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Abundance and body size of the invasive snail *Physa acuta* occurring in Burdwan, West Bengal, India

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Abstract
The abundance and body size of the population of the invasive snail *Physa acuta* Draparnaud, 1805 (Gastropoda: Hygrophila: Physidae) was assessed from recently established population in Burdwan, India. On the basis of the shell length and body weight of the snails the body size variations were portrayed. The results suggest that the relative abundance of the snails in the sewage drain systems varied among the different sizes and with the months significantly (P<0.05 level) revealed through logistic regression. Correlations among the different body size variables were observed, with the shell length (x)-body weight(y) relationship being: $y = 0.004x^{4.165}$ and the shell length(x) - shell weight (y) relationship being: $y = 0.106x^{2.298}$. Availability of the invasive snail *P. acuta* in the study area indicates the geographical range expansion in eastern India since its first report from Kolkata in 1995. Monitoring the freshwater habitats should be adopted to note the extent of invasion of the freshwater snail *P. acuta* in India.

Keywords: Invasive snail, *Physa acuta*, abundance, logistic regression, length-weight relationship

1. Introduction
The North American sewage snail *Physa acuta* has spread globally as an invasive species. In India, the spread of the snail *P. acuta* has been recorded from Kolkata, India [1] and subsequently from Assam [2, 3], where these snails are known to inhabit the sewage drains and adjacent puddles. While the snail *P. acuta* inhabits freshwater bodies like lakes and ponds in other regions of the world [4-8], in India, the snails are still restricted to the sewage drain habitats. However, the geographical range expansion of the snail is possible, even if it restricts to a particular habitat. This proposition is substantiated by the observations of the snails in the sewage drains in Burdwan, India, which is in close proximity to Kolkata. Occurrence of the snail *P. acuta* in the concerned geographical area prompted us to evaluate the relative abundance and morphology to characterize the population structure in the habitats. Population ecology and life history traits like body size [9-11] and fecundity [12] are considered as crucial parameters for understanding the colonization and spread of invasive species including freshwater mollusca [13-15]. Monitoring of the *P. acuta* population at the local scale would enable highlighting the relative abundance and possibilities of colonization and establishment in the concerned habitats. Invasive species exhibit geographical range expansion that augments the abundance and subsequent dominance within the habitats. Continuous monitoring enables assessment of the expansion of the population in the invaded habitats. Thus, in compliance with the significance of population monitoring of invasive species, an attempt was made to evaluate the population structure of the freshwater snail *P. acuta*. The results would justify the establishment of the invasive snail in the sewage drain habitats with a range expansion at the geographical scale. The present study is a pioneer effort to characterize the abundance and body size of the invasive snail *P. acuta* in India.

2. Materials and methods
2.1 Study sites
The study on the distribution of the snails were made using Burdwan as a focal study area with an extended studies on the distribution of the snails in and around Kolkata and the adjoining area. Initial study included survey of the recorded [1, 16] and prospective habitats including the ditches, bogs and sewage drains in the study sites. Collection of the snails was made following

random sampling of the habitats that were found positive for the invasive snail *P. acuta*. Further, the habitats were sampled biweekly since 2012 to 2014 and the data on the abundance and the positive habitats were used as input variables to portray the relative spread across the geographic area concerned.

2.2 Assessment of abundance and size of *P. acuta*

The snails were collected from the sewage drain and associated puddles, using a net fitted with a long handle. The net was dredged along the length of the sewage drain and the collected snails were placed in plastic bags containing water from the drain and tap water mixed in a ratio of 1:1. In the laboratory the collected snails were emptied in glass aquaria (32 X 36 X 38 cm) containing tap and pond water mixed in equal proportion for rearing and maintenance. Following removal of detritus and decomposing wastes collected in the process, the snail population were segregated into different size classes, based on the shell length (in mm) and the numbers against each size class were recorded. With a class interval of 1mm, 12 size classes of the snails were constructed (considering shell height of 1 mm through 12 mm). The snail population based on the size classes was recorded for all the months except May when the sewage drains remained completely dry.

