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Biometric evaluation of the freshwater snail *Pila globosa* (Swainson, 1822) from the river Ganga in Bihar, India

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ABSTRACT

Length-frequency distribution, morphometry, length-weight relationship, relative condition factor and sex ratio of the apple snail *Pila globosa* (Swainson, 1822) were studied from the river Ganga. A total of 922 specimens were collected during July 2020 to June 2021, ranging from 3.53 to 62.99 g and from 16.49 to 59.78 mm (shell length). Length-frequency distribution revealed that more than 50% of male and female specimens were within 35 to 45 mm in length and only a few (1.58%) female specimens were observed in the higher length (>55 mm). Females have significantly longer shells and are heavier than males. Shell length has the strongest correlation with shell width (r=0.974) and the least with penultimate whorl length (r=0.855). Length-weight relationship, indicated that the species has negative allometric growth (b<3). The relative condition factor was 1.07 for males, 1.00 for females and 1.01 for pooled data. Sex ratio (M:F) statistically deviated from the hypothetical ratio of 1:1, indicating a heterogeneous snail population (1:1.9, p<0.05).

Keywords: Ganga River, Growth pattern, Sex ratio, Shell morphology

Introduction

The family Ampullariidae consists of omnivorous gastropods with distinct globular shells that have been distributed throughout the tropical regions of South America, Africa and the Asian continent (Hayes *et al.,* 2015; Cowie, 2015). Five species of Ampullariidae from the genus *Pila* are found in the Indian subcontinent (Rao, 1989). The apple snail *Pila globosa* (Swainson, 1822) has ethno-medicinal properties and is also used as a bio-indicator for monitoring the health status of aquatic ecosystems (Bhattacharya *et al.,* 2016).

Length-frequency distribution provides timely information about the growth, maturity and age composition of the species (Ahmed *et al.,* 2016). Morphometric measurements are frequently used to identify and characterise the snail species (Schniebs *et al.,* 2013; Okabe and Yoshimura, 2017). It also helps determine whether a stock is heterogeneous or homogeneous based on the interrelationship between different length variables (Ahirwal *et al.,* 2017). Length-weight relationship is a useful tool to assess the growth, maturity, and general well-being of organisms (Le-Cren, 1951; Froese, 2006, Prajapati *et al.,* 2022; Sarma *et al.,* 2022). The condition factor provides information on the health status of a species and the community as a whole and can be used to compare the physiological robustness of fish on a numerical basis (Oni *et al.,* 1983). Knowledge of the reproductive traits of the organism is essential for conservation and maintaining their populations in an ecosystem (Breton *et al.*, 2018).

P. globosa is widely available in ponds, tanks and other derelict water bodies of Bihar and substantially contributes as a source of protein and income for underprivileged communities. It is also used as an animal feed additive in freshwater aquaculture in this region due to the high protein content of their meat. Some studies have been carried out earlier on its length-frequency distribution, morphometry, length-weight relationship and condition factor from India and adjacent countries (Saha *et al.,* 2016; Panda *et al.,* 2021).

However, information on length-frequency distribution, morphometry, length-weight relationship, relative condition factor and sex ratio of *P. globosa* has not been documented in the riverine system of India. Hence, the present study was conducted to establish baseline data on important biological parameters of the Indian apple snail from the river Ganga in Bihar, India.

Materials and methods

Sampling site

In the present study, a total of 922 specimens (16.49-59.78 mm shell length range and 3.42-62.99 g shell weight range) caught from the river Ganga (Bihar) were collected from the Digha fish market (25°40'8.4"N; 85°0'18"E) through monthly samplings during the period July 2020-June 2021. Shell length was measured along an axis passing through the apex to the bottom of the shell, to the nearest 1 mm using a digital vernier caliper (Insize-0/150 mm) and weight was recorded to the nearest 1 g using a digital balance (WENSAR TM-MAB 220).

