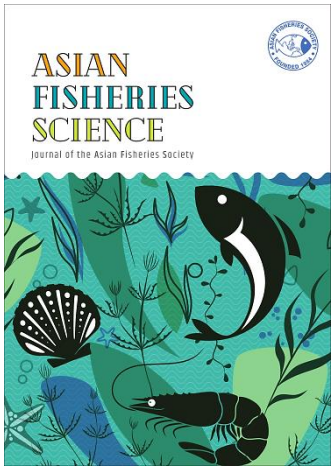

Ovarian Cycle of Freshwater Pearl Mussel, *Lamellidens marginalis* (Lamarck, 1819) Collected from a Culture Pond in Bangladesh

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Abstract

The current study was undertaken to investigate the ovarian cycle of freshwater pearl mussel, *Lamellidens marginalis* (Lamarck, 1819) collected monthly from a rearing pond in Bangladesh. Gonadal maturation was determined from 2 to 3 mm thick slices of dorso-ventral section cut from the middle of the mussels for histology. The overall male-female sex ratio, 1:1.08 did not deviate significantly from the 1:1 ratio as expected (χ^2-test). *Lamellidens marginalis* females exhibited a prolonged spawning period from February to November when water temperature remains above 22 °C. Oogenesis occurred round the year although the majority of the females were in early and late-developing stages during January and February. Four distinct peaks in maturity indices (MIs) of *L. marginalis* females were noted during October, February, June and August when most of the females were ripe and ready for spawning. Condition indices (CIs) ranged from 0.42 ± 0.23 (September 2016) to 0.85 ± 0.25 (October 2015) by mean (± SD) exhibiting five peaks over 12-month study period. Further study should be designed to investigate the timing and brooding pattern of this freshwater unionid species.

Keywords: unionidae, histology, maturity index, condition index

Introduction

Shellfish constitutes a significant part of the world fishery today. Bivalves alone contributed 11.6 % to global aquatic production during 2016 and ranked 3rd position just next to finfish and seaweeds (FAO 2018). Some bivalves such as pearl oysters and mussels produce natural pearls. In Bangladesh, molluscs are extensively used for the production of lime and feed for poultry, shrimp and fish.

The quantity of reproductive output and the time and duration of spawning are crucial for understanding life history, population dynamics, for proper management of a particular species. Numerous techniques have been used to assess the reproductive condition of bivalve molluscs (Choi et al. 1993; Chung et al. 2001; Park and Choi 2004; Uddin et al. 2007; 2013). The suitability of the methods for the estimation of reproductive output is principally related to the reproductive strategy of the species (Murua and Saborido-Rey 2003). Thus, prior knowledge on the reproductive biology of the species is essential. As gonad is an integral part of the visceral mass and cannot be separated from the main body in bivalves except in scallops, the gonadosomatic index (GSI) cannot be estimated directly. Instead, histological methods coupled with changes in condition indices over time have been most widely and frequently used, since histology provides visual information about gonadal tissues (Lango-Reynoso et al. 2000). The gametogenic status can be graded based on the microscopic appearance of gonadal tissues through histological preparations (Lango-Reynoso et al. 2000; Uddin et al. 2007). The gonadal activity of *Lamellidens marginalis* (Lamarck, 1819) are bradytictic in nature (Ghosh and Ghose 1972). Freshwater mussel, *L. marginalis* is a prolific breeder, and it spawns round the year (Misra et al. 2010; Behera et al. 2014). Gaikwad and Kamble (2013) reported that water temperature between 20 to 25 °C is most suitable for spawning activity of *L. marginalis* in a lotic habitat of India. In

another study, Ghosh and Ghose (1972) reported that spermatogenesis of *L. marginalis* occur in all months of the year with the highest activity during July to January in contrast, the ovary of this species is active for about 10 months from April to January. However, the gonadal phases and spawning intensity of this species vary with populations (Misra et al. 2010).

