight was recorded with an electronic balance (Model: eki 600-2n, Korea). Gonadal smears and gills were taken in labelled Petri dishes to determine the sex from smears and the presence of glochidia in gills. The shells were exposed to sun-dry, and the dry shell weight (DSWT) was noted. The CI was calculated as the ratio of wet tissue weight to DSWT, as described by Uddin *et al.* (2010).

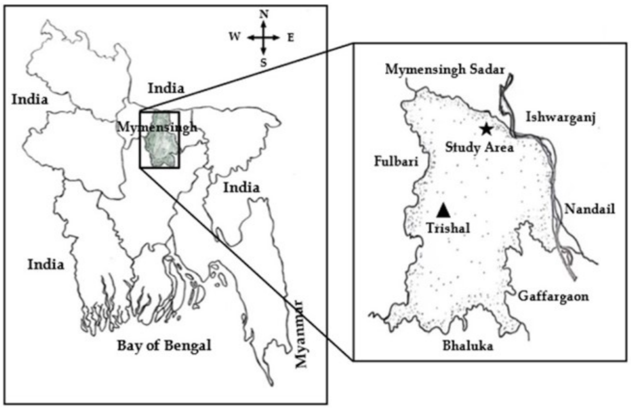

FIGURE 1 Sampling site of *Lamellidens marginalis* at Darikathal Beel, Trishal, Mymensingh, Bangladesh.

2.2 Glochidia larval occurrence

The gonadal smears were examined under a microscope (Novel Biological Binocular Microscope XSZ-107T, China) to identify sex. The presence of spherical-shaped eggs in the gonadal smears indicated females. The occurrence of testicular materials, such as spermatogonia, spermatids or spermatozoa in the form of small granules, indicated males. Freshwater mussel *L. marginalis* exhibits internal fertilisation. The male releases sperm directly into the water, where it enters the female through the incurrent siphon. Following internal fertilisation in females, the fertilised eggs grow into a larval stage known as a glochidium (plural: glochidia) in the supra-branchial chamber of the female. Prior to discharge, the glochidia develop inside the gill of the female mussel for 2 – 4 weeks, where they are constantly flushed with oxygen-rich water. The released glochidia larvae temporarily parasitise fish by attaching themselves to the fins or gills of the fish. Therefore, the gills of females were examined under a microscope to determine the timing of glochidia larval occurrence over an annual cycle.

2.3 Monitoring of water quality parameters

Environmental parameters of the study area, such as temperature, dissolved oxygen (DO), and pH, were recorded monthly during sampling. Water temperature and DO were recorded from three different locations of the beel at each sampling date using a direct reading DO meter (model: Lutron, PDO 519, Taiwan). The pH of the beel's water was recorded using a pH meter (model: pHep® pH meter, Hanna Instruments, USA) on the spot.

2.4 Data analysis

All the data obtained from the study were analysed statistically and expressed as a mean (± SD) using MS Excel. A chi-square test (χ^2-test) was run to determine whether any deviation occurs between the ratio of male to female from 1 : 1. Pearson's product-moment correlation was performed to follow the degree of correlation between the CI and the percentage of females with glochidia larvae.

3 | RESULTS

3.1 Water quality parameters

Monthly changes in water quality parameters, such as temperature, DO, and pH, of the habitat varied considerably, as shown in Figure 2. Water temperature fluctuated from 15.8 to 30.4°C with a mean value of 23.6 ± 4.7°C. Over the 12 months, the maximum temperature was noted in August and the minimum was reported in January. The DO concentration in the sampling site varied from 5.48 mgL^{-1} to 8.33 mgL^{-1} and the values remained above 5 mgL^{-1} over the study period. Mean DO concentration was 6.97 ± 0.74 mgL^{-1} during the study period. The waterbody remained slightly alkaline throughout the investigation period, with a pH range between 7.25 and 7.83 having a mean value of 7.53 ± 0.19.

3.2 Biometric measurements

Biometric measurements such as SL, SW, shell SH, soft tissue wet weight (TWWT) and (DSWT) are reported in Table 1. Of the 240 *L. marginalis* specimens analysed, the mean SL ranged from 69.76 mm to 94.13 mm, the mean SH varied from 21.82 mm to 41.20 mm, the mean SW differed from 21.55 mm to 52.33 mm, the mean TWWT varied from 5.64 g to 18.38 g and the mean DSWT ranged from 7.98 g to 32.0 g.