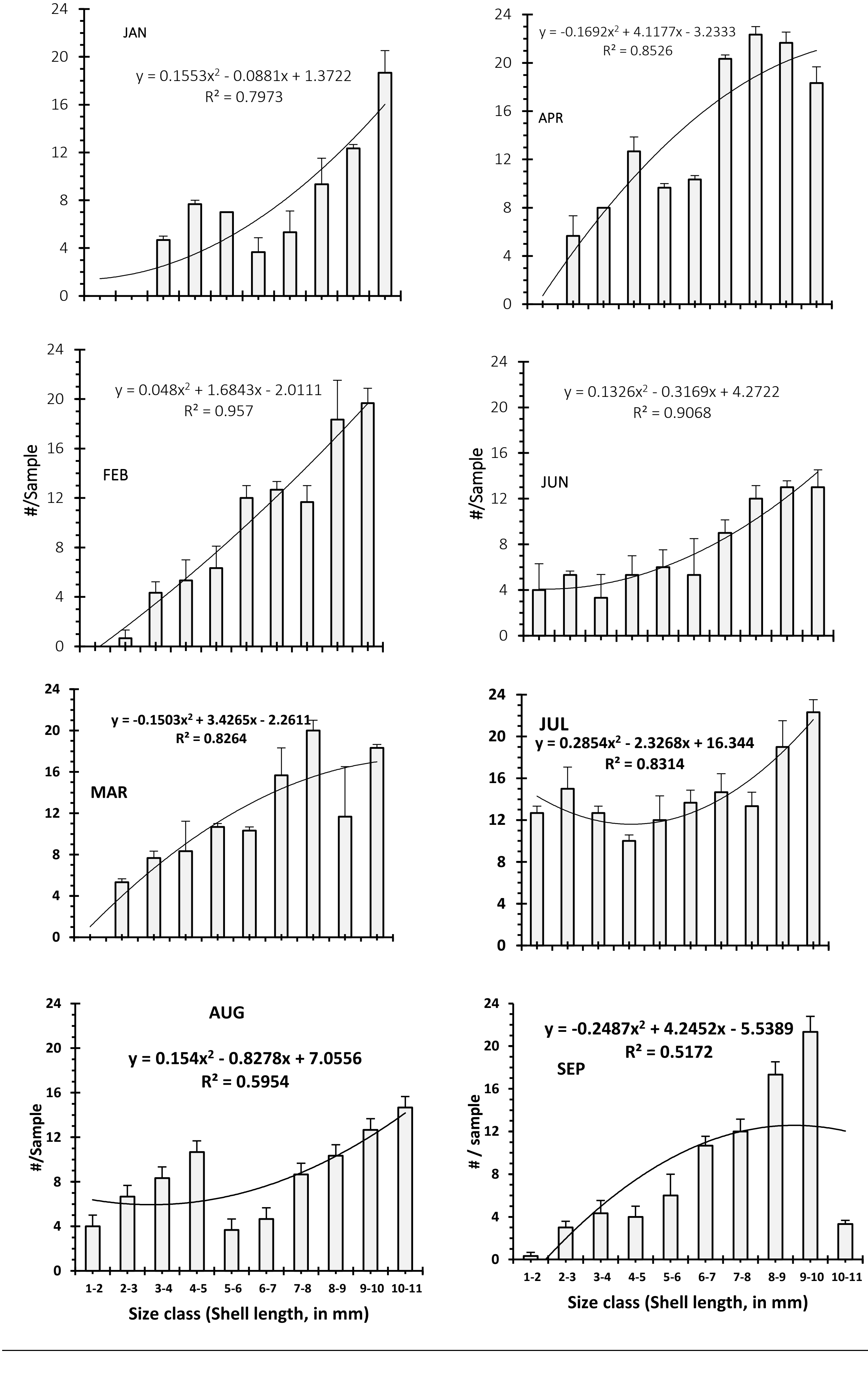
The shell length and body weight (wet) of selected *P. acuta* were measured to portray the body size and shape for the population. For each of the snail, the shell length (from the apex to the tip of the last whorl in mm) and shell breadth (width of the last whorl) were measured using a vernier caliper (Insize, Brazil) and recorded to the nearest 0.1mm. Body weight of the individual was recorded using a pan balance (Citizen, India) to the nearest 0.1mg. Following death of the individual snail, the shell height and the shell weight were further measured, and compared to deduce the weight of the soft tissue. At least 250 snail individuals were considered for the study with emphasis to include the variability in the population. In all instances, a snail considered once was not used further to avoid pseudo-replication.

