Growth of *Allopeas gracile* under starvation: does it comply with von Bertalanffy growth equation?

Sabarni Chakraborty, Abhijit Sikary, Tanisha Ghosh, Ashmita Chakraborty, Suparna Mandal, Gargi Nandy, Gautam Aditya

Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Kolkata 700019, India

Corresponding Authors e-mail: gautamaditya2001@gmail.com; sabarnichakraborty.9@gmail.com; nandygargi@gmail.com; abhijitsikary55@gmail.com; chakrabortyashmita48@gmail.com; tanisha2500@gmail.com; suparnamandal46@gmail.com

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Abstract. The growth of the land snails is a primary indicator of the fitness at the individual and population levels. Considering starvation as a limiting factor of growth, the adaptation of the land snail *Allopeas gracile* (Hutton 1834) (Gastropoda: Subulinidae) was assessed using von Bertanlaffy growth equation. As a starvation treatment, the laboratory reared 0-day old snails were fed at a gap of 1 to 6 days (six levels of starvation treatment) or fed regularly (control). In all instances, the shell length and the biomass (indicators of growth) increased with the time interval complying with the von Bertalanffy growth equation, but the magnitude of maximum shell length, $L_{maximum}$, and the asymptotic length, L_∞, decreased, while the growth rate coefficient (k) increased, as the duration of starvation period extended. The k and L_∞ were represented as functions of the starvation gap (T) and substituted in classical von Bertalanffy equation, representing a better fit model explaining the growth. The results indicate that the food availability is a crucial factor determining the pace of growth in the land snail *A. gracile*. As an extension, the present model can be used for the portrayal of the growth of the macroinvertebrates under varying level of starvation.

Keywords: graceful awl snail, starvation, von Bertalanffy growth function, asymptotic length L_∞, growth rate coefficient (k).

1. Introduction

The von Bertalanffy growth function (VBGF) proposed by Ludwig von Bertalanffy in 1934 and 1957, is one of the most extensively used equation to predict age from body size or body mass. The equation represents growth of an individual, related to increase in size (or length) due to catabolism and increase in weight due to anabolism. Though the metabolic processes are contrasting in terms of energy expenditure and use of biomolecules, both of them conjointly facilitate the growth procedure. The equations proposed by von Bertalanffy (1934, 1957) assumes a favorable condition in terms of resource availability, temperature and other factors. But in reality, organisms may face adverse conditions for a long time (as in habitat destruction can lead to food scarcity) or it can change rhythmically with seasons (seasonal change in humidity, temperature, rainfall etc.), which may affect their growth negatively. In this paper, we focus on the change in growth pattern along with the parameters affecting it, with starvation.

The graceful awl snail *Allopeas gracile* (Hutton 1834) (Gastropoda: Sublininae) is a small land snail species, taken as model organism for this experiment for its cosmopolitan distribution. Although an indigenous species of tropical North America (Gude, 1914; Pilsbry, 1948), *A. gracile* has spread across the globe by means of commercial use as aquarium pets to several parts of the world. *A. gracile* is found in North America (Roth, 1997; Capinera, 2017), Asia (Naser, 2010; Ramakrishna & Dey, 2010; Sang & Nhuong, 2014), Australia (Murphy & Shea, 2015), Africa (Lange & Mwinzi, 2003; Rowson et al., 2011), Europe (Rahman et al., 2016).

In India, *A. gracile* is found abundantly across different states. It has been found in almost all types of terrestrial habitats in Western Ghats (Mavinkurve et al., 2004; Aravind et al., 2005, 2008; Raheem et al., 2014), New Delhi (Sarma et al., 2007) and Kolkata (Ramakrishna & Dey, 2010). It is a common agri-horticultural pest (Raut & Ghose, 1984) and prefers moist environments such as shady and damp regions of soil, moist parts of garden, tree roots, rear sides of flowering pots, topsoil in and around host food plants, moss laden bricks and woods (Mitra et al., 1976; Raut & Ghose, 1984; Jambari et al., 1999; Nandy & Aditya, 2022a, b; Nandy et al., 2022). *A. gracile* feeds mostly on fallen and decomposing leaves of various plant species and tissue status (Raut & Ghose, 1984; Capinera, 2017). This snail species also shows necrophagy, feeding on dead snails and other animal protein (Mitra & Biswas, 1974; Mitra et al., 1976). The pestiferous nature of the snail *A. gracile* has been reported in Malaysia on vegetables and tobacco plants (Jambari et al., 1999), and in India, according to Raut and Ghose (1984) it is probably the most widely distributed of the various molluscan pests. Though it is considered to be a minor pest of potted plants in India (Mitra & Biswas, 1974; Mitra et al., 1976), in USA *A. gracile* shows a wide range of dietary choices and growth pattern (Capinera, 2017).