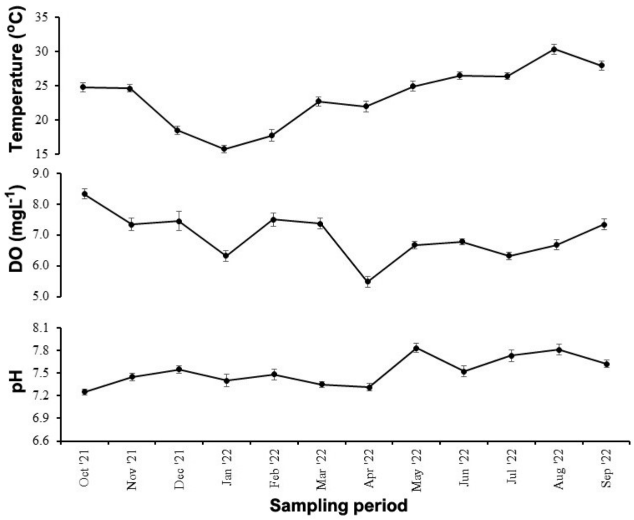

FIGURE 2 Monthly changes in mean values (± SD) of water temperature, dissolved oxygen (DO), and pH of the sampling site.

3.3 Sex ratio

Of 240 *L. marginalis* analysed, 117 were male and 123 were female. The male-female sex ratio was 1 : 1.03. The χ^2-test conducted for the study showed no significant deviation in the sex ratio of males to females from the expected 1:1 ratio. No sexually indifferent mussel was identified from the analysed specimens over the study period.

3.4 Occurrence of glochidia in female *L. marginalis*

Glochidia larvae were found in the supra-branchial chamber of female *L. marginalis*, and they were attached to the gills of female mussels (Figure 3). Monthly changes in the occurrence of glochidia larvae in *L. marginalis* are shown in Table 2. No females contained glochidia larvae in January and February when the water temperature remained below 18°C. Glochidia larvae were present in all females in March and April, indicating that no larval discharge might have occurred by April. The absence of glochidium larva in a remarkable number of females (seven out of nine) in May might indicate that the larvae were released from the females by May when the water temperature was above 24°C. Larval existence remained low from May to September, implying prolonged glochidial discharge during that time at 24.95 to 30.37°C. All females contained glochidia from October to December, signalling no larval discharge during this period. As no glochidium larvae were reported in females during January, all larvae might have been released before the sampling in January. All the females, whether carrying glochidia or not, were identifiable by observing the gonadal smears under a microscope, with visible eggs indicating that the animals were not castrated due to parasitic infestation.

TABLE 1 Biometric measurements of *Lamellidens marginalis* (mean ± SD) collected from Darikathal Beel, Trishal, Mymensingh, Bangladesh.

Month	*N*	Shell length (mm)	Shell height (mm)	Shell width (mm)	Tissue wet weight (g)	Dry shell weight (g)
Oct 2021	20	69.76 ± 13.16	35.34 ± 7.45	21.55 ± 5.16	5.64 ± 3.77	7.98 ± 3.46
Nov 2021	20	73.35 ± 6.28	36.03 ± 4.19	22.68 ± 2.73	7.09 ± 1.56	11.30 ± 5.45
Dec 2021	20	70.49 ± 2.27	21.82 ± 0.66	35.12 ± 1.77	6.93 ± 0.48	8.83 ± 0.96
Jan 2022	20	82.52 ± 5.42	41.20 ± 3.53	26.35 ± 2.76	10.64 ± 1.90	20.94 ± 7.08
Feb 2022	20	87.95 ± 4.62	31.10 ± 2.47	47.80 ± 2.30	12.49 ± 3.70	28.70 ± 7.58
Mar 2022	20	82.57 ± 4.46	27.33 ± 2.40	43.35 ± 1.85	16.54 ± 4.99	21.62 ± 4.62
Apr 2022	20	85.35 ± 4.87	27.78 ± 2.56	44.08 ± 2.55	18.38 ± 3.47	26.48 ± 5.45
May 2022	20	85.27 ± 6.61	29.56 ± 2.20	45.58 ± 5.0	11.05 ± 3.59	32.0 ± 7.87
Jun 2022	20	85.05 ± 4.16	28.73 ± 2.85	40.67 ± 2.50	16.22 ± 3.41	30.04 ± 7.56
Jul 2022	20	83.12 ± 9.0	25.61 ± 5.39	41.22 ± 3.99	11.34 ± 3.34	21.81 ± 12.17
Aug 2022	20	89.05 ± 10.03	27.34 ± 5.39	45.45 ± 6.31	14.26 ± 5.13	31.10 ± 17.43
Sep 2022	20	94.13 ± 8.36	36.09 ± 4.09	52.33 ± 4.91	13.50 ± 3.52	23.68 ± 13.31