2.3 Data analysis

Population structure of the snail based on the size classes were analyzed following a generalized linear model to deduce about the differences in the proportional representation of the size classes over the different months in a year. Assuming generalized linear model (GLM), the data on the relative abundance of each size class of *P. acuta* collected over the one year period, was subjected to a regression following binomial GLM using a logit link with size class and month as predictors. In the binomial GLM, the response variable 'proportion of snails' is assumed to follow binomial (n, p) distribution with n trials (collection samples within a month) for each combination of explanatory variables. The probability parameter p is here a linear combination of explanatory variables. A logit link was used and parameters were estimated through maximum likelihood using the software XLSTAT [18]. The logistic equation is provided in the form of: (y) Abundance = 1/ (1+ exp (- (a +b_1x_1 +b_2x_2))), where x_1 and x_2 represents months and size classes of *P. acuta*. A Chi square value (Wald's chi-square) was used to deduce the significance of the estimated parameters of the model that includes month, and size class. Further, a mixed model ANOVA was applied to justify the differences in the relative abundance of the different size classes over time (months). A regression analysis [19] was used to relate the variables representing the body size of the snails. Thus shell length (SL in mm), shell breadth (SB in mm), body weight (BW in mg) of living snails (208 specimens) were considered for the purpose. Extrapolation of the regression (power) equation between shell length and the shell weight of dead snails (226 specimens) the weight of the soft tissue (W_{ST}, in mg) of living snails was obtained. The shell weight of living snails (W_{SHELL}, in mg) was deduced by subtraction of the soft tissue weight from the body weight. The collection of the living and the dead snails were carried out at different time and habitat within Burdwan to comply with the norms of replication. The purpose of the analysis was to portray the body size of *P. acuta* occurring in Burdwan for further comparison with populations in other invaded areas like Kolkata, India.

3. Results

The proportional representation of each size class of the snails varied significantly suggesting the dynamic nature of the population in the invaded habitats. In all instances throughout the months the differences in the proportional representation of the individuals in the size classes mark the efficacy of the invasive snail to establish in the habitats concerned (Fig 1). The proportional abundance of the different size classes varied could be related to the months and the size class as: (y) abundance = 1 / (1 + exp(-(-2.84 - 0004*(x_1) Month + 0.07*(x_2) Size class))). Following the norms of the GLM, the contribution of the size class as explanatory factor remained significant at $p < 0.05$(intercept= − 2.838 ± 0.329; Wald $\chi2$ = 74.5; P<0.001; month = 0.0004±0.0001; Wald $\chi2$ = 0.0001; not significant; size class = + 0.067 ± 0.034; Wald $\chi2$ = 3.935; P <0.047). However, the relative numbers of the different size classes (Fig 1) differed significantly with the months (Table 1.1). The results of the two analysis indicate that the proportional representation of the different size classes vary within the population but remain constant with the months. The result of ANOVA suggests that the absolute number of the population however vary with the months, possibly reflecting the environmental regulation of the population of the snail *P. acuta*. The morphometric parameters like the shell length (SL, in mm), shell breadth (SB in mm), SL/SB ratio, body weight (BW), weight of soft tissue (W_{ST} in mg) and the shell weight of dead snails (W_{SHELL} in mg) were used as variables to define and classify the population of *P. acuta*. The correlations among these variables are shown in Table 2, and the regression of the shell height and the body weight of living *P. acuta* (sample size of 208 living specimens; Body weight (y) = 0.004* Shell length (x) $^{4.165}$; R^2 = 0.989) is shown in Fig 2a. Extrapolation of the regression (power) equation between shell height and the shell weight of dead snails (226 specimens, Shell weight (y) = 0.106* Shell length (x) $^{2.298}$; R^2 = 0.989, Fig 2b), the weight of the soft tissue (W_{ST}) was obtained. The morphological variables including the shell length/ shell breadth ratio were significantly positively correlated (Table 2), indicated by the Pearson product moment correlation coefficient, (r), values. The regression equations among the different variables are shown in Fig 3, bear high values of coefficient of determination (r^2), excepting for the relation of the shell length and body weight against the shell length/shell breadth ratio. The morphological estimates and the differences in the size classes of *P. acuta* suggest considerable heterogeneity in population existing in the concerned habitats.