Freshwater pearl mussel, *L. marginalis* is abundant in inland water-bodies such as lakes, rivers and ponds of Bangladesh (Dan et al. 2001). In Bangladesh, mussels deliberately collected from natural sources are predominantly used for the production of lime, poultry and aquafeeds. Technologies could be developed for culturing *L. marginalis* in ponds to ensure the conservation of the natural stock as well as meet the demand of rapidly expanding poultry and aquafeeds. In Bangladesh, most of the farming families in the villages, own ponds near their houses which are used for domestic purposes, such as washing clothes, bathing, as a source of drinking water, and also for cultivating fish. On the bottom of these ponds, mussels can be grown to provide an additional source of income for the families. No attempt has been made for freshwater mussel culture due to lack of proper knowledge on the timing of juvenile fall from natural stock or lack of hatchery for mussel in Bangladesh. To establish the freshwater mussel hatchery system for the timely supply of adequate spats, knowledge on their reproductive strategy, spawning time and duration as well as the precise information on favourable ecological conditions for their reproduction is indispensable. In the above context, the current study aims to furnish the above information by studying the annual ovarian cycle and condition index of *L. marginalis* from a farming pond of Bangladesh through biometry and histology.

Materials and Methods

Sampling

The adult freshwater pearl mussels *L. marginalis* having shell length over 60 mm were collected from a farming pond of Bangladesh Fisheries Research Institute (BFRI), Mymensingh, Bangladesh from October 2015 to September 2016. Mussels collected from a natural population were stocked in the pond as a part of pearl culture project. The rectangular pond having a surface area of 80 m^2 and the sources of water were rainfall and underground water from deep tube-well with an average depth of 1.5 m. The bottom mud of the pond was silty, and the pond was well-exposed to sunlight. The pond was fertilised with organic (500 $kg.ha^{-1}$ $month^{-1}$ as compost) and inorganic fertilisers (50 $kg.ha^{-1}$ $month^{-1}$ as urea and 25 $kg.ha^{-1}$ $month^{-1}$ as TSP) and lime was applied on monthly basis at the rate of 25 $g.m^{-2}$. Mussels were collected monthly from the bottom of the pond by hand-picking. After collection, 20 adult mussels (shell length over 60 mm) were sent to the Aquatic Ecology Laboratory of Bangladesh Agricultural University for biometric analysis and histology. After measuring the shell length using digital slide callipers (Heng Liang, China), the mussels were dissected out, and tissue wet weight recorded with an electronic balance (Eki 300-2n, EKI Series Balance). A transverse section (2 to 3 mm) of each mussel was cut for histology. The shells were allowed to sun-dry and dry shell weight recorded. Condition index as the ratio of wet tissue weight to dry shell weight of individual mussels was calculated as outlined by Uddin et al. (2010).

Monitoring of water quality parameters

Water quality parameters of the pond such as temperature, dissolved oxygen (DO), pH and total alkalinity were monitored monthly during sampling. Water temperature (°C) and DO ($mg.L^{-1}$) were taken from three different locations of the pond at each sampling date using a direct reading DO meter (Lutron, PDO 519, Taiwan). The pH of the pond water was recorded by using a direct reading pH meter (pHep® pH meter, Hanna Instruments) in triplicate at every sampling date. Total alkalinity of the pond water samples was measured by titration method in Pearl Culture Laboratory of Bangladesh Fisheries Research Institute (BFRI).

Histology

The section taken from the soft tissue was fixed in Davidson's solution for 48 h. The routine histological procedure was followed to prepare the slides and observed under the microscope to identify the sex and gametogenic stages. Only female individuals were selected for the current study and categorised into five stages as applied for the study of the reproductive cycle of bivalves in many studies: early developing; late-developing; ripe; spawning; and spent. The presence of undifferentiated mussels in the samples was also noted.

Maturity index

For the determination of maturity index (MI), gametogenic stages of the female mussels were graded into different numerical ranking scores of a maturity scale of 0 to 4 as early developing (2), late-developing (3), ripe (4), spawning (2), spent (1) and undifferentiated (0) modified from Sunila (1981). The maturity index of analysed female mussels was calculated by dividing the sum of the numerical ranking score by the total number of females analysed for each month.

Statistical analysis

All the collected data were analysed statistically and expressed as (Mean ± SD) using SPSS statistical

package version 22. A chi-square (χ^2 -test) was performed to test the null hypothesis of 1:1 sex ratio of males and females. Simple correlation analysis was performed to identify a possible significant correlation between condition index (CI) and maturity index (MI) as well as water temperature, DO, pH and total alkalinity with CI and MI separately using Pearson Product Moment Correlation Coefficient. Significance was expressed at 5 % level.