In Indian context, the growth of *A. gracile* in terms of the shell length and biomass complies with the von Bertalanffy growth equation, but temperature may affect some aspects (Nandy & Aditya, 2022b). The growth of *A. gracile* complies with the von Bertalanffy growth equation with the supply of food *ad libitum* independently of density (Nandy & Aditya, 2022a). However, in conditions where the availability is not consistent, a change in the growth pattern may be expected. Since, growth of the snails as well as in other invertebrates, is dependent on the constant accumulation of the resources, a deviation from such condition will impact the growth (Brockelman & Sithithavorn, 1980; Liebsch & Becker, 1990; Reddy & Rao, 1985;

Meyer et al., 1986; Lavarías et al., 2023). In order to substantiate this proposition, the individual growth of the snails subjected to varying levels of starvation was evaluated for compliance with the von Bertalanffy growth equation. *A. gracile* was chosen as the model snail because of its diverse ecological roles. The results of the study will assist in quantifying the prospective changes in *A. gracile* growth according to food availability. A deviation from the generalized von Bertalanffy growth equation will provide insights about the adaptability of *A. gracile* to adverse condition and sustenance of growth.

2. Material and methods

2. 1. Collection and rearing of the snail

Adults of *A. gracile* were handpicked from the area beside the post office of Ballygunge Science College, University of Calcutta, Kolkata, India. The collected snails were kept in a white plastic container and brought to the laboratory for rearing, in room temperature (27 ±2ºC) and >80% relative humidity and moist soil (Nandy & Aditya, 2022a, b). In the laboratory the living snails were segregated in 10 different 400 ml transparent plastic containers with perforated lids, with 15 snails in each container. The shells of the dead snails, if any, were removed. Excess snails were kept in a medium sized terrarium (34 X24 X13cm^3) with a thin layer of moist soil and a perforated plastic sheet cover. The snails kept in the containers and the terrarium, were fed cucumber slices *ad libitum*. The rearing containers and the terrarium were cleaned every day of the snails' fecal matter and excess food. After the first few days (~5days) of getting acclimatized to the new controlled environment, the snails started laying eggs. With sexual maturity the number of eggs laid per individual, increased. They generally laid eggs in the soil crevices and on the backside of the uneaten cucumber slice. Every 24 hour, the eggs (F_1) laid by the parental progeny were collected and kept in a separate white container with perforated lid and moist soil layer. The date of egg collection, the number of eggs collected and the hatching time were noted as well as the hatching time of the eggs were written down on the body of the container. The process was repeated throughout the experiment, from the initial collection during March 2023 to the end of the experimental period of August 2023.

One hundred and twenty-six F_1 progeny of *A. gracile* of same age and size were selected for the experiment and were reared under seven different feeding regime, each with three replicates, each containing six snails. An additional four replicates with similar density and the treatments as well as another pool of twelve snails each maintained as a source for supply of the snails to maintain the constant density of six snails in case of death of snail individuals.

This comprised the experimental setup (Table 1). The feeding regime comprised of: (a) regular food, representing the control set and (b) treatment sets (six sets), where individuals were starved for 1-day to 6-days period, respectively (treatments), to observe the effect of food availability on growth. The snails were measured for length and weight at the very beginning, then considered for the experiment for 12 weeks, with the above defined feeding gaps (starvation) for each treatment set.