Length-frequency distribution and morphometry

The length-frequency distributions for male and female were plotted separately at 5 mm intervals. Morphometric characters studied include the seven continuous shell characters such as Shell length (SL), Shell width (SW), Aperture length (AL), Aperture width (AW), Whorl length (WL), Penultimate whorl length (PWL) and Spire length (SPL), as well as six operculum characters such as Operculum length (OL), Operculum width (OW) and distance from the nucleus to the Right margin of the operculum (RC), Left margin of the operculum (LC), Upper margin of the operculum (TC), to Lower margin of the operculum (BC); all these measurements were taken using digital vernier caliper made along imaginary straight lines (Fig. 1).

For the compared morphometric characters, linear regression equation was fitted using least square method (Snedecor and Cochran, 1967) and the relationship were represented as Y = a + b X. Based on linear regression model intercept and correlation coefficients were worked out

Length-weight relationship and relative condition factor

The length-weight relationship (LWR) was determined separately for male (n=362), female (n=560) and combined sexes (n=922) using the equation as $W= {}_aL^b$ (Le-Cren, 1951), where W is shell weight (g), L is shell length (mm), *a* is the intercept and b is the regression coefficient.

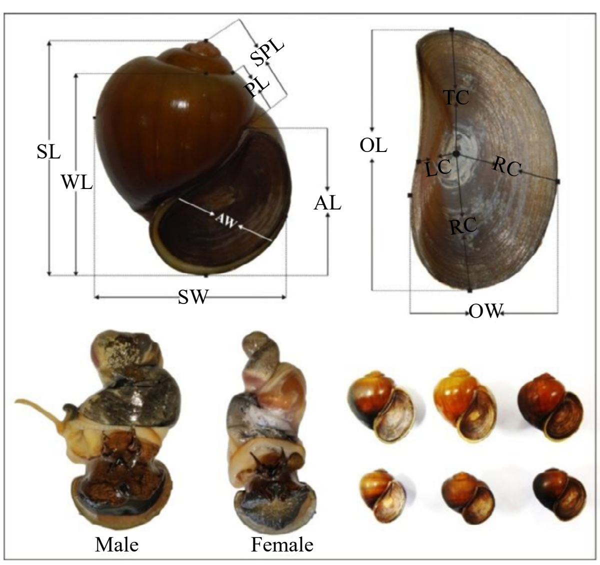

Fig. 1. Morphometric measurements made on shell and operculum of Indian apple snail

ANCOVA was performed in order to determine variations in exponent *b* value between the sexes (p=0.05). To test deviation of *b* value from isometry, t-test was employed and the calculated value was compared with the t-table value for n-2 degrees of freedom (p = 0.05) (Snedecor and Cochran, 1967). Generally, snails are considered to grow isometrically when their estimated exponent value *b* is close to 3. However, if b values deviate from 3, snail growth becomes either negative (b<3) or positive (b>3) allometric. The relative condition factor of an individual snail was computed using the formula as $Kn=W/{}_aL^b$, where W is the observed weight (g) and the value of *a* and *b* obtained from length-weight relationship equation.

Sex ratio

Morphologically, male and female identification in snails is very difficult. Hence, all the specimens were dissected without damaging the reproductive organs. The male has an elongated penile structure or flagellum (4.25-49.45 mm) under the penis sheath, whereas the female has coiled ovary of yellow to reddish-orange colour (Fig. 1). The number of male and female specimens encountered during each month's sampling was used to calculate the sex ratio. To study the homogeneity of the distribution of male and females, the value of sex ratio obtained were subjected to chi-square test using the formula $X^2=(O-E)^2/E$, where observed and expected number of specimens was denoted as O and E, respectively (Snedecor and Cochran, 1967).