Results

Water quality parameters

The vital water quality parameters such as temperature, dissolved oxygen (DO), pH and total alkalinity were monitored monthly over the study period, and the mean values (± SD) are shown in Fig. 1. Water temperature was found to vary from 19.23 to 29.57 °C. The highest water temperature was recorded in August whereas the lowest temperature in January. The DO concentration in the waterbody fluctuated from 4.82 to 7.62 mg.L^{-1}, and the mean value was 6.26 ± 0.68 mg.L^{-1}. The waterbody remained alkaline throughout the study period with a pH varying from 7.72 to 8.75. The values of total alkalinity of the pond water ranged from 98.1 to 240.5 mg.L^{-1}, and the mean value was 181.55 ± 12.11 mg.L^{-1}.

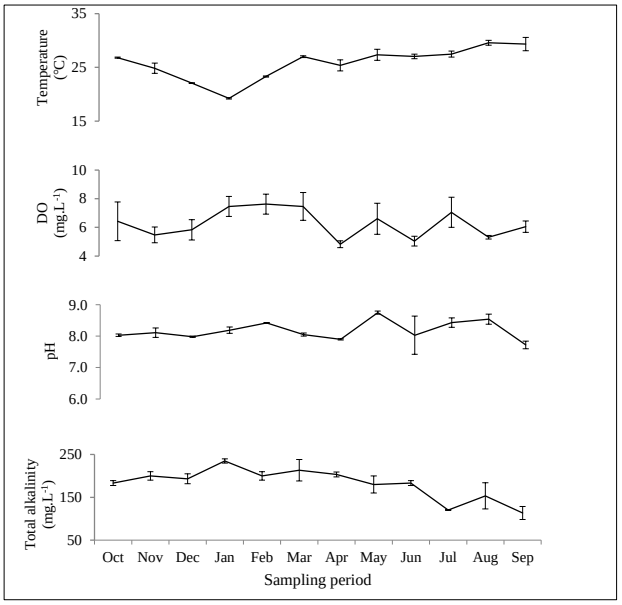

Fig. 1. Monthly changes in mean values (± SD) of water temperature, DO, pH and total alkalinity of the sampling site.

Biometry

Biometric measurements such as shell length (SL), soft tissue wet weight (TWWT) and dry shell weight (DSWT) are shown in Table 1. Out of 240 mussels analysed, mean SL ranged from 62.38 to 94.53 mm SL, TWW varied from 9.18 to 33.54 g and DSW differs from 14.63 to 35.75 g.

Sex ratio

Histology revealed that out of 240 mussels analysed, 115 samples were male and 125 were female and no sexually indifferent mussel was identified (Table 1). The overall male-female sex ratio was 1.00:1.08, which did not deviate significantly from the 1:1 ratio as expected (χ^2- test) with a slight dominance of females over the male.

Ovarian cycle of Lamellidens marginalis

Histological assessment of ovarian transects denoted oogenesis throughout the year as shown in Fig. 2.

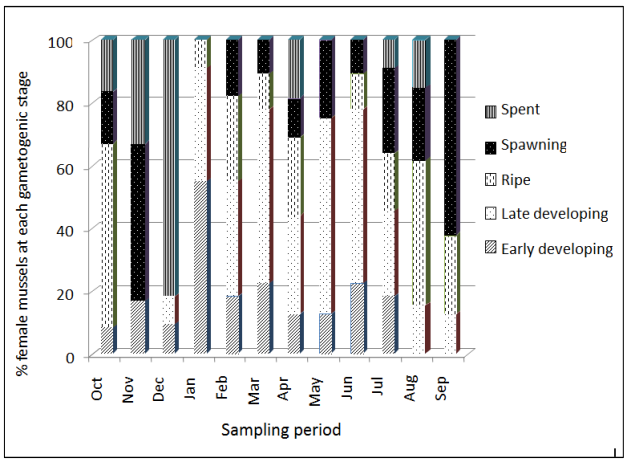

Fig. 2. Temporal distribution of ovarian stages of *Lamellidens marginalis* collected from the sampling site.

