

Article

Baseline Morphometric Assessment and Sexual Dimorphism of Three Freshwater Mollusks in a Tropical Floodplain Wetland

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Abstract

This study examined the morphometric variability and sex-related patterns in three freshwater mollusk species, *Pila globosa*, *Lamellidens marginalis*, and *Parreysia corrugata* from the Hakaluki Haor ecosystem, Bangladesh. All species showed strong linear relationships between shell length and body weight ($p < 0.001$). The R^2 values indicated moderate association in *P. globosa* (0.57) and *L. marginalis* (0.59) and a high association in *P. corrugata* (0.80). Most traits were similar across sexes; however, females of *P. corrugata* had significantly higher body weight and slightly wider shells, suggesting mild sexual dimorphism. Condition indices were species-specific, with males generally higher, notably in *P. corrugata* ($p < 0.05$). Violin box plots showed minor sex differences in *P. globosa* aperture and spire, with overlap. PCA revealed that size traits were major contributors, explaining 60–75% of the variation. Females of *P. globosa* scored higher on the size axis, indicating sexual size dimorphism, while *L. marginalis* and *P. corrugata* showed significant overlap. Overall, results highlight strong allometric scaling, limited species-specific sexual dimorphism, and body size as the main determinants of morphology.

Keywords: molluscan shellfish; Hakaluki Haor; condition indices

1. Introduction

Freshwater mollusks are an ecologically significant and evolutionarily diverse group of invertebrates that play critical roles in the structure and functioning of aquatic ecosystems [1–3]. Their contributions to nutrient cycling, organic matter decomposition, and trophic dynamics make them essential bioindicators of environmental health in wetlands, rivers, and lakes [4–6]. Among freshwater gastropods and bivalves, the apple snail *P. globosa* and the mussels *L. marginalis* and *P. corrugata* are particularly important throughout South and Southeast Asia, largely because of their ecological roles within the trophic web and freshwater biodiversity [7,8]. These species inhabit a wide range of habitats and show morphological variation shaped by ecological gradients, hydrological fluctuations, and anthropogenic pressures. Understanding morphometric attributes and sexual dimorphism in these mollusks is therefore crucial for assessing population structure, adaptive strategies, and environmental responses in dynamic aquatic systems [9,10]. Hakaluki Haor, one of the largest natural wetlands in Bangladesh, is a unique ecological landscape defined by seasonal flooding, diverse microhabitats, and high productivity [11]. This tropical floodplain wetland supports a rich assemblage of aquatic organisms, including numerous molluscan species that are integral to local fisheries and livelihoods [12,13]. Although the three species examined in this study are currently classified as “Least Concern” by the IUCN [14], Hakaluki Haor is facing increasing environmental pressures from agricultural



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expansion, pollution, overexploitation of aquatic resources, and hydrological changes [15]. These pressures may drive morphological shifts within molluscan populations, reflecting both phenotypic plasticity and potential long-term evolutionary responses.

Morphometric analyses, both traditional and geometric, provide valuable insights into intra- and interspecific variation driven by genetic, developmental, and environmental factors [16]. For gastropods such as *P. globosa*, shell form is shaped by habitat stability, predation, water chemistry, and reproductive behavior. Similarly, in bivalves such as *L. marginalis* and *P. corrugata*, shell dimensions and contour features often reflect ecological stressors, sediment characteristics, and hydrological regimes [17]. Variation in shell morphology affects not only survival and reproductive success but also plays a key role in taxonomic classification, conservation efforts, and stock assessments [18]. Despite their ecological and economic importance, especially as food sources, biofilters, and ecosystem engineers, comprehensive morphometric studies of these mollusks are limited in tropical floodplain wetlands in Bangladesh.

Sexual dimorphism in freshwater mollusks helps explain species biology and population dynamics [19]. Although many gastropods and bivalves show subtle or overlapping sexual traits, differences in shell size, shape, or soft-body features may arise from variations in energy use, reproductive effort, or ecological niche separation [20]. This information aids species identification, reproductive biology, and conservation strategies. Differences in shell shape and soft-tissue characteristics between males and females can affect their growth, habitat preferences, and ability to withstand environmental stress [21,22]. Identifying these dimorphic characteristics is crucial for accurate species assessment and sustainable management, particularly as freshwater mollusks face challenges like habitat destruction and overharvesting [4,9]. Documenting sexual differences in *P. globosa*, *L. marginalis*, and *P. corrugata* enhances conservation efforts by aiding population monitoring, guiding breeding programs, and predicting how species may respond to ecological changes in vulnerable wetlands. This study examines specific morphometric traits and sexually dimorphic features of these species collected from Hakaluki Haor. By analyzing shell measurements, form variation, and sex-related traits, this study aims to enhance understanding of population structure and adaptation in these species. The results provide essential baseline data for future ecological monitoring, conservation, and resource management in tropical floodplain wetlands. Moreover, documenting morphological diversity in this dynamic ecosystem improves our knowledge of molluscan biology and their responses to rapidly changing environmental conditions.

2. Materials and Methods

2.1. Study Sites and Sample Collection

Sampling was conducted in Hakaluki Haor (Figure 1), a vital wetland in Sylhet Division, northeastern Bangladesh. Situated roughly between 24°35'–24°45' N and 92°00'–92°08' E, it ranks as one of the country's most ecologically significant floodplain wetlands [23,24]. The region comprises shallow basins, seasonal floodplains, and interconnected water bodies that fluctuate with the monsoon [15]. Its hydrology is primarily influenced by the Kushiya River, especially during heavy rains. Between May and July 2025, samples of bycatch from traditional fishermen using a drag net (0.5–1.00 cm mesh) were collected from different locations within the Haor. Sampling took place from the late pre-monsoon through the monsoon, capturing the flood's onset and the floodplain's uniform expansion during the monsoon. Hydrological data were collected with a YSI multiparameter meter; water depth was measured with a tape measure; and water transparency was evaluated using a Secchi disk.