JAN
y = 0.1553x2 - 0.0881x + 1.3722
R² = 0.7973
APR
y = -0.1692x2 + 4.1177x - 3.2333
R² = 0.8526
FEB
y = 0.048x2 + 1.6843x - 2.0111
R² = 0.957
JUN
y = 0.1326x2 - 0.3169x + 4.2722
R² = 0.9068
MAR
y = -0.1503x2 + 3.4265x - 2.2611
R² = 0.8264
JUL
y = 0.2854x2 - 2.3268x + 16.344
R² = 0.8314
AUG
y = 0.154x2 - 0.8278x + 7.0556
R² = 0.5954
SEP
y = -0.2487x2 + 4.2452x - 5.5389
R² = 0.5172
#/Sample
/ sample
1-2
2-3
3-4
4-5
5-6
6-7
7-8
8-9
9-10
10-11
Size class (Shell length, in mm)

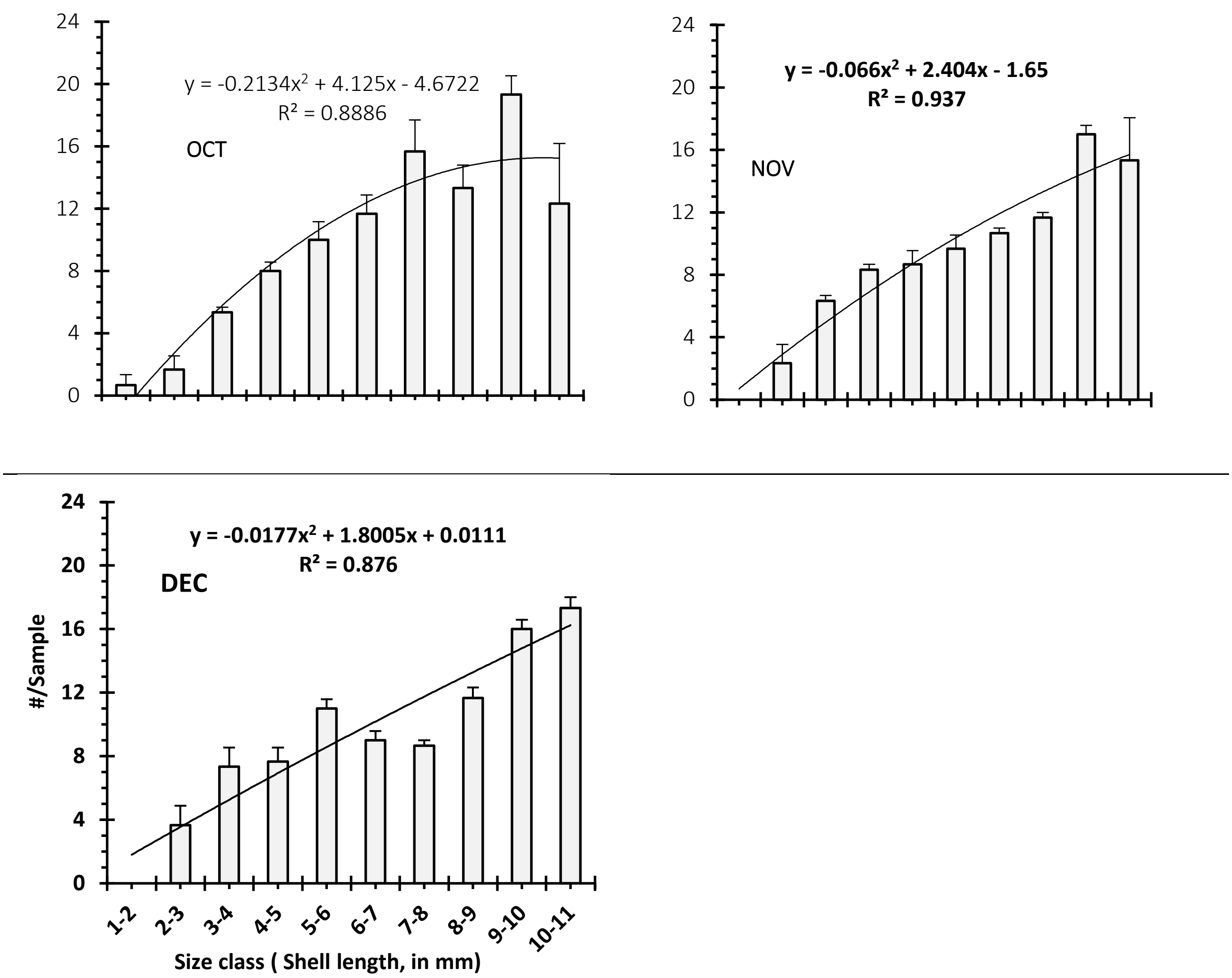


Figure 1: The relative abundance of different size classes of the snail *P. acuta* sampled from the sewage drain and associated puddles in Burdwan, India between 2012 and 2014. Best fit regression (Polynomial regression) is shown against each month.

Table 1: The results of repeated measures ANOVA to justify the differences in the relative abundance of the size classes of the invasive snail *P. acuta* in the samples collected from Burdwan, India between 2012 and 2014. The values in bold indicate significance at $P < 0.001$ level. The analysis was made assuming sphericity as revealed through Mauchly' sphericity test. Here shell length (SL) and months are considered as the explanatory variables. The partial η^2 explains the extent of variations explained by the variable.

Effect	Wilks' λ	F	Hypothesis df	Error df	Partial η^2
SL	0.010	**151.727**	9	14	0.990
SL * Month	0.00003	**4.380**	90	105.211	0.691
Tests of Between-Subjects Effects					
Source	Sum of Squares	df	Mean Square	F-value	Partial η^2
Month	1558.224	10	155.822	**12.697**	0.852
Error	270	22	12.273		
Tests of Within-Subjects Effects					
SL	6912.133	9	768.015	**135.532**	0.860
SL * Month	2562.867	90	28.476	**5.025**	0.696
Error(SH)	1122	198	5.667		
Tests of Within-Subjects Contrasts					
SL	6690.844	1	6690.844	**1089.822**	0.980
SL * Month	553.986	10	55.399	**9.023**	0.804
Error(SH)	135.067	22	6.139		

Figure 2: The relation between shell length (SL in mm) and the body weight (BW, in mg)) (a) of living specimens and dead shell (W_{SHELL}, in

mg) (b) of *Physa acuta* collected from the sewage drains and associated puddles of Burdwan, India.