On every seventh day, the shell length (to the nearest 0.1 mm) was measured using a divider and a scale for the first three weeks and subsequently using a digital vernier caliper (Insize, Brazil). The corresponding body weight (to the nearest 0.1mg) of the surviving snails was measured using a pan balance (Citizen®, India), and recorded for further analysis. Owing to the fact that the shells of the *A. gracile* subjected to longer duration of starvation were fragile and could not be handled for the measurement in vernier calipers, a divider and a scale were used to measure the length. The snails which died during the course of the experiment were measured for the shell length and recorded. Incidentally, for 4-day to 6-day starvation periods particularly the numbers of snails surviving at the end of 12 weeks were less than 18 (n= 6X3 replicates). The eggs laid in the sets of containers were collected and kept in a separate container each day. However, the fecundity was not included in the analysis and thus, the eggs laid during the course of the experiment were not subjected to any analysis. This work was carried out as an extension to the published research work of Nandy and Aditya (2022a, b).

	Treatment	Replicates							Replacement to d to g
		Data			Supportive for (a to c)				
		a	b	c	d	e	f	g	pool
Experimental design	Control	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6 7 8 9 10 11 12
	1-day	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6 7 8 9 10 11 12
	2-day	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6 7 8 9 10 11 12
	3-day	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6 7 8 9 10 11 12
	4-day	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6 7 8 9 10 11 12
	5-day	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6 7 8 9 10 11 12
	6-day	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6	1 2 3 4 5 6 7 8 9 10 11 12

Table 1. The experimental design adopted to evaluate the effects of starvation on the growth of the snail *A. gracile*. Numerical in the table represent the number of snails in the replicates. The number of replicates used in the experiment against the treatments (1-day through 6-day gap of starvation). The experimental replicates were kept constant at a density of 6 individuals and the pool density of 12 snails were kept in a larger container. In case of death, the snails from the supportive were replaced to the data replicates, whereas the death in the supportive were replaced to the supportive replicates. The experiment was repeated twice during the whole period. In all instances, the data on the shell length (in mm) and the body weight (in mg) were taken for the snails in replicates a through c. Using the data on the length and body weight, the compliance of the growth to the von Bertalanffy growth function was assessed. Further the relationship of the starvation with the fecundity was evaluated with the starvation extent as a factor.

2. 2. Theoretical framework for growth analysis with VBGF

The classical equation proposed by von Bertallanffy in 1934, was

$$\frac{dm}{dt} = E - km \ldots\ldots\ldots\ldots\ldots\ldots 1$$

where, E is the anabolism constant, k is the catabolism constant and m is body-mass (von Bertalanffy, 1968). Here, only the growth rate and asymptotic length were incorporated in the equation, to deduce age at certain time, from certain length or weight of the catch, which, on integration becomes,

$$m = \frac{E}{k} - \left(\frac{E}{k} - m_0\right)e^{-tk} \ldots\ldots\ldots 2$$

when integrated from m_0 to m, and time from zero to t_0, or,

$$m = \frac{E}{k}\left(1 - e^{-k(t-t_0)}\right) \ldots\ldots\ldots\ldots 3$$

when integrated as m=0 to m, and time being t=t_0 to t.

In further studies, the quantity E was dissociated into two parameters, k and L_∞ and the VBGF differential form for length, was

$$\frac{dl}{dt} = k(L_\infty - L),$$

which upon integration becomes the proposed von Bertalanffy model (1957) of growth equation,

$L_{t=} L_\infty(1 - e^{-k(t-t_0)}) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 4$, for shell length in mm at time t, and

$BW_t = BW_\infty(1 - (e^{-k(t-t_0)})^b \ldots\ldots\ldots\ldots\ldots 5$, for body weight in mg at time t,

where L_t is the shell length in mm and BW_t is the bodyweight in mg, at a specific time t, L_∞ is the asymptotic length and k representing the growth rate coefficient describing the pace of reaching L_∞ by an organism, and t_0 is hypothetical time when body-length is equal to zero. The three parameters incorporated were not considered as function of factors (such as temperature, humidity, geographic position, food, individual metabolic rate etc.), which later were observed by various scientists to vary (Pagalay et al., 2016; Vincenzi et al., 2016; Gündoğdu & Çevik, 2018; Nandy & Aditya, 2022a, b) with different factors. Our study focuses on effect of starvation on growth, considering k and L_∞ to be functions of starvation, denoted by time gap in number of days (T) of provision for food.