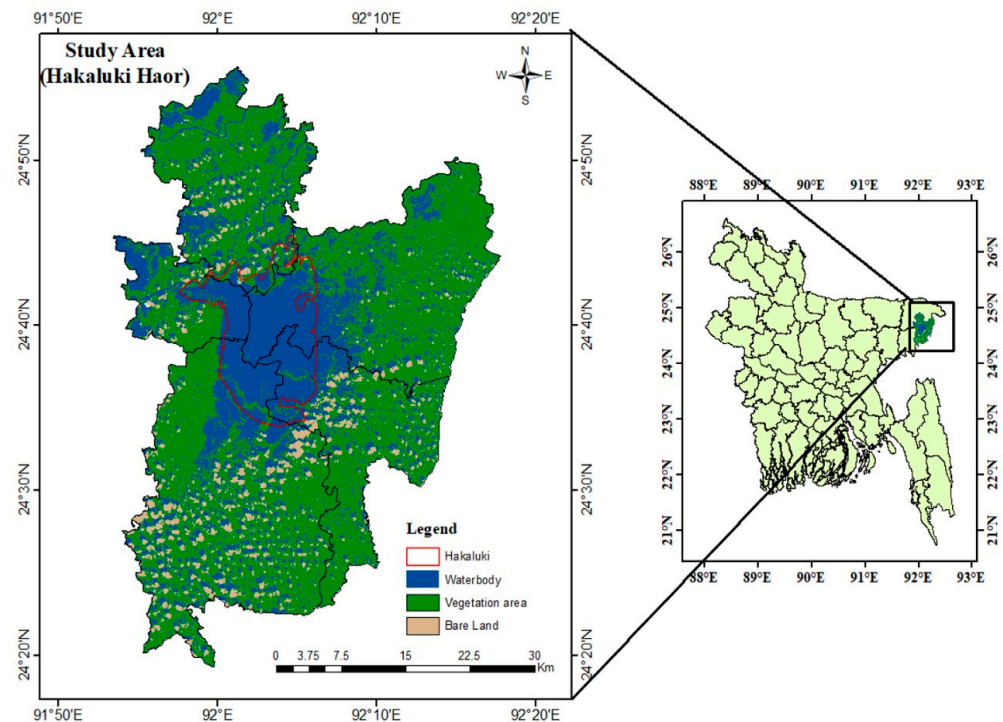


Figure 1. Hakaluki Haor floodplain ecosystem in Bangladesh.

2.2. Measurement Tools for Length-Weight Relationships and Condition Indices

Species were identified and confirmed based on the previous description [25–27] and keys mentioned in <https://molluscabase.org/>. Specimens were carefully placed between the caliper jaws to ensure firm but gentle contact, and their lengths, widths, and heights were measured to 0.01 mm precision. To minimize stress, exposure of live specimens was kept to a minimum. For weighing, individuals were briefly dried, then weighed on a calibrated precision balance, with their mass recorded to 0.001 g. All instruments were calibrated to zero before use, and each measurement was repeated three times to improve accuracy and minimize observer error. Animals were subjected to euthanasia in ice slurry for 1 h to collect and weigh soft tissue. Mussel shells were sundried for at least 4 h before the condition index (CI) measurement [28]. For snails, the condition index is calculated as the percentage ratio of soft tissue wet weight to total weight [25,29], whereas for mussels, it is the percentage ratio of soft tissue wet weight to dried shell weight (oven-dried for 3 h at 50 °C after complete removal of all soft tissue) [30]. Males and females were identified by visual observation of sperm or ova cells in the gonad under the microscope following dissection [28,31]. Only individuals with confirmed sex were included in the present dataset.

2.3. Statistical Analysis

Data were statistically analyzed using IBM SPSS version 26. The significance of regression coefficients was assessed using an ANOVA F-test, and slopes were constructed from log-log regressions on transformed data. However, Shapiro–Wilk tests indicated non-normality ($p < 0.05$) for most variables, so nonparametric methods were applied. Specifically, differences between groups and between sexes were evaluated using the Mann–Whitney U test at a significance level of $p < 0.05$. Graphs and principal component analysis (PCA) biplots were generated in OriginPro 2026 (64-bit) SR110.3.0.197 (Learning Edition).

3. Results

3.1. Water Quality Properties

Table 1 summarizes the main water quality parameters, including temperature, DO, conductivity, TDS, pH, transparency, and sampling water depth. The temperature was approximately 25.82 ± 2.88 °C, and the dissolved oxygen level was 4.96 ± 0.24 ppm.

Table 1. Major water-quality parameters at sampling sites (values are mean $\pm$ standard deviation).

Parameters	N	Values
Temperature (°C)	12	25.82 ± 2.88
Dissolved O ₂ (ppm)		4.96 ± 0.24
Conductivity (μ S/cm)		162.58 ± 4.97
Total dissolved solids (ppm)		31.29 ± 0.81
pH		6.45 ± 0.34
Transparency (cm)		9.55 ± 1.58
Water depth (m)		1.67 ± 0.12

3.2. Regression Analysis Between Length and Weight Parameters

Figure 2 illustrates the regression correlation between length and weight across three mollusks, showing a linear relationship in which weight increases with length, and these correlations are highly significant ($p < 0.001$). For *P. globosa*, the model with $R^2 = 0.57$ suggests that length explains about 57% of the variation in weight; a 0.1 cm increase in length corresponds to an approximate 0.168 g increase in weight. *L. marginalis* exhibits the steepest slope with $R^2 = 0.59$, with weight rising by roughly 0.358 g per 0.1 cm, indicating the fastest mass gain per unit length, although its variability is similar to *P. globosa* and the measured length range is narrow. *P. corrugata* offers the best fit, with $R^2 = 0.80$, where weight increases by about 0.247 g per 0.1 cm, and the data points are tightly clustered around the line, ensuring reliable predictions within the sample range. Confidence bands are relatively narrow and parallel, supporting the stability of these estimates within the observed lengths; avoid extrapolating beyond these ranges.