At the beginning of the study in October, most of the mussels were ripe and ready for spawning (58.3 %) whereas some spawning spent and early developing females were also observed during this month. Major spawning event occurred by November as half of the females were spawning detected through histology observation. No spawning activity of female was noted during December and January when the water temperature remained below 22 °C. Early developing and ripe females dominated in January and spawning commenced in February when the water temperature was increasing from the annual minima. Spawning continued from February to end of the study during September although the intensity of spawning remarkably varied over time. Major spawning pulses were noted during May, and July to September when more than 23 % females were in the spawning stage. Late developing mussels were found throughout the year except October and November having domination from January to March and May to July.

Maturity index (MI)

Over 12-month data analysis, the MI of female *L. marginalis* ranged from 1.27 to 3.0. Four distinct peaks in MI of female freshwater pearl mussels were noted during the study period. Pearson Product Moment Correlation Coefficient revealed a moderate positive correlation between MI and CI (r = 0.436; $P > 0.05$). The

Table 1. Number of mussels and their shell length (SL, mm), tissue wet weight (TWW, g), dry shell weight (DSWT, g) and condition index (calculated only for females). Data represent monthly mean and standard deviation.

Month	N = 20		SL	TWWT	DSWT	CI
	Male	Female				
Oct '15	8	12	72.4 ± 10.6	23.54 ± 3.70	27.59 ± 5.52	0.85 ± 0.25
Nov '15	14	6	82.5 ± 11.1	14.40 ± 2.92	29.94 ± 9.01	0.51 ± 0.17
Dec '15	9	11	87.4 ± 4.9	18.72 ± 3.43	31.93 ± 6.93	0.60 ± 0.12
Jan '16	9	11	89.8 ± 7.0	20.49 ± 4.44	35.75 ± 10.03	0.70 ± 0.33
Feb '16	9	11	93.1 ± 5.5	19.09 ± 5.70	34.63 ± 6.15	0.55 ± 0.12
Mar '16	11	9	90.7 ± 6.7	19.33 ± 5.54	30.44 ± 11.13	0.66 ± 0.15
Apr '16	4	16	78.9 ± 5.8	10.75 ± 3.95	18.62 ± 8.86	0.61 ± 0.20
May '16	12	8	81.2 ± 7.3	11.00 ± 5.01	20.25 ± 11.60	0.59 ± 0.18
Jun '16	11	9	94.5 ± 6.6	18.22 ± 3.19	26.11 ± 7.94	0.74 ± 0.18
Jul '16	9	11	80.5 ± 5.8	9.18 ± 3.31	14.63 ± 5.44	0.68 ± 0.22
Aug '16	7	13	84.7 ± 3.6	14.69 ± 3.94	20.30 ± 4.02	0.72 ± 0.16
Sep '16	12	8	88.7 ± 2.6	15.75 ± 1.71	22.88 ± 5.36	0.42 ± 0.23

first and highest peak in MI was reported in October when the majority of females were in ripe stage (58.3 %), and the value was inconsistent with CI of the mussels. The MI dropped suddenly in November signifying the major spawning event of the females which is in agreement with histology and CIs. The lowest MI was noted in December when a majority of females (81.8 %) were in spent stage. The onset of new maturation period can be distinguished with increasing MI from 1.27 to 2.91 between December and February. MIs downturned again till May when spawning females occurred steadily in samples. Another peak in the value of MI was marked in June logical with CIs. The MIs dropped in July and peaked again in August while it declined again in September, and the events that happened were persistent with the changes in CIs (Fig. 3). Among the water quality parameters, water temperature showed strong positive correlation with MI (r = 0.616; $P < 0.05$). However weak association was noted between DO and MI (r = 0.069; $P > 0.05$), pH and MI (r = 0.166; $P > 0.05$), and total alkalinity and MI (r = - 0.265; $P > 0.05$).

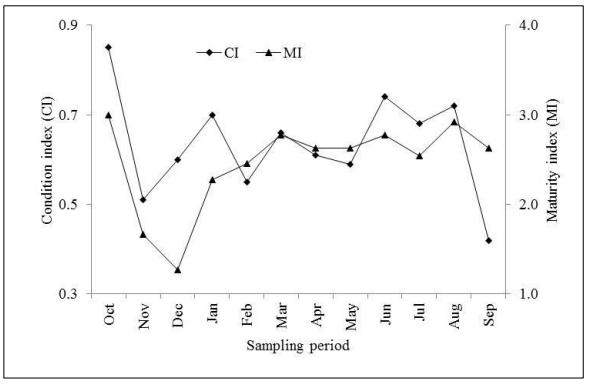

Fig. 3. Monthly changes in mean values of condition index (CI) and maturity index (MI) of female *L. marginalis* collected from the sampling site.