Results

Length-frequency distribution

In the present study, the shell length of male snails ranged from 24.39 to 51.94 mm (average 37.80±0.26 mm) and weight ranged from 4.77 to 38.94 g (average 17.06±0.34 g) and female size ranges were 16.49-59.78 mm shell length (average 39.04±0.31 mm) and 3.53 to 62.99 g (average 19.06±0.42 g). Females had significantly longer shells (t=2.847, df=922, p<0.05) and were heavier (t=3.387, df=922, p<0.05) than those of males. Length-frequency distribution revealed that more than 50% of the male and female specimens were within 35 to 45 mm shell length range, beyond which, there was a decline in the numbers of both sexes. Only a few female specimens (1.58%) were observed above 55 mm, while no specimens of this length were observed for males (Fig. 2). Month-wise length-frequency distribution of males and females (Fig. 3) showed that the dominant female sizes were 35, 40 and 45 mm, accounting for 30.2, 38.8 and 33.8% of the total population in pre-monsoon, monsoon and post-monsoon seasons, respectively. Similarly, dominant sizes among males were 35, 40 and 40 mm, which constituted around 34.1, 45.1 and 50.5% of the total

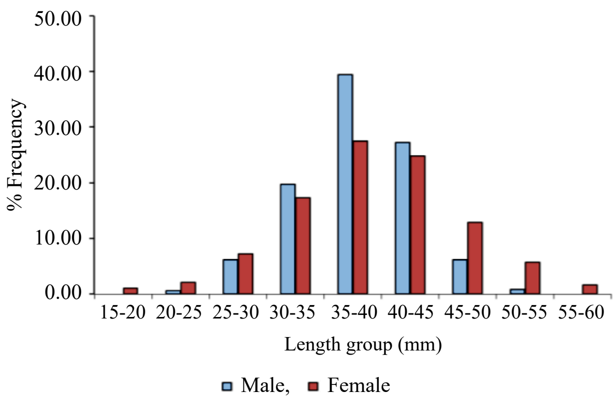

Fig. 2. Length-frequency distribution for male and female individuals of *P. globosa*

population in pre-monsoon, monsoon and post-monsoon, respectively. The maximum size of females was observed to be 51.29, 59.78 and 57.56 mm in the pre-monsoon, monsoon and post-monsoon seasons respectively, while for males the maximum size in each season was 48.61, 51.94 and 47.88 mm.

Morphometry

Morphometric analysis was done for 229 specimens ranging in length from 23.52 to 59.78 mm and weighing between 3.53 and 57.98 g. The maximum coefficient of variation was observed for penultimate whorl length and minimum for aperture length (Table 1).

Linear regression performed for SL against the SW, AL, AW, WL, PWL, SPL, OL and OW indicated that SL has the greatest degree of association with SW (r=0.974) and the least degree of association with PWL (r = 0.855). Linear regression between OL and OW, RC, LC, TC and BC indicated that the OL has maximum degree of association with TC and lowest with OW (Table 2).

Length-weight relationship and relative condition factor

Monthly length-weight data for male, female and sexes pooled were grouped into pre-monsoon (February-May), monsoon (June-September) and post-monsoon (October-January) for estimating the seasonal LWR. The estimated regression coefficient values for females were 2.56, 3.05