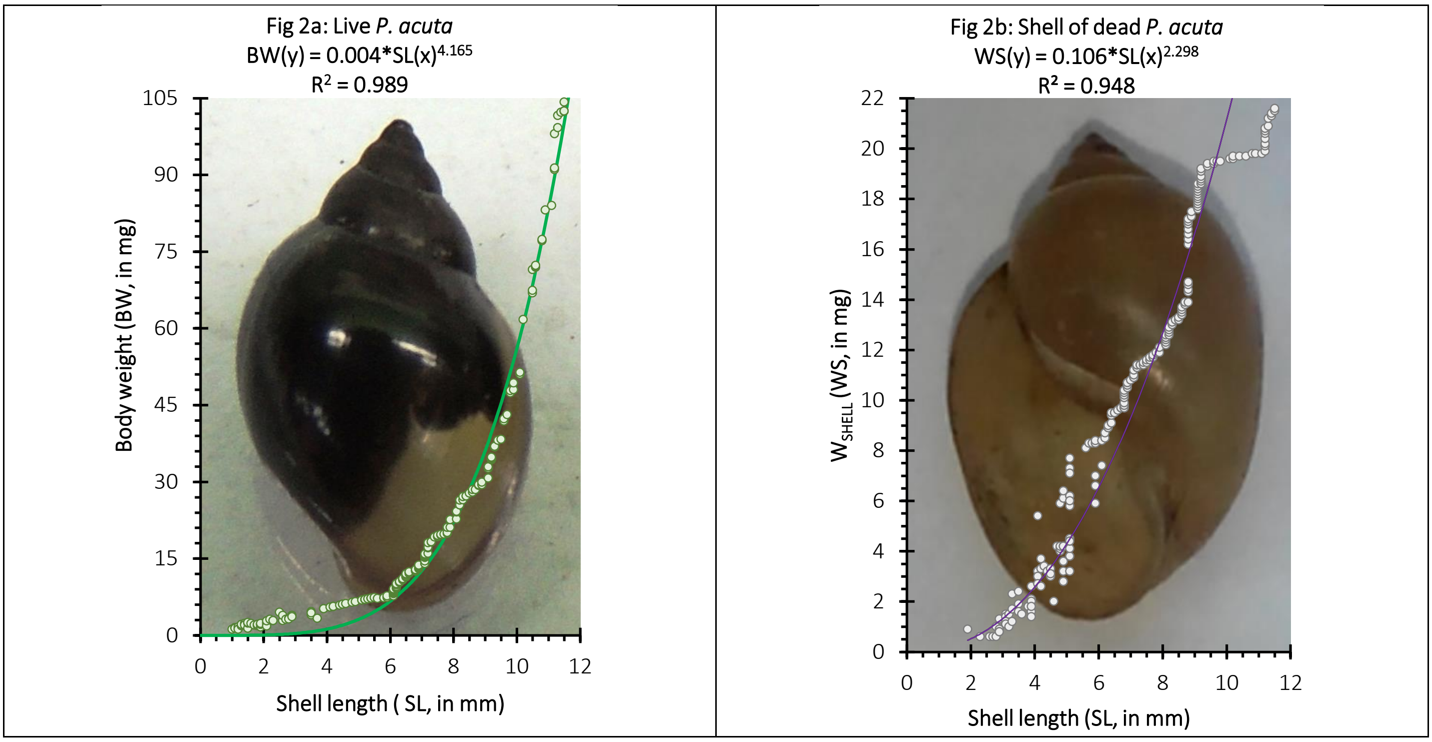


Table 2: The correlation coefficients among the different variables representing the morphology of the snail *Physa acuta* collected from the sewage drains and associated puddles of Burdwan, India. (living *P. acuta* n= 208; Shells of dead *P. acuta* n =226); SL- Shell length (in mm); SB- Shell breadth (in mm); BW- Body weight (in mg); W_{SHELL} – Weight of shell (in mg); W_{ST} =Weight of soft tissue (in mg); SL/SB ratio – Shell length and shell breadth ratio;(The values of W_{SHELL} and W_{ST} were derived from extrapolation of the equation in 2b to the shell length presented in Fig 2a)