TABLE 2 Observation of glochidia larvae in monthly specimens of *Lamellidens marginalis*.

Month	*N* = 20		No. of females with glochidia	No. of females lacking glochidia	% of females with glochidia (%)
	Male	Female			
Oct 2021	8	12	12	0	100
Nov 2021	4	16	16	0	100
Dec 2021	7	13	13	0	100
Jan 2022	8	12	0	12	0
Feb 2022	13	7	0	7	0
Mar 2022	13	7	7	0	100
Apr 2022	15	5	5	0	100
May 2022	11	9	2	7	22.22
Jun 2022	13	7	3	4	42.86
Jul 2022	7	13	6	7	46.15
Aug 2022	11	9	2	7	22.22
Sep 2022	7	13	11	2	84.61
Total	117	123	77	46	

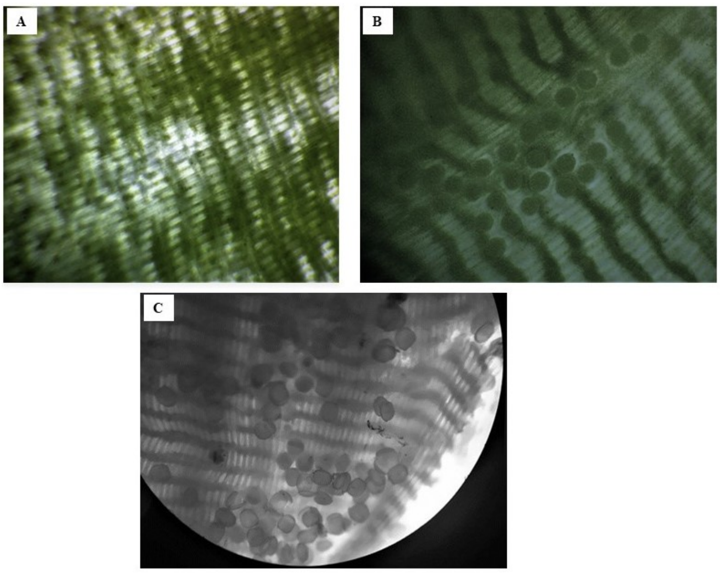

FIGURE 3 Occurrence of glochidia under the microscope in the gill chamber of female *Lamellidens marginalis*. A, gill chamber without glochidium; B, the occurrence of glochidia in females; C, morphologically developed glochidia in females.

3.5 Condition index

In the current study, remarkable temporal fluctuations were clearly visible in CIs, as shown in Figure 4. Mean CIs varied from 0.36 to 0.79 over the study period. Pearson's product-moment correlation coefficient indicated that the CI has a strong positive correlation (r^2 = 0.87) with the percentage of females with glochidia larvae in their gills. The highest CI peak was recorded in December (0.79), when all the females (100%) had glochidia in their gill chambers, while the lowest mean CI was recorded in May (0.36) when only 22.22% of females were identified with glochidia in their gill chambers. The second CI peak noted in March (0.76) was also lined up when all females exhibited glochidia larvae in their gills. CIs increased steadily from October to December, and the values decreased sharply till February. A sudden increase in CIs was noticed from February to March, followed by a consistent decrease until May. CIs increased gradually after May and continued till July (0.62). The CI values decreased slightly in August and then accelerated again till the remainder of the study period.