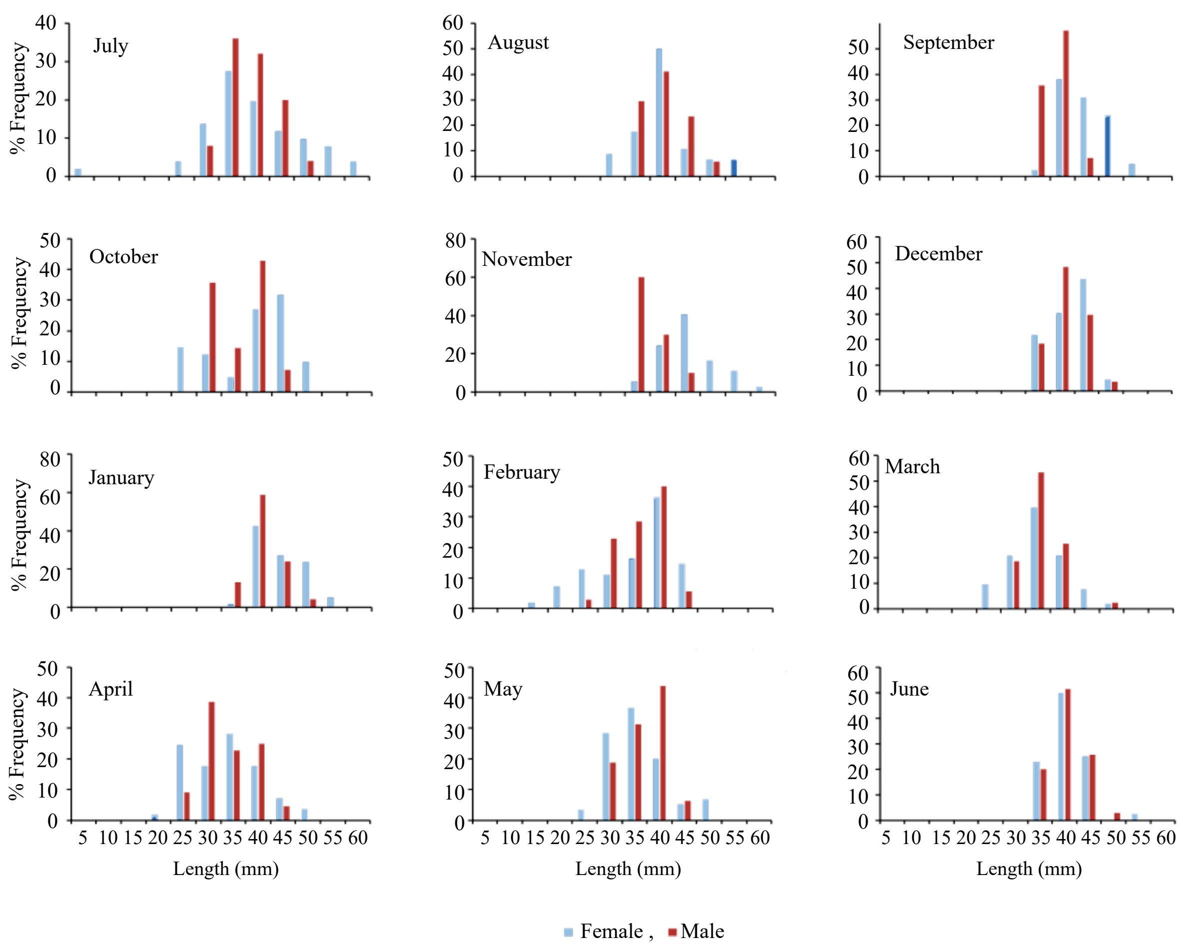

Fig. 3. Month-wise length-frequency distribution for male and female *P. globosa*

Table 1. Descriptive statistics of various morphometric characters of *P. globosa*

Shell characters	Min	Max	Mean±SE	Median	Mode	SD	CV%
SL	23.52	59.78	40.61±0.39	40.56	44.49	6.53	16.04
SW	22.68	58.89	39.07±0.39	38.98	46.95	6.80	17.40
AL	18.98	43.64	31.05±0.26	31.27	28.55	4.51	14.52
AW	10.99	33.49	18.84±0.19	18.86	17.86	3.26	17.30
WL	21.71	53.46	37.05±0.34	37.02	40.06	5.86	15.82
PWL	4.69	19.64	09.31±0.12	09.36	08.89	2.07	22.26
SPL	9.24	31.73	16.12±0.19	16.04	16.99	3.30	20.45
OL	10.15	42.15	28.41±0.26	28.64	25.65	4.55	16.02
OW	9.41	50.11	16.91±0.19	16.64	16.11	3.35	19.82
RC	6.92	21.64	13.23±0.12	13.24	14.31	2.11	15.95
LC	2.27	6.18	04.01±0.04	03.95	3.91	0.72	18.00
TC	3.92	19.14	13.14±0.12	13.13	13.21	2.07	15.77
BC	9.05	31.38	15.18±0.15	15.21	14.55	2.55	16.82