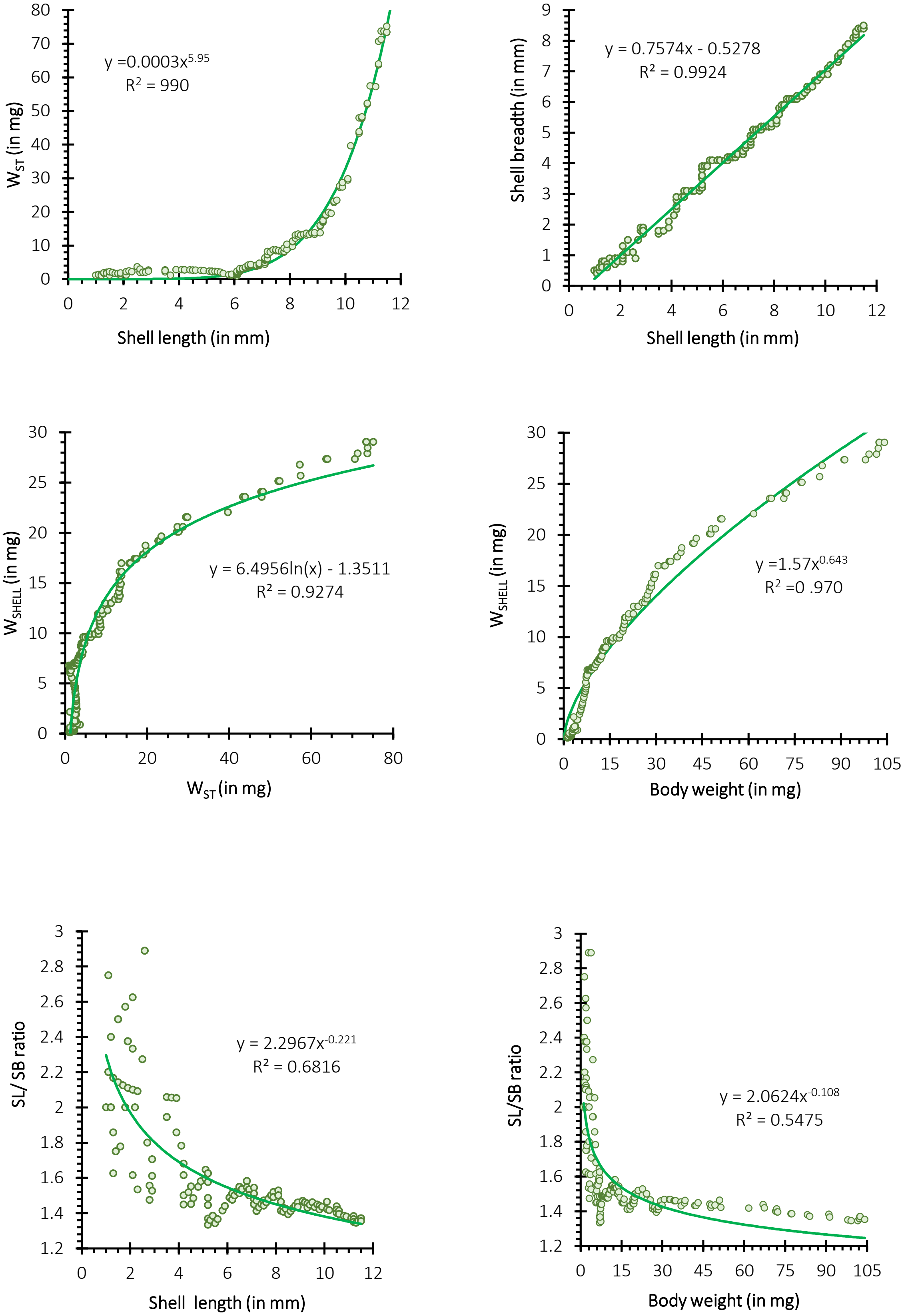
Variables	SL	SB	BW	W_{SHELL}	W_{ST}
SB	**0.996**				
BW	**0.818**	**0.833**			
W_{SHELL}	**0.953**	**0.957**	**0.944**		
W_{ST}	**0.729**	**0.748**	**0.989**	**0.884**	
SL/SB ratio	**-0.733**	**-0.758**	**-0.438**	**-0.593**	**-0.353**