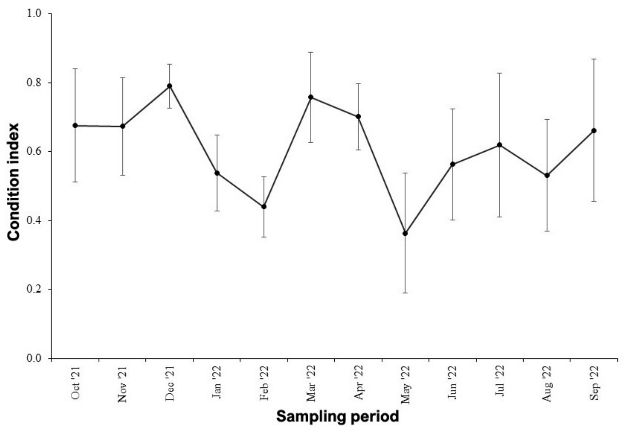

FIGURE 4 Monthly changes in condition index (mean ± SD) of *Lamellidens* marginalis collected from Darikathal Beel, Trishal, Mymensingh over the study period.

4 | DISCUSSION

To our knowledge, this is the first report on temporal patterns of glochidia larval occurrence in freshwater mussel (*L. marginalis*). The study revealed that *L. marginalis* females displayed glochidia larval occurrence in the gill chamber for 10 months from October to December and March to September. The larval occurrence could be related to spawning events of the *L. marginalis* species, as they spawn throughout the year in tropical environments, although the degree of spawning activities varies seasonally (Behera *et al.* 2014). This larval calendar was also supported by Siddique *et al.* (2020), who reported that natural populations of *L. marginalis* spawn year-round except during December when the water temperature remains very low. In unionids, fertilisation occurs internally in the modified gill chamber of the female, where the embryos advance into glochidia larvae (Mackie 1984). Therefore, glochidia larvae are likely to be present in the marsupia of females over the prolonged spawning period of mussels.

A decline in the percentage of females containing glochidia is an indication of glochidial release into the environment. All females had glochidia larvae from October to December and March to April, but a sudden decline was observed in the percentage of females containing glochidia in May (22.22%), which was an indication of glochidial release. Glochidial discharge continued till September. Glochidial shedding is generally related to the environmental set-up and availability of host fishes in the habitat. Glochidia are more often expelled by females under favourable environmental conditions such as suitable temperature, available host fish species and so on (Helfrich *et al.* 2019; Sangsawang *et al.* 2019; Vikhrev *et al.* 2019). Hastie and Young (2001) suggested that certain environmental cues trigger the shedding of glochidia larvae from the branchial brooder. This suggests that the environmental conditions could be favourable from May to September for glochidia larvae to lead a parasitic mode of life in fish. Glochidia larval discharge initiated by May could be associated with the spawning season of the host fish species, as outlined by Haggerty and Garner (2000). We reported that glochidial release commenced in May, which is similar to the glochidial release of *Lampsilis cardium* and *Potamilus alatus* in the upper Mississippi river (Holland-Bartels and Kammer 1989). Soler *et al.* (2018) reported that the glochidial release of *Margaritifera auricularia* occurs in April, which is also in close conformity with our study.

In our study, we observed no glochidium larva in the gills of females in January and February, when the water temperature remained below 18°C. On the other hand, glochidia were observed in the gills of the females in the remaining 10 months when the water temperature ranged from 18.54°C to 30.37°C. Glochidial release occurred from May to September when the water temperature varied from 24.95°C to 30.37°C. Even slight changes in certain water quality variables determine the time of gametogenesis and spawning of bivalves (Baba *et al.* 1999; Uddin *et al.* 2007; Barber and Blake 2016). The single most critical environmental factor influencing the maturation and spawning of *L. marginalis* is temperature (Gaikwad and Kamble 2013; Niogee *et al.* 2019; Siddique *et al.* 2020). Our findings reflected that glochidial discharge from *L. marginalis* could be temperature dependent and might occur within certain temperature limits.

Aquatic animal reproduction success depends on the concentration of DO in the water. It has been reported that major spawning pulses of *L. marginalis* were often associated with relatively higher DO levels in the environment (Gaikwad and Kamble 2013; Siddique *et al.* 2020). In the current study, we observed DO levels ranging from 5.48 to 8.33 mgL^{-1} throughout the study period, which were within the suitable range for *L. marginalis* growth, gametogenesis, spawning, glochidia larvae development and larvae release for leading a parasitic mode of life, as supported by Gaikwad and Kamble (2013) and Helfrich *et al.* (2019).