*All measurements in mm

Table 2. Linear regression of various morphometric characters of *P. globosa*

Parameters	*a*	*b*	*r*	*p*
SL and SW	0.339	0.988	0.974	0.049
SL and WL	2.021	0.859	0.965	0.000
SL and SPL	0.084	0.457	0.943	0.000
SL and OW	1.212	0.382	0.942	0.000
SL and AL	0.081	1.391	0.941	0.006
SL and AW	1.631	0.423	0.928	0.000
SL and OL	3.320	0.617	0.921	0.000
SL and PWL	0.398	0.249	0.855	0.011
OL and TC	1.704	0.403	0.884	0.000
OL and BC	1.676	0.475	0.849	0.000
OL and RC	2.674	0.372	0.803	0.000
OL and LC	2.041	0.116	0.731	0.000
OL and OW	0.981	0.527	0.716	0.026

and 2.98 in the pre-monsoon, monsoon and post-monsoon seasons respectively; the corresponding estimates for males were 2.84, 2.94 and 2.81 (Table 3). ANCOVA did not indicate any significant differences in the regression coefficients between the sexes in the three seasons and between the seasons ($p>0.05$). Hence, the data were pooled and LWR was estimated as $W=0.0348L^{2.84}$ for males and $W=0.0452L^{2.88}$ for females. ANCOVA indicated no significant differences in the regression coefficients for both sexes ($p>0.05$). Hence, a common equation was established with data for sexes pooled, as $W=0.0428L^{2.86}$.

t-test showed that the estimated regression coefficient value ($b=2.86$) significantly ($p<0.05$) deviated from the isometric value of 3, indicating that the species exhibits negative allometric growth.

Month-wise relative condition factor (Kn) was found to be highest for male in January and in December for female (Fig. 4). The estimated average Kn values were 1.07±03 for males and 1.00±02 for females respectively, which did not show significant difference ($p>0.05$).

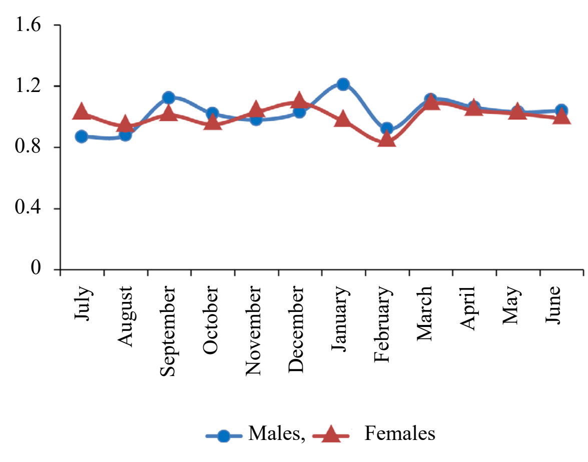

Fig. 4. Month-wise relative condition factor for male and female *P. globosa*

Table 3. Values of the regression coefficient for seasonal length-weight relationship estimated for *P. globosa*

	Pre-monsoon			Monsoon			Post-monsoon		
	Sample	b	r^2	Sample	b	r^2	Sample	b	r^2
Male	171	2.84	0.929	89	2.94	0.795	102	2.81	0.918
Female	223	2.56	0.919	180	3.05	0.874	157	2.98	0.938
Sexes pooled	394	2.63	0.921	269	2.96	0.841	259	2.95	0.935

Sex ratio

Analysis of the month-wise sex ratio indicated that there was no significant difference between the distribution of males and females from December to July, while the proportion of males and females varied significantly from August to November ($p<0.05$). Overall month-wise sex ratio for the entire study period was 1:1.9, which deviated significantly ($p<0.05$) from the expected ratio of 1:1, suggesting heterogeneity in the apple snail populations in the Ganges River in Bihar (Table 4).