Condition index (CI)

Condition indices are generally used to express the physiological status of the bivalves which often follows the gonadal cycle of the species. In the current study, CI was calculated only for the females as a ratio of wet tissue weight to dry shell weight. Remarkable temporal variations were evident in CIs exhibiting five peaks over the 12-month study period (Fig. 3). The highest peak of CI was noted during October when the majority of the females (58.3 %) were ripe and ready for spawning as evidenced from histology. Condition indices decreased 40 % by November when spawning individuals occupied 50 % females in the histological preparations. An increasing trend in CIs was noticed after November and second peak was seen in January. Condition indices declined in February while the values increased again by March showing another peak. CIs decreased steadily after March which was inconsistent with the reduction of gonad weight due to energy loss and release of gametes during spawning. The values of CIs peaked again in June and declined in July however it peaked again in August. A sudden decline in CIs occurred by September. Although CI had moderate positive correlation with MI (r = 0.436; $P > 0.05$), this estimate was weakly correlated with water temperature (r = - 0.003; $P > 0.05$), DO (r = 0.027; $P > 0.05$), pH (r = 0.179; $P > 0.05$) and total alkalinity (r = 0.211; $P > 0.05$).

Discussion

Water quality parameters

Spawning of gametes in bivalves is the cumulative result of endogenous and exogenous factors. Water quality parameters such as temperature, salinity, food availability, light, lunar phase, dissolved oxygen, pH potentially trigger the spawning of bivalve molluscs (Barber and Blake 1991). Temperature is the single most critical environmental cue which regulates maturation and spawning of bivalves (Baba et al. 1999). Many species of scallops initiate gametogenesis and spawning when the water temperature increase from the annual minima or

decrease from the yearly maxima (Barber and Blake 1991; Uddin et al. 2007). In the current study, we recorded the water temperature ranging from 19.25 °C to 29.57 °C over a 12-month study period. No spawning female was reported during December and January when the water temperature remained below 22 °C. It may indicate that *L. marginalis* females collected from the rearing pond undergo spawning in this habitat when the water temperature goes above 22 °C. It may be possible as the spawning of *Corbicula japonica* was reported extremely low due to low water temperature and salinity in Lake Abashiri, Hokkaido, Japan (Baba et al. 1999). In contrast to the present study where the spawning was observed at temperatures above 22 °C, Gaikwad and Kamble (2013) reported that the optimum spawning temperature of *L. marginalis* from Panchganga River, Maharashtra, India, was from 20 to 25 °C. Such discrepancy may be explained by the latitudinal differences between the two locations and type of habitats as the gametogenesis and spawning period, egg size and fecundity of bivalve species which typically vary with latitudinal ranges (Barber and Blake 1991). The duration of the spawning period is often negatively correlated with latitude (Sastry 1979; Thompson et al. 1996).

Dissolved oxygen (DO) is an essential parameter for successful spawning of aquatic animals. Gaikward and Kamble (2013) reported that spawning of *L. marginalis* occurred from July to September when DO level was relatively higher than the remaining months of the year. In the current study, we observed DO level more than 4.82 $mg.L^{-1}$ throughout the year indicated that the values were acceptable for spawning of *L. marginalis*.

The pH is another important factor that influences gametogenesis and growth of bivalve molluscs (Hornbach and Childers 1987). The pH of the aquatic environment affects the deposition of Ca and the availability of dissolved CO_2 for conversion to $CaCO_3$ in mollusc shells, mainly through its effect on local water hardness (Wilbur 1964; Dussart 1976; Sutcliffe and Carrick 1973). The diversity and abundance of bivalve molluscs are negatively affected in acidic waters due to reduction in Ca uptake, deposition and maintenance of shells at high concentrations of H^+ (Mackie and Flippance 1983; Hornbach and Childers 1987). Hincks and Mackie (1997) also reported that zebra mussel, *Dreissena polymorpha* (Pallas, 1771) were absent in European lakes with pH values below 7.3. In the present study, the bottom water of the pond was alkaline having pH more than 7.7 round the year indicated that the values were suitable for growth and reproduction of mussels.