$$k = f(T),\ L_\infty = g(T)$$

The lengths and the difference between successive lengths (lengths measured for successive weeks) shows linear regression, of equation $y = mx + c$, where y is the difference between two successive increases in lengths, x is the length measured, *m* is slope and *c* is the intercept. The *k* and L_∞ values are to deduce from the observed values of length of the snail.

$$k = \{\ln(m+1)\} * (-1) \ldots\ldots\ldots 6$$

As the increase in length reduces with increasing length, i.e., with age, rate of growth reduces, as the individual approaches asymptotic length, so, the slope is always negative, which makes the magnitude of *k* to be negative. But, growth rate of length can never be negative, so, it is multiplied with "-1" to make the magnitude positive.

$$L_\infty = \frac{c}{m} * (-1) \ldots\ldots\ldots\ldots\ldots\ldots\ldots 7$$

As the slope is always negative, the ratio is multiplied with "-1" for the same reason stated above.

The observed values (from equation 6 and 7) of these parameters are to be compared with the expected values (from regression analysis of *k* and L_∞ with time gap(T)) later in this paper.

The relation between length and body-weight was necessary to deduce asymptotic weight at the same length, to put it into equation 2.

$$BW_t = a.L_t^b \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 8 \text{ (Froese, 2006)}$$

a and *b* are the regression coefficients, where, *a* is multiplier and *b* is exponent. For assessing the body weight, the term in parentheses in equation 1, is raised to the power of b, and

$$BW_\infty = a.L_\infty^b \ldots\ldots\ldots\ldots\ldots\ldots\ldots 9$$

We are considering equation 3 for our analysis, along with an approach towards variation in growth rate and length infinity parameters, whether they are affected by our concerned way of treatment i.e., starvation.

2. 3. Statistical analysis:

The data on the shell length (SL, in mm) and body weight (BW, in mg) of the snail in each starvation level over the time period were subjected to Kruskal-Wallis test with Steel-Dwass-Critchlow-Fligner multiple comparison, using *XL*STAT software (Addinsoft, 2010). Since the data did not comply with normality (Zar, 1999), application of Kruskal-Wallis test was made. For the data on the observed and expected values of shell length and body weight for each week, a paired t-test was used to justify the fit to the VBGF model. Various regression models were applied to justify the relationship of starvation levels to the growth features of the snail using either the coefficient of determination (R^2) or Akaike Information Criterion (AIC) to justify best. The Akaike Information Criterion (AIC) is a mathematical tool for comparing different model fit for the same dataset. The smaller value of AIC is considered better fit of the model i.e., the model with smaller AIC has a better predictive ability (Akaike, 1974). The

Kruskal-Wallis test was applied to the data on the observed, expected and modified values (following the VBGF model) of the shell lengths in the thirteen weeks for each of the starvation levels. The purpose was to justify that the shell length values did not vary significantly among the three types.

3. Results

Observations on the shell length and the body weight of *A. gracile* showed considerable variations in increment in relation to starvation. While a linear pattern of the increment in the shell length and the body weight was observed, the changes in the shell length and the body weight were negatively linked with the extent of starvation, during the 12-week time period of observation. The snails *A. gracile* showed the highest shell length and body weight (shell length in mm, range 8.1 – 14.9; mean 10.68 ± 0.547 SE; body weight in mg, range 29.92 – 106.98; mean 57.72 ± 6.51 SE, n = 15) when they were fed regularly. The gradual increase in both length and weight, even after subjected to different levels of starvation are shown in Figures 1 and 2, with the help of Box and Whisker plots, with the outliers marked in some instances. The observed values of length and body weight for seven different food regimes, were subjected to Kruskal-Wallis test (as the data did not comply to normality) with feeding gap as explanatory variable (K=1330.115, df=90 for shell length and K=1306.618, df=90 for body weight) indicating presence of significant differences ($P<0.05$). As reflected in the multiple comparison following Steel-Dwass-Critchlow-Fligner methods, the different age classes varied significantly ($P < 0.05$ level) in terms of increment in the shell length or body weight for the different treatment groups (starvation interval).