Discussion

Individual shell and operculum morphology of snails are determined by their genetic make-up, but intra-specific variation in these characters can be attributed to the ontogenetic variations in growth in the juvenile and adult life stages (Chiu *et al.,* 2002). Shell morphology of snails is also influenced by ecological factors, such as the long-term stability of habitats (Dupouy *et al.,* 1993), the presence of predators (Dewitt *et al.,* 1999), physical characteristics of environments and patterns of habitat use (Antonucci *et al.,* 2012). In the present study, more than half the population of male and female specimens sampled were within 35 to 45 mm in length and females were found to have longer shells and were heavier than males. Analysis of morphometric characters showed that the shell length and operculum length have a varying strength of correlation with other measured characters. The comparative growth of morphometric characters in relation to shell length was found to be highest for shell width and least for penultimate whorl length, whereas operculum length better correlates with the upper margin of the operculum and least with operculum width.

The exponent value in the LWR of an organism also depends upon the health status of the population, their gonadal maturity, spawning, environmental condition and availability of food in the habitat (Froese, 2006; Prajapati *et al.,* 2022; Sarma *et al.,* 2022). Seasonal length-weight relationships indicated that the exponent value for both sexes is quite high during the monsoon, which may be due to more suitable environmental conditions and better availability of favourable food items for growth and maturation of gonads. Overall exponent value for pooled data indicated that the species has negative allometric growth, which agrees with the earlier report on *P. globosa* from Bangladesh waters (Saha *et al.,* 2016). The relative condition factor values for all the specimens ranged from 0.84 to 1.21, with an average of 1.01 and a maximum Kn value was obtained for males (1.07). Monthly changes in the relative condition factors for both sexes could be attributed to the availability of food items and variations in the temperature. It also depends upon the prevailing ecological condition, spawning stress and reproductive potential of the species (Richter, 2007; Prajapati *et al.,* 2022).

Knowledge of the sex ratio in different months is essential for obtaining information on the seasonal segregation of the male and female sexes. Females were significantly more prevalent from August to November which may be due better environmental conditons for breeding in these months. In the present study, the estimated overall sex ratio was 1:1.9, which is quite similar with earlier observation on the freshwater banded snail *Viviparus bengalensis*, where the ratio is 1:1.2 (Raut, 1981).

The present study was carried out to establish the first detailed comprehensive baseline data on the freshwater Indian apple snail, *P. globosa*, from the river Ganga in Bihar. These findings will be useful for future studies on its spawning and reproductive behaviour in the region. More systematic studies need to be undertaken on its life

Table 4. Month-wise sex ratio in sampled population of *P. globosa*

Months	Males (%)	Females (%)	M:F	$\chi2$ (df=1)	p value	Significance*
July	34.25	65.75	1: 1.92	3.62	0.06	NS
August	27.42	72.58	1: 2.65	6.32	0.01	S
September	25.45	74.55	1: 2.93	6.63	0.01	S
October	25.93	74.07	1: 2.86	6.26	0.01	S
November	21.74	78.26	1: 3.60	7.35	0.01	S
December	55.71	44.29	1: 0.79	0.35	0.55	NS
January	42.59	57.41	1: 1.35	1.19	0.28	NS
February	38.89	61.11	1: 1.57	2.22	0.14	NS
March	43.88	56.12	1: 1.28	0.73	0.39	NS
April	43.56	56.44	1: 1.30	0.84	0.36	NS
May	44.86	55.14	1: 1.23	0.57	0.45	NS
June	42.68	57.32	1: 1.34	0.88	0.35	NS
Overall	38.73	61.27	1:1.90	23.31	0.00	S

*NS-Not significant ($p>0.05$), S-Significant ($p<0.05$)

stage-wise nutritional profiling to understand the nutrient dynamics in the life cycle of the snail.

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