Calcifying organisms have difficulty in forming and maintaining their calcareous exoskeleton in conditions with low $CaCO_3$ concentrations (Orr et al. 2005). Natural populations of molluscs in six lakes of south-central Ontario, Canada were affected by low alkalinity in terms of growth and reproduction (Rooke and Mackie 1984; Servos et al. 1985). No veliger of zebra mussel, *D. polymorpha* was seen at alkalinity of lower than 40 $mg.L^{-1}$ (Hincks and Mackie 1997). The total alkalinity in the current study ranged from 98.1 to 240.5 $mg.L^{-1}$ in the experimental pond throughout the 12-month study period. The relatively higher total alkalinity of pond water might be associated with the application of lime at the rate of 1 kg.40 m^{-2} monthly and the values were suitable for growth and reproduction of mussels.

Ovarian cycle

The current study showed that *L. marginalis* females exhibit a prolonged spawning period over 10 months of the year from February to November with three spawning peaks in May, and from July to September, and November in the investigated lentic habitat. Out of five ovarian maturity stages detected, at least three stages were observed at each sampling date implied that oogenesis of *L.marginalis* is a continuous process throughout the year. Due to bradytictic nature of *L. marginalis* (Ghosh and Ghose 1972) it may be possible to exhibit gonadal activity and spawning over a prolonged period in the tropical region. Behera et al. (2014) observed that freshwater mussels, *L. marginalis* is a prolific breeder and it spawns round the year with a peak spawning activity from July to September. In another study, Ghosh and Ghose (1972) reported an active ovary of this species over 10 months of the year from April to January. In the present study, the spawning of females was observed for 10 months with three spawning pulses. The little discrepancies with the above studies might be possible due to different geographic locations as the degree and number of spawning phase of this species differ significantly from one population to another (Misra et al. 2010). No spawning females were detected during December and January when the water temperature remained below 22 °C. This finding differs with Gaikwad and Kamble (2013) who reported optimum water temperature of 20 to 25 °C for spawning activity of *L. marginalis* in a lotic habitat of India. Such differences could be attributed by type of habitat, population or geographic position.

Maturity index (MI)

The condition index represents more precisely the stages of the gametogenic process. However, the use of MIs helped to understand the series of events during the reproductive cycle (Etchian et al. 2004). Four distinct peaks in MIs of *L. marginalis* were noted during October, February, June and August when most of the females were ripe and ready for spawning. More than one cycle was also noted for different species of bivalves in a year (Roseberry et al. 1991; Etchian et al. 2004). The onset of new oogenesis period can be inferred with increasing MI from December to February. Such increment in MIs of *Maya*

arenaria due to gametogenesis was also reported by Gauthier-Clerc et al. (2006).

Condition index (CI)

A steep decline in CIs generally reflects the release of gametes during spawning (Seed and Suchanek 1992). However, CIs do not always indicate the quantity of gametes as some storage tissues also fill the mantle (Duinker et al. 2008). Such distinction has great practical value for fishery managers for decision making on the declaration of harvest ban period of the particular population. *Lamellidens marginalis* revealed primary peak during October 2015 and secondary peak during June 2016 when most of the mussels were in ripe condition along with good number of spawning females. Moreover, the decrease of condition index observed in November and July for *L. marginalis* coincided with the period of spawning and releasing of gametes of the females. This phenomenon is characteristic of bivalves, which generally lose their weight following the emission of gametes (Smaoui-Damak et al. 2006). The third peak CI was noted during January without any spawning during this month. It would be a useful message for the managers to harvest quality mussels without interrupting the peak spawning activity of this species in this habitat.

Conclusion

The present study reports the ovarian cycle of freshwater pearl mussels, *Lamellidens marginalis* (Lamarck, 1819) from a rearing pond in Bangladesh. A prolonged spawning period of the females was observed lasting over 10 months of a year. Major spawning event occurred during November, February, May, and July to September. Four distinct peaks in MIs of females were noted during October, February, June and August when most of the females were ripe and ready for spawning as reflected from histology. Mean CIs ranged from 0.42 ± 0.23 to 0.85 ± 0.25 exhibiting five peaks over the 12-month study period. From gonadal phenology and condition index data, December and January could be the best time for harvesting better quality mussels without interrupting the peak spawning of mussels. For better understanding about the life history of this species, further study should be designed to identify the timing and brooding pattern of this freshwater unionoid species.

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