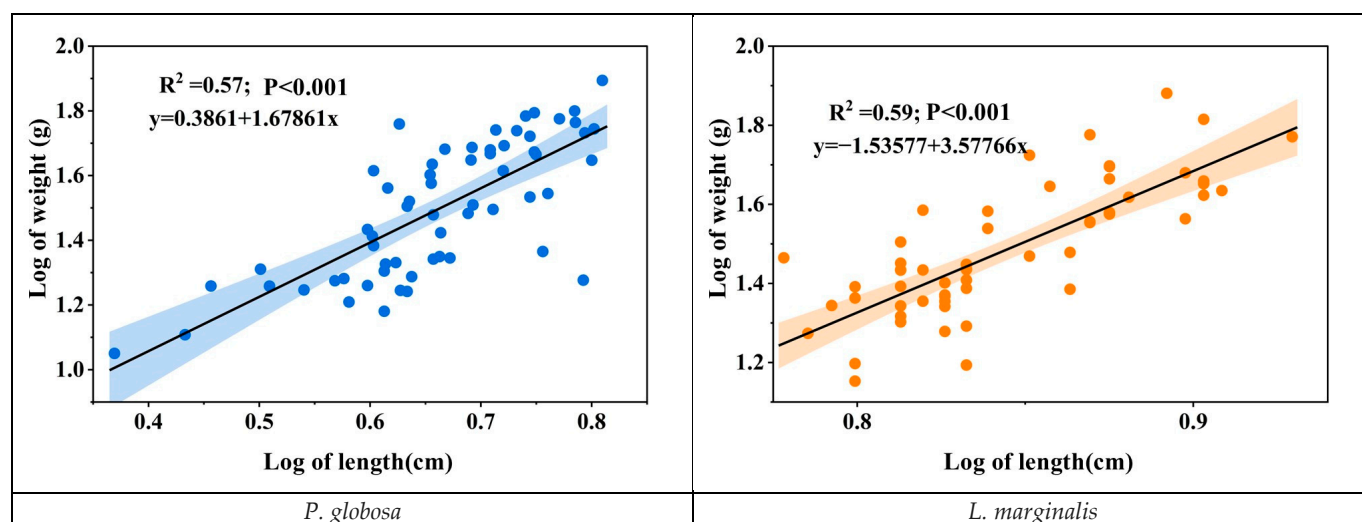


Figure 2. Cont.

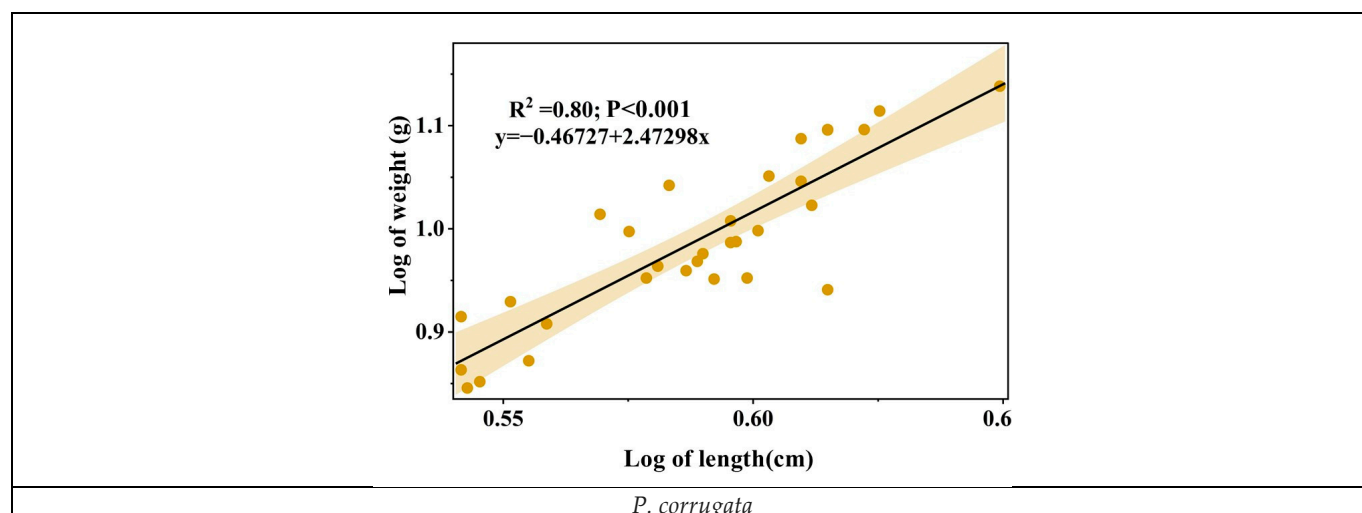


Figure 2. Correlation plot showing the relationship between length and weight for *P. globosa*, *L. marginalis*, and *P. corrugata* collected from the Hakaluki Haor floodplain ecosystem.

3.3. Sex-Specific Length and Weight Measurements

Most male–female morphometric comparisons across the three mollusk species do not show significant differences, as determined by the Mann–Whitney U test ($p = 0.05$; Table 2). For shell length, no significant differences were detected between males and females in *P. globosa* (4.69 ± 0.14 vs. 4.72 ± 0.20 cm; $p = 0.93$, effect size = 0.01), *L. marginalis* (6.93 ± 0.10 vs. 7.08 ± 0.11 cm; $p = 0.33$, effect size = 0.13), or *P. corrugata* (3.74 ± 0.09 vs. 3.89 ± 0.05 cm; $p = 0.20$, effect size = 0.03), indicating comparable shell growth in both sexes. Similarly, total body weight did not differ significantly in *P. globosa* ($p = 0.75$) and *L. marginalis* ($p = 0.44$), although females consistently exhibited slightly higher mean values. However, *P. corrugata* showed a significant difference in total weight (8.77 ± 0.61 g in males vs. 10.04 ± 0.39 g in females; $p = 0.04$) with a moderate effect size (0.35), suggesting biologically meaningful dimorphism. Soft tissue wet weight remained statistically similar across sexes in all species ($p > 0.05$), further supporting the lack of strong dimorphism. In shell width, no significant differences were found for *P. globosa* and *L. marginalis*, whereas *P. corrugata* exhibited a significant difference (2.46 ± 0.16 vs. 2.52 ± 0.16 cm; $p = 0.02$, effect size = 0.39), indicating wider shells in females. Dried shell weight showed no difference in *L. marginalis* but was marginal in *P. corrugata* (3.85 ± 0.29 vs. 4.84 ± 0.20 g; $p = 0.05$, effect size = 0.48). *P. globosa* and *L. marginalis* lack sexual dimorphism, whereas *P. corrugata* exhibits moderate female-biased size differences.

Table 2. Sex-specific measurements of length and weight parameters in *P. globosa*, *L. marginalis*, and *P. corrugata* collected from the Hakaluki Haor (values are mean $\pm$ standard error).

Species	Sex	N	Mean	p-Value	Effect Size	95% Confidence Interval	
						Lower Bound	Upper Bound
Shell length (cm)							
<i>P. globosa</i>	Male	32	4.69 ± 0.14	0.93	0.01	4.40	4.98
	Female	29	4.72 ± 0.20			4.31	5.14
<i>L. marginalis</i>	Male	32	6.93 ± 0.10	0.33	0.13	6.73	7.14
	Female	29	7.08 ± 0.11			6.85	7.31
<i>P. corrugata</i>	Male	12	3.74 ± 0.09	0.20	0.03	3.55	3.93
	Female	22	3.89 ± 0.05			3.78	4.01

Table 2. Cont.

Species	Sex	N	Mean	p-Value	Effect Size	95% Confidence Interval	
						Lower Bound	Upper Bound
Weight (g)							
<i>P. globosa</i>	Male	32	35.02 ± 2.48	0.75	0.04	29.97	40.07
	Female	29	35.43 ± 3.41			28.44	42.42
<i>L. marginalis</i>	Male	32	31.62 ± 2.36	0.44	0.10	26.80	36.43
	Female	29	33.86 ± 2.39			28.96	38.76
<i>P. corrugata</i>	Male	12	8.77 ± 0.61	0.04	0.35	7.43	10.11
	Female	22	10.04 ± 0.39			9.24	10.84
Soft tissue wet weight (g)							
<i>P. globosa</i>	Male	32	18.15 ± 1.06	0.21	0.16	15.98	20.31
	Female	29	17.29 ± 2.08			13.02	21.55
<i>L. marginalis</i>	Male	32	8.36 ± 0.46	0.55	0.08	7.62	9.09
	Female	29	8.13 ± 0.42			7.26	9.00
<i>P. corrugata</i>	Male	12	1.21 ± 0.37	0.65	0.08	0.97	1.44
	Female	22	1.17 ± 0.22			1.07	1.26
Shell width (cm)							
<i>P. globosa</i>	Male	32	3.67 ± 0.58	0.93	0.01	3.46	3.88
	Female	29	3.65 ± 0.77			3.36	3.94
<i>L. marginalis</i>	Male	32	3.49 ± 0.36	0.85	0.02	3.36	3.62
	Female	29	3.58 ± 0.49			3.39	3.76
<i>P. corrugata</i>	Male	12	2.46 ± 0.16	0.02	0.39	2.36	2.56
	Female	22	2.52 ± 0.16			2.45	2.59
Dried shell weight (g)							
<i>L. marginalis</i>	Male	32	12.44 ± 0.74	0.40	0.11	10.93	13.95
	Female	29	11.48 ± 0.59			10.27	12.69
<i>P. corrugata</i>	Male	12	3.85 ± 0.29	0.05	0.48	3.21	4.49
	Female	22	4.84 ± 0.20			4.41	5.26

Differences are established between males and females of the same species based on the Mann–Whitney U test at $p < 0.05$; 0.1: small effect size, 0.3: moderate effect size; and 0.5 and above: large effect size.

3.4. Additional Length-Based Parameters Between Male and Female *P. globosa*

Figure 3 compares base length, aperture length and width, and spire length between male and female *P. globosa*. The violin shapes indicate unimodal distributions, similarly spread across both sexes, for all traits. None of these parameter differences is statistically significant according to the Mann–Whitney U test ($p < 0.05$). Males have slightly higher spire lengths (2.40 ± 0.75) compared to females (2.28 ± 0.61), but the high p -value (0.94) indicates no difference. Conversely, females tend to have marginally larger base lengths (3.31 ± 0.67) than males (3.22 ± 0.60), although this is also insignificant ($p = 0.49$). Similarly, females generally show greater aperture dimensions, with aperture length (3.48 ± 0.67) and width (2.09 ± 0.54) exceeding those of males (3.36 ± 0.51 and 1.92 ± 0.38), but these differences are not statistically meaningful ($p = 0.24$ and 0.11).

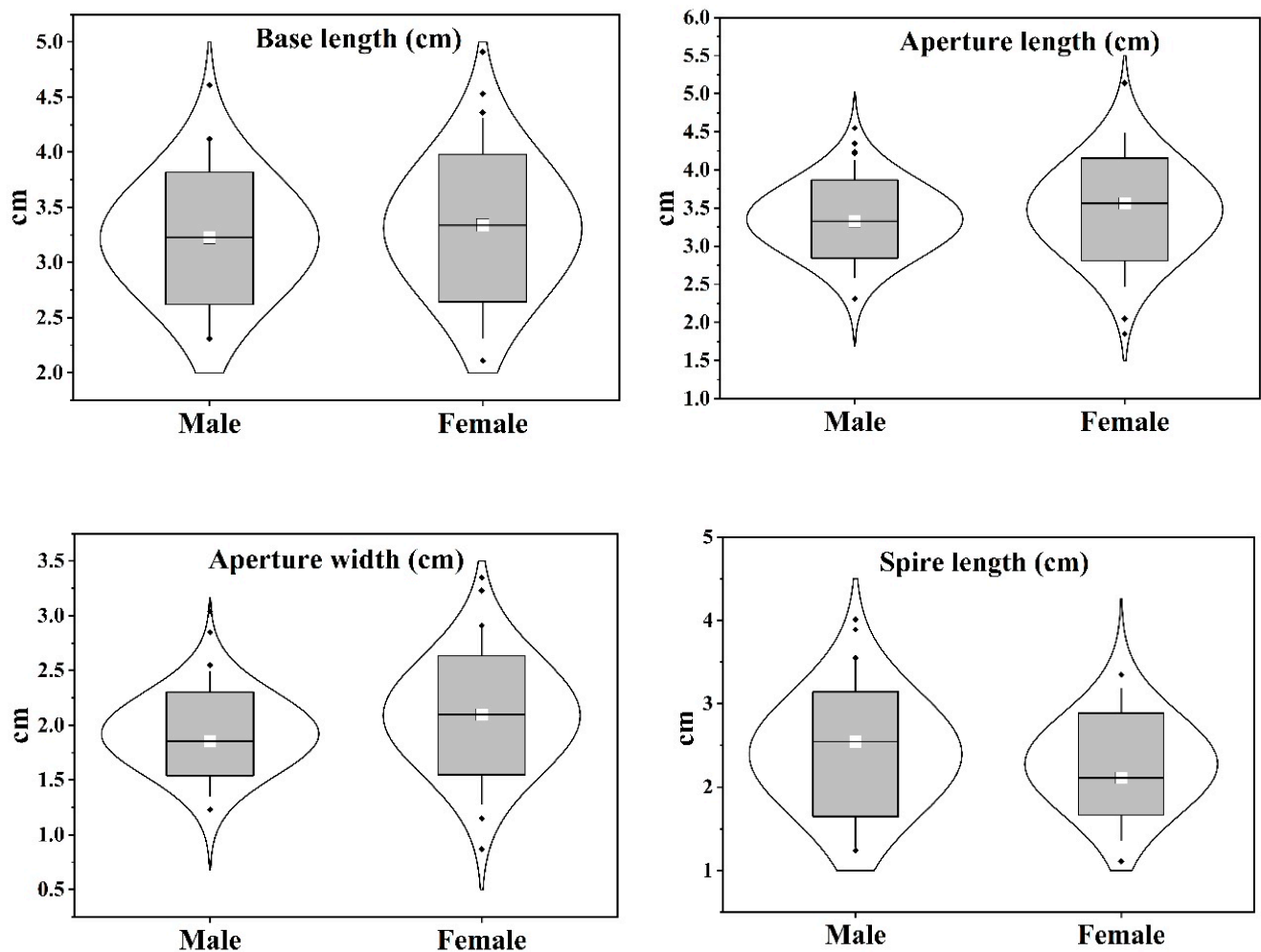


Figure 3. Comparison of additional length-based morphometric parameters between male and female *P. globosa* (white rectangular box indicates the mean, and whiskers show standard deviation).

3.5. Condition Index of Males and Females

Figure 4 features boxplots comparing the condition indices of males and females across three mollusk species. The data suggest species-specific patterns, with higher condition indices in males, except for *L. marginalis*, where the sexes are more similar. In *P. globosa*, males exhibit a wider range and higher median values, with the male boxplot showing greater variability and several upper outliers, suggesting that some individuals are in significantly better condition. Females display a more condensed distribution with moderately lower medians. In *L. marginalis*, the condition indices of both sexes are more similar, with overlapping interquartile ranges and comparable medians, though males show a slightly broader spread and a few low outliers, suggesting greater variability. For *P. corrugata*, males again have higher median condition indices, with a distribution that is elevated and more variable, including several high-outlier points. Female indices tend to be lower ($p < 0.05$), with narrower ranges and medians, indicating a generally lower and more uniform physiological condition than in males.

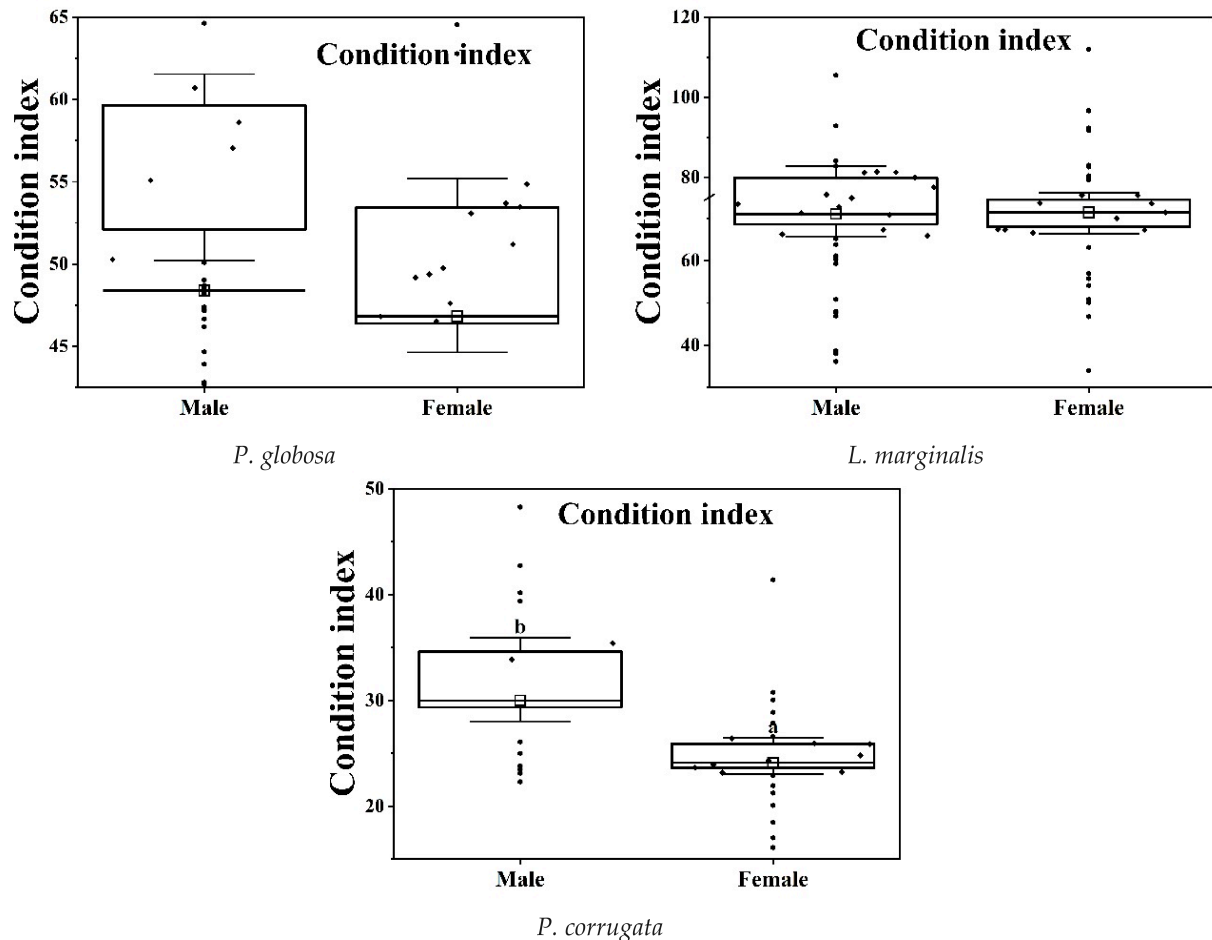


Figure 4. Comparison of the condition index between male and female *P. globosa*, *L. marginalis*, and *P. corrugata* collected from the Hakaluki Haor (white rectangular box shows the mean value, and whiskers represent the standard deviation).

3.6. Principal Component Analyses

Principal component analyses revealed consistent patterns of morphometric variation among the three freshwater mollusks studied (Figure 5). In *P. globosa*, PC1 (63.52%) primarily represented overall size, with positive loadings indicating increased shell dimensions, aperture size, and body mass; females scored higher, highlighting clear sexual size dimorphism. PC2 (12.03%) described shape differences between soft-tissue mass and spire length versus aperture and base width, though significant overlap between sexes suggested only minor shape distinctions. In *L. marginalis*, PC1 (60.01%) similarly reflected shell size and weight, while PC2 (17.46%) captured variation in soft-tissue mass. Male and female distributions overlapped substantially, indicating limited morphological divergence between sexes. For *P. corrugata*, PC1 (75.47%) explained most of the variation, representing overall size and shell mass, whereas PC2 (14.67%) distinguished individuals by their soft-tissue wet-weight-to-dry-shell-weight ratios. Both sexes showed extensive overlap, with few males exhibiting high PC2 scores. Overall, size accounts for most of the variation, with minor differences in shape between the sexes.