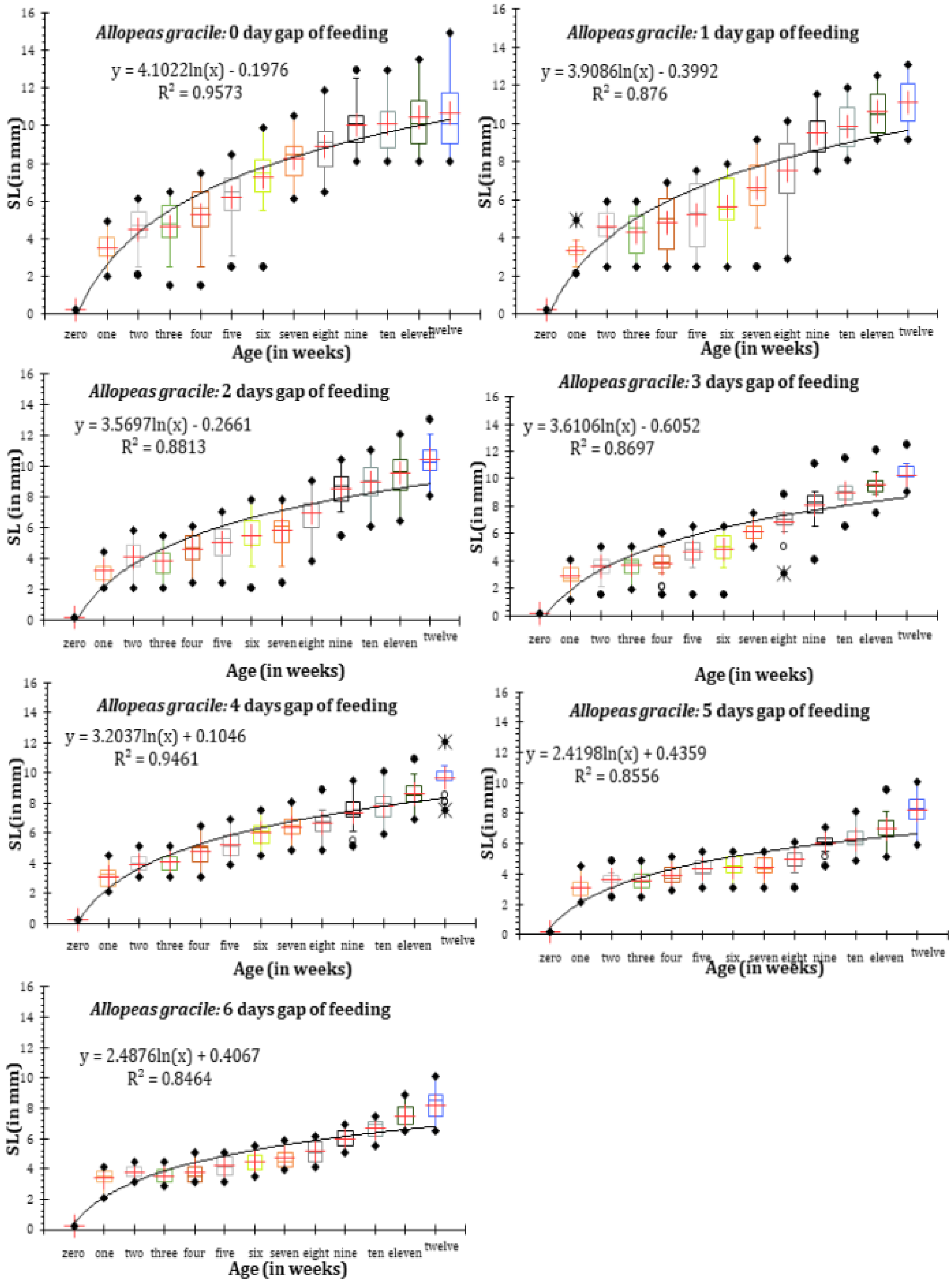


Figure 1. The boxplot representation of the increment in the shell length in each week for the snails *A. gracile* exposed to different levels of fasting. The filled circles represent the extreme values, the open circles represent outliers, the cross represent the mean value, the bar inside the box represents the median value with the upper and lower quartiles spread in the box. A regression equation and the coefficient of determination are mentioned along with the regression line in each graph.

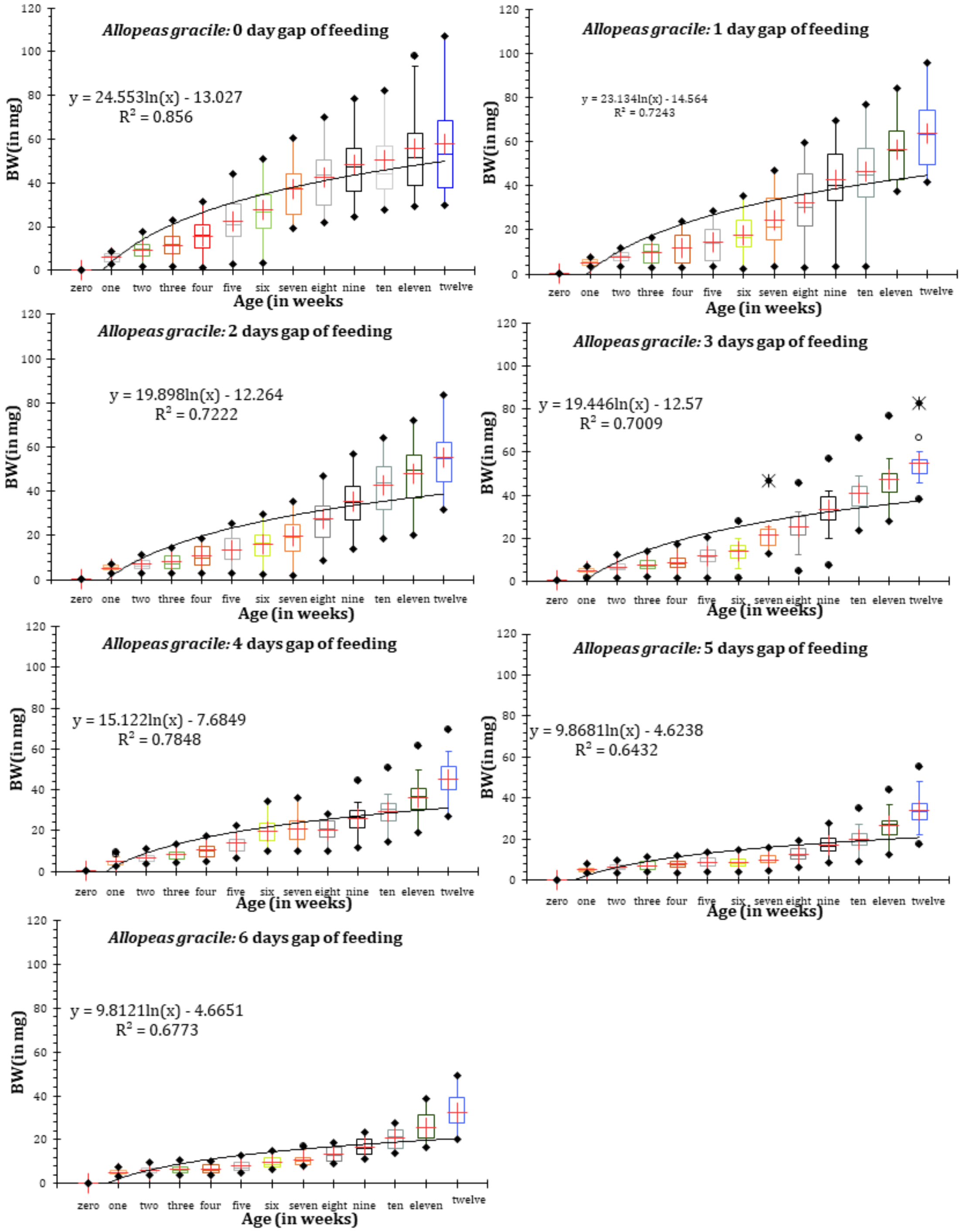


Figure 2. The boxplot representation of the increment in the body weight in each week for the snails *A. gracile* exposed to different levels of fasting. The filled circles represent the extreme values, the open circles represent outliers, the cross represent the mean value, the bar inside the box represents the median value with the upper and lower quartiles spread in the box. A regression equation and the coefficient of determination are mentioned along with the regression line in each graph.

The expected values of length and weight for seven cohorts, were evaluated with observed values after putting in equation 4 and 5, respectively (Figs 3 and 4). In all instances growth curves of the shell lengths and body weight in relation to age fitted the von Bertalanffy growth model. The statistical comparison of observed and expected values of shell length and body weight, by two-tailed t-test, compelled to accept null hypothesis (H_0: no significant difference between observed and expected values) showing even with starvation, the extent of concerned variable may reduce, but the growth pattern of von Bertalanffy, is not deviated (Table 2).

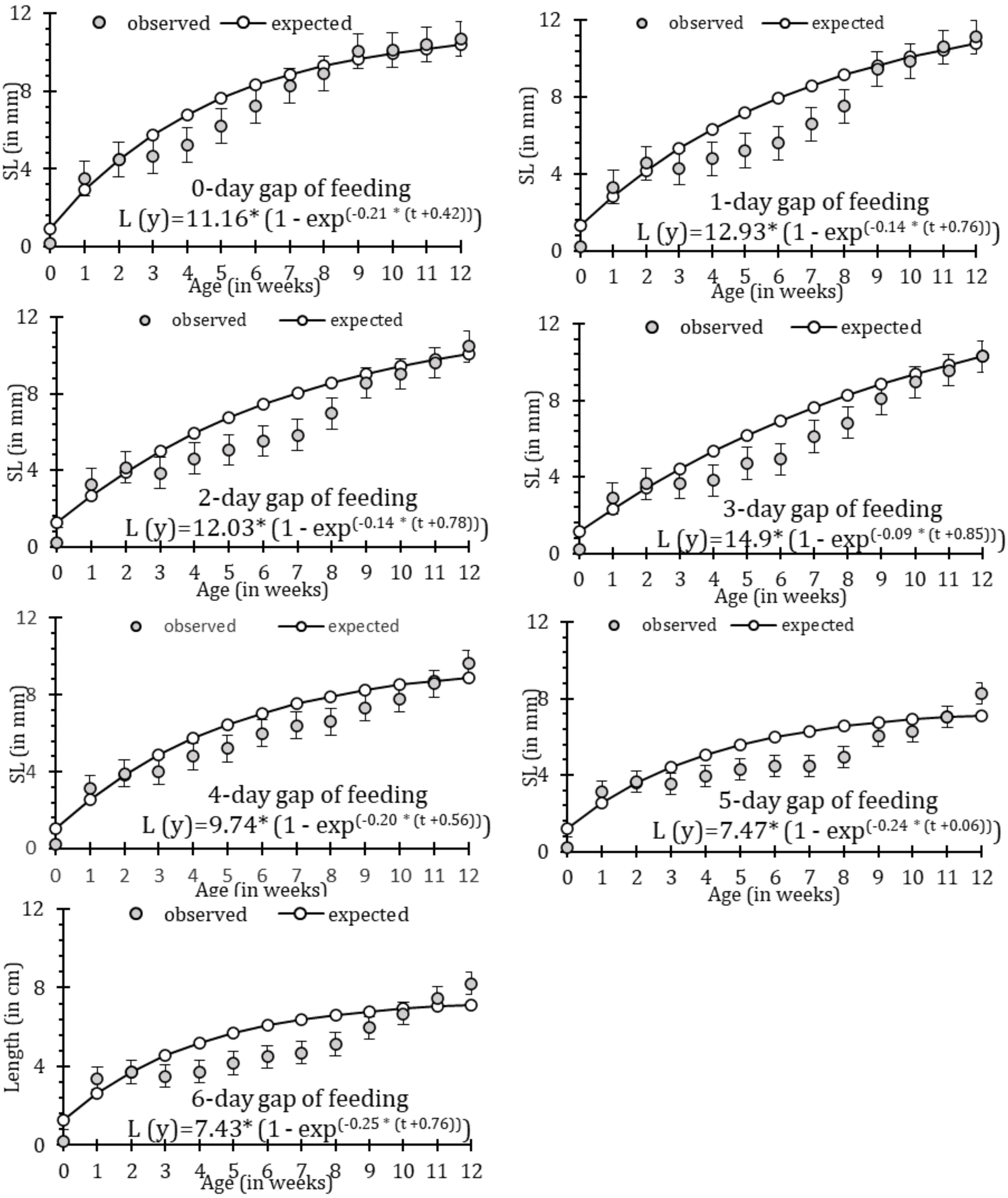


Figure 3. The compliance of the shell length (SL, in mm) increment with age (in weeks) with the von Bertalanffy growth equation for the snail *A. gracile* exposed to varying level of gap of feeding (seven levels of fasting, 0 through 6 day)

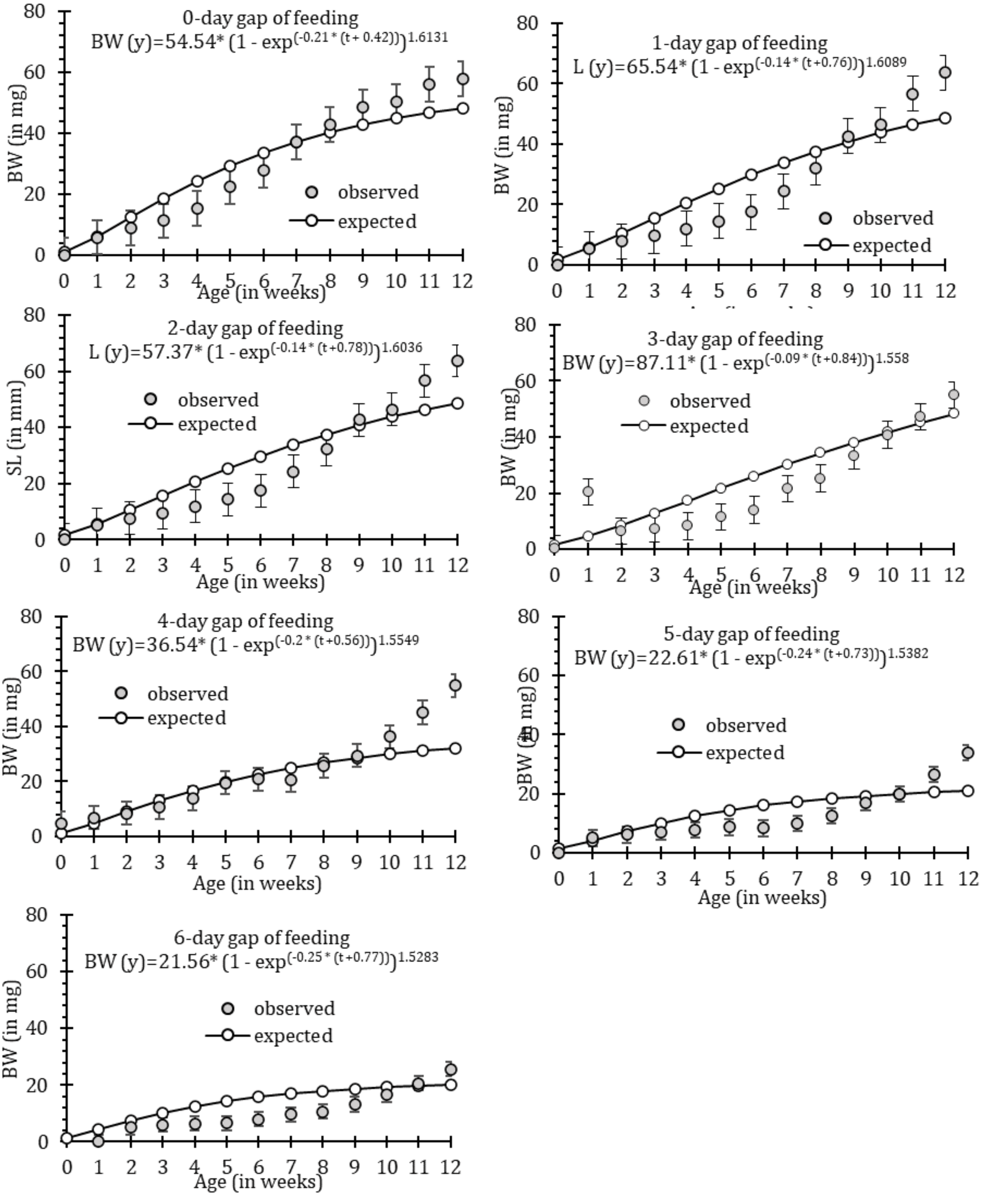