Furthermore, pH is a crucial factor for bivalves, and alkaline water favours better formation of calcareous shells (Jones 1981). The filtration rate, growth, gametogenic phenology and spawning of bivalves are influenced by pH (Hornbach and Childers 1987; Islam *et al.* 2020). In the current study, the pH ranged from 7.25 to 7.83. The alkaline water of the habitat might indicate a suitable condition for the physiological activities of *L. marginalis*. Hincks and Mackie (1997) also noted that European lakes with pH levels below 7.3 were devoid of the zebra mussel *Dreissena polymorpha*. Similar pH conditions were also reported by Siddique *et al.* (2020) and Niogee *et al.* (2019), where the bottom water was alkaline, with a pH of more than 7.7 throughout the year, indicating favourable conditions for the growth and reproduction of *L. marginalis*.

Unlike fishes, in the case of bivalves, except scallops, the gonad is mingled with the visceral mass and cannot be isolated completely from the body. Therefore, it is practically difficult to measure the reproductive health of the bivalves in terms of the gonadosomatic index, as is done in fish. In that context, the CI is widely used in bivalves to express the nutritional and reproductive health of the animal (Seed and Suchanek 1992; Smaoui-Damak *et al.* 2006; Uddin *et al.* 2010). In our study, remarkable variations in the CI values were noticed, with two distinct peaks in December and March. The highest mean CI was recorded in December, when all females had glochidia in their gill chambers, while the lowest mean CI was recorded in May, when 22.22% of females had glochidia in their gill chambers. In *L. marginalis* from a lentic environment in Mymensingh, Siddique *et al.* (2020) identified three peak CI periods of *L. marginalis* obtained from a perennial habitat in January, April and July. Meanwhile, Niogee *et al.* (2019) reported two CI peaks of *L. marginalis* in June and October from a mussel culture habitat. The little discrepancy in peak CIs with the above data might be attributed to environmental and habitat factors. When making decisions regarding the proclamation of a harvest ban period for a certain population, fisheries managers can greatly benefit from this differential in practice. In bivalves, CIs generally increase with gametogenesis, peak when ripe, and decline when the gametes are released during spawning (Seed and Suchanek 1992; Siddique *et al.* 2020). However, the somatic growth of the animal and energy loss during spawning could also contribute to the CI values. In freshwater unionid bivalves, internal fertilisation occurs in, and the embryos develop into mature larvae, called glochidia, in the gill chamber of females (Mackie 1984). Therefore, a decline in CIs in *L. marginalis* might not indicate the release of ova due to spawning, but a drop in CIs could indicate the release of mature glochidia larvae from the brooding females. The correlation coefficient between CIs and the percentage of females with glochidia larvae (r = 0.87) also supported the above statement. Thus, unlike other bivalves, glochidial occurrence could be a vital factor in the fluctuation of CIs in freshwater unionid mussels.

In conclusion, the temporal pattern of glochidia larval occurrence and release from *L. marginalis* was reported from monthly collected specimens from a wetland over a year. Sexes separate in *L. marginalis* and the male-to-female sex ratio was 1.00:1.03. Temperature is thought to play a vital role in glochidia larval occurrence and discharge from the females to the environment. Glochidia larvae occurred in the supra-branchial chamber of female mussels from March to December, whereas the larval stage was absent in females in January and February when the water temperature was below 18°C. Glochidial discharge occurred from May to September from the gill chambers of female *L. marginalis* to the environment, as some females lacked glochidia larvae during these months when the water temperature remained above 24°C. The CI varied from 0.36 to 0.79, with two distinct peaks in December and March. Glochidia larval's presence or absence could be a crucial factor in CI fluctuations of *L. marginalis* and other unionid bivalves, in addition to gonadal maturation, spawning activities, and somatic growth. Current findings could be useful for the development of hatcheries and larval rearing systems of *L. marginalis* in captivity and the collection of good quantities of juveniles from the wild for commercial farming of this highly commercial species.

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CONFLICT OF INTEREST
The authors declare no conflict of interest associated with this publication.

AUTHORS' CONTRIBUTION
MAS research design, sample collection, laboratory analysis, statistical analysis and draft manuscript (manuscript writing); SB sample collection, laboratory analysis and manuscript editing; MSH research design and manuscript editing; NAS laboratory analysis, data analysis and manuscript editing; MJU research design, research supervision, critical reviewing and finalisation of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of the study will be made available on a reasonable request from the corresponding author.

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