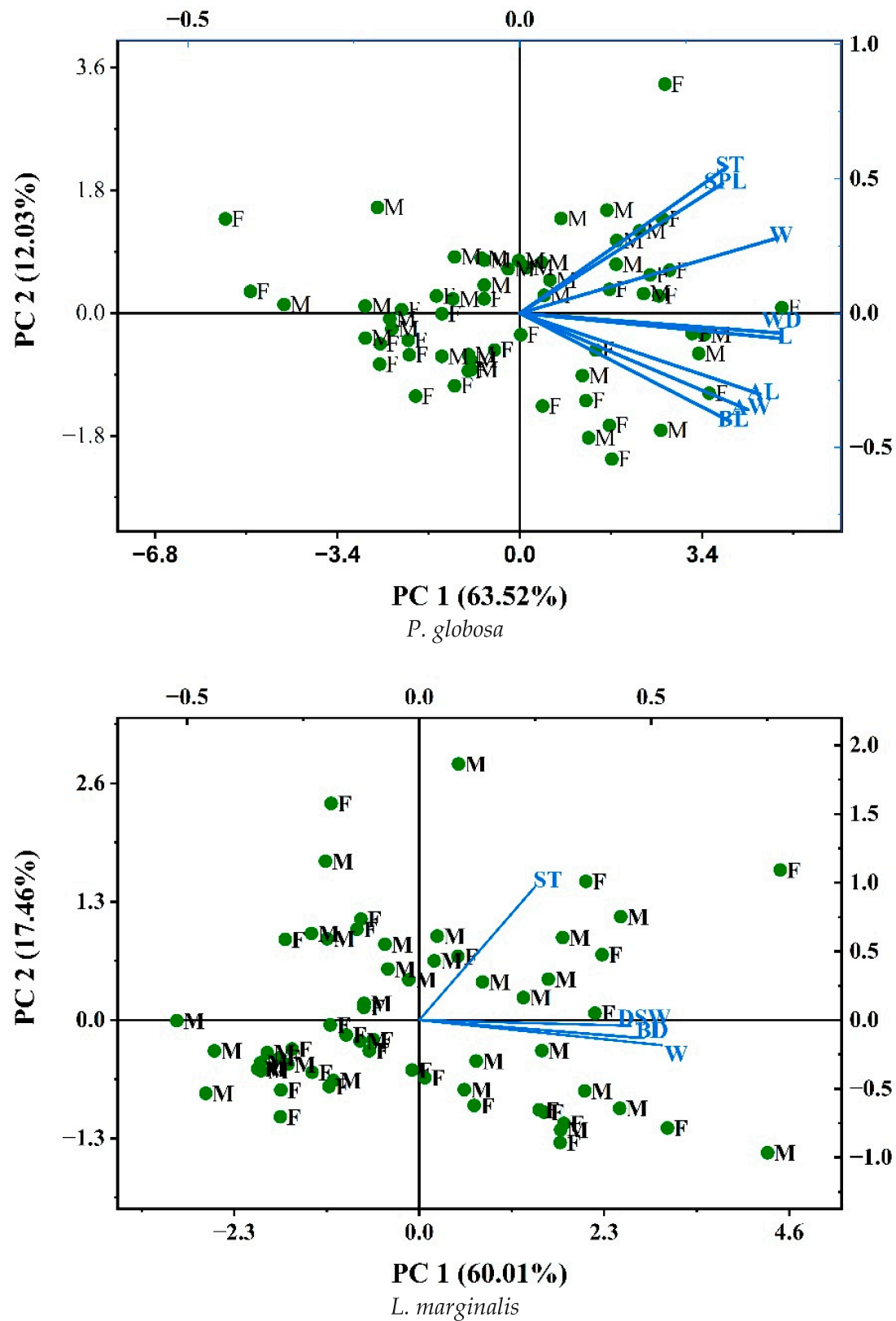


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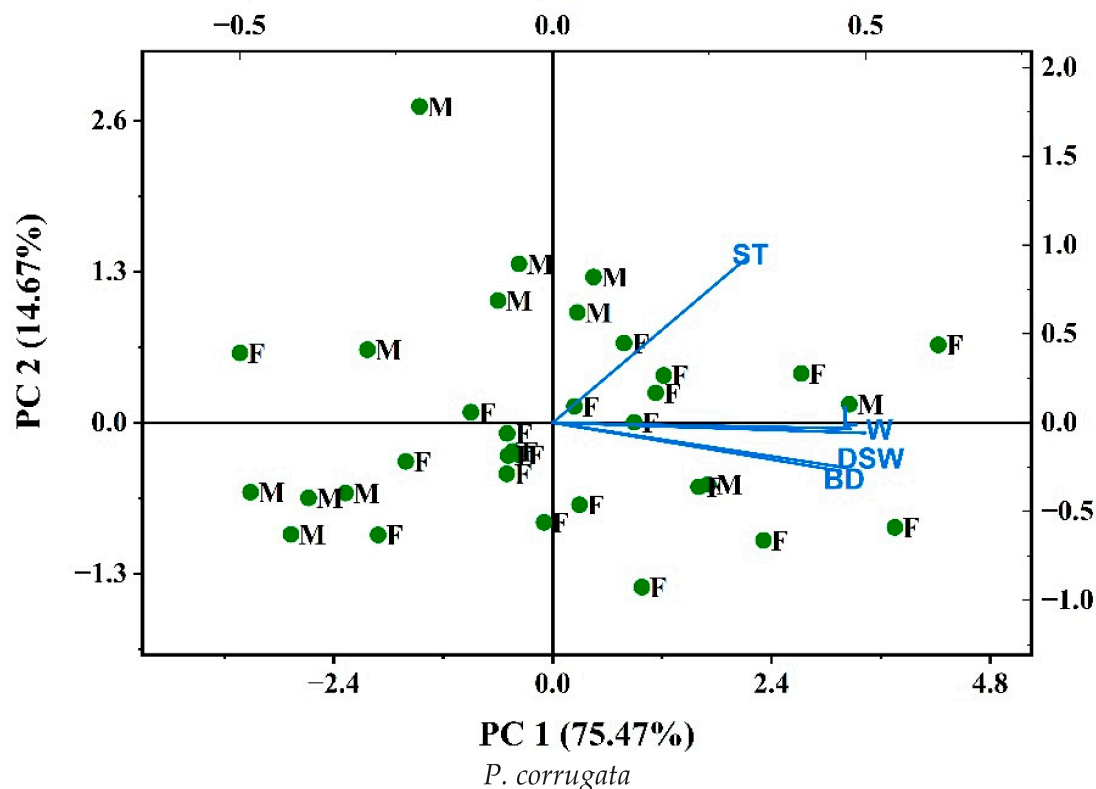


Figure 5. Principal component analysis of different morphometric traits between male and female of the study mollusk (ST—soft tissue weight (g), SPL—spiral length (cm), W—weight (g), WD—shell width (cm), L—length (cm), AL—aperture length (cm), AW—aperture width (cm), BL—base length (cm), BD—shell depth (cm), L—length (cm), DSW—dry shell weight (g), M—male, F—female).

4. Discussion

Current multi-species analyses reveal a strong allometric relationship between size and mass, with high-quality length–weight regressions ($R^2 \sim 0.80$) and narrow confidence intervals within observed size ranges. These patterns align with classic allometric theory for mollusks and are supported by empirical studies showing excellent fits for bivalve length–weight models across various environments and species [32,33]. Notably, the high R^2 for *P. corrugata* (0.80) reflects findings in Indian unionids that identified tight length–weight relationships and seasonal variations in scaling exponents, highlighting the predictability of biomass from shell metrics across the full ontogenetic range [27,34]. Conversely, the slightly lower but significant R^2 values for *P. globosa* and *L. marginalis* suggest that, for gastropods and some unionids, variability due to habitat, developmental stage, or tissue-to-shell allocation warrants caution when applying these models beyond the sampled size range [25,35]. The weaker correlations may stem from differences in measurement parameters, reproductive status, and the presence of mixed-size or mixed-age groups. Furthermore, variations in shell shape and growth patterns likely contribute to variability in weight at a given length.

Differences in slopes between species are biologically significant in mollusk morphometrics [36]. The maximum mass gain per unit length in *L. marginalis* matches aquaculture and field research, where it often shows negative to near-isometric allometry ($b \approx 2.5\text{--}3.0$), depending on culture phase and conditions [37]. This indicates a flexible distribution of energy between the shell and somatic tissues [38]. The close clustering of *P. corrugata* supports previous findings that morphometric measures are reliable predictors in this species and related ones, likely because shell and dry-shell weight are closely linked to total mass in stable river environments [39,40]. Our linear models follow the recommended log–log

allometric approach, highlighting the importance of bias-aware fitting and inference within specified ranges.

Shell base length, aperture size, and spire length together influence *P. globosa*'s mobility, protection, and stability [41]. A larger base increases internal space and helps the snail anchor securely, while longer or wider apertures allow greater extension of the foot for movement and feeding, but may slightly reduce defensive closure [42]. Spire length shapes the shell's internal structure, balancing weight distribution and strength. These morphometric traits determine how effectively the animal moves, feeds, resists predators, and maintains stability in its habitat [43]. Overall, the plots suggest weak sexual dimorphism: small shifts in central tendency without clear, consistent separation.

Sex-specific comparisons show minimal sexual dimorphism in most traits, except for two notable cases in *P. corrugata*: females tend to have higher body weight and slightly wider shells [27]. This subtle dimorphism aligns with the existing unionid literature, which generally finds sex differences to be weak or context-dependent, often appearing in mass-related traits near reproductive periods [44]. In *P. globosa*, minimal sex differences in linear dimensions align with regional surveys reporting broadly conserved shell proportions across seasons and locations, despite a wide ecological range [20]. *P. corrugata* shows significant male–female differences because females invest more in reproduction, including egg production and brooding, which increases body mass and slightly widens the shell [45]. This difference could point to a fecundity advantage, as females generally allocate more energy to reproductive tissues, resulting in higher body mass [46]. However, additional evidence, such as gonad weight or reproductive success, is needed to confirm this. This leads to significant variation in weight and shell dimensions, unlike *P. globosa* and *L. marginalis*, which show minimal sexual dimorphism.

Condition index (CI) patterns were species-specific, with males generally higher, significantly so in *P. corrugata*. CI is a well-established integrator of energy reserves relative to shell or size, and its sensitivity to environmental quality and reproductive cycle is extensively documented in bivalves [47]. Meta-analyses and protocols alike stress that CI responds to food availability and culture intensity, supporting our inference that male-biased CI reflects differential resource allocation or timing relative to gametogenesis [26]. Comparable sex overlap in *L. marginalis* echoes recent lake and farm studies reporting seasonal CI fluctuations but limited, persistent sex separation, again suggesting that environmental factors and spawning schedules can mask dimorphism outside peak reproductive windows. In Bangladesh, from May to July, the rainy season begins, boosting the food supply for freshwater mollusks and triggering their active reproduction [25,26]. During this period, females devote much of their energy to producing and nurturing gametes [48]. Therefore, reproductive effort reduces energy available for body maintenance, leading to lower body reserves and a lower condition index.

The multivariate structure derived from PCA organizes these univariate patterns. In all three species, PC1 accounts for 60–75% of the variance, representing an overall size–mass axis with consistently positive loadings for linear and mass traits—mirroring the typical “size axis” observed in molluscan morphometrics. The secondary axis (PC2), which contrasts soft tissue with shell dimensions, is biologically meaningful: allometric research indicates that investment in tissue versus shell varies with hydrodynamics, trophic level, and life history, creating orthogonal gradients of shape and allocation relative to size. For *P. globosa*, females' higher placement on PC1 (larger size) combined with males' tendency toward PC2 (longer spires and more soft tissue) aligns with regional studies showing negative allometry and environmental plasticity in subtle, often secondary differences in aperture and base traits. The extensive intersexual overlap in *L. marginalis* and *P. corrugata*

PCs supports previous findings that sex accounts for little morphometric variation after size is considered, even with modest mass dimorphism.

5. Conclusions

This study establishes consistent allometric relationships and largely overlapping morphometric patterns across *P. globosa*, *L. marginalis*, and *P. corrugata*. Length–weight models proved robust within sampled ranges, particularly for *P. corrugata*, while sexual dimorphism was generally limited, emerging most clearly in female-biased mass and shell width in *P. corrugata*. Condition index patterns further highlighted species-specific physiological differences, with males often showing higher condition indices. PCA confirmed body size as the dominant driver of morphological variation, with shape-related differences representing only minor secondary axes. However, there are some limitations to consider. Relying on cross-sectional data limits our ability to infer growth patterns or seasonal reproductive effects. The narrow length ranges in certain species may also affect the applicability of regression slopes. Moreover, environmental factors such as habitat variation, food availability, and hydrological conditions were not directly measured, though they are likely to affect morphometry and condition. Future longitudinal studies that incorporate habitat data would enhance the ecological and conservation relevance of these findings.

Freshwater mussels and snails are threatened by pollution, dams, and exploitation, yet Bangladesh lacks comprehensive conservation programs for them. Monitoring efforts are mainly confined to short-term academic surveys and regional studies, with no standardized national initiatives. Consequently, the absence of long-term monitoring hampers effective conservation planning and impedes understanding of population trends in these species.

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Institutional Review Board Statement: Current institutional research ethics standards apply only to vertebrates and therefore do not apply to invertebrates, particularly freshwater snails and mussels. In addition, the sample was sourced from the discarded bycatch of commercial and traditional fishermen; no institutional animal ethics approval is required for this purpose.

Data Availability Statement: Data will be made available upon reasonable requests to the corresponding authors.

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Conflicts of Interest: The authors declare no conflicts of interest.

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