4. Discussion

The snail *P. acuta* was observed in the sewage drains and the associated puddles with varying relative abundance in the different months. The representation of the size classes varied in the months significantly, indicating the dynamic nature of the population. The monthly and size class level variations were observed in the *P. acuta* populations reflecting the effects of the environmental condition, including resource availability [20, 21]. Variations in the population structure is observed for *P. acuta* in the invaded habitats both in lentic and lotic condition in different geographical locations [22-27]. Although similar variations in the size class distributions over time and space was observed in the present instance, the populations of *P. acuta* were restricted to the sewage drain habitats. Invasive species exhibit high population density at a local scale owing to higher fecundity and lower resistance to environmental conditions. Perhaps the high density and consistent availability of the population of *P. acuta* in the sewage drains is a result of high reproduction by relatively high population density. Sewage drain systems form an interconnected network of channels having links with other freshwater ecosystems like ponds, lakes and canals. While extensive survey of the freshwater habitats of the concerned geographical area and Kolkata did not record *P. acuta* from the ponds and lakes. Earlier studies have shown that the native malacophagous leech [28] and the water bugs [29, 30] can act against the colonization of *P. acuta* in the local water bodies. The vulnerability of the snail *P. acuta* to the predators are equal to the native prey like *Lymnaea luteola* [30]. Consistent high population density of the snail *P. acuta* along with the links of the sewage drains may facilitate the colonization in the accessible ponds and lakes in near future [31, 32].

Heterogeneity of the population is reflected through the differential representation in the size classes of *P. acuta*. The size classes were represented through the shell length (SL, in mm), which is also a means of presenting the body size of the snail.

Figure 3: The relations among the morphometric variables of *P. acuta* collected from Burdwan, India. W_{SHELL} = Weight in mg of the dead shell only. W_{ST} = Weight in mg of the soft tissue. The best fit regression equations are shown in the figures.



Ontogenic changes in the morphology of the snail can be deduced through the relation of the shell length and body weight at different ages. At a spatial scale, the presence of the snails of different age groups would be reflected through the corresponding variations in the shell length and body weights. Thus the heterogeneous size classes observed in the present instance is a reflection of the snails of different age classes, though the variations in the shell size and body weight of a specific age class is also included. The relationships between shell length and body weight (wet) is almost an universally accepted measure of representing the body size in the aquatic snails [9-11-20, 21]. In the present instance, both the living and dead specimens were considered for the morphometric analysis of the body size of the invasive snail *P. acuta*. The relationship between the shell weight and shell length of the dead snails were utilized to deduce the shell weight and the soft tissue weight of the living snails. The regression equations comply with the theoretical and empirical observations on different snails and mussels. Although bivariate regression equations have been considered (Figs 2 and 3), use of multiple predictors are recommended for the assessment of the morphological variables and biomass for aquatic snails, whether dry, ash free dry or wet weight are being considered as response variables) [32, 33]. Nonetheless, the length-weight relation of the present instance complies with the allometric equation (power regression equation was the best fit) as predicted for the aquatic snails. Further assessment using the data on the growth of *P. acuta* can be carried out to validate the proposed relations among the weight and the length. The shell morphometry and the body size of snails including *P. acuta* exhibit plasticity under the varying environmental conditions, including the presence of predators. As a consequence, the body size of *P. acuta* may vary with the geographical locations, recognized as ecotypes, which needs to be judged for the population of the snails in Burdwan and Kolkata, along with other populations found elsewhere in India. However, biomonitoring of the aquatic habitats should be prioritized to understand the habitat and range expansion of *P. acuta* in Indian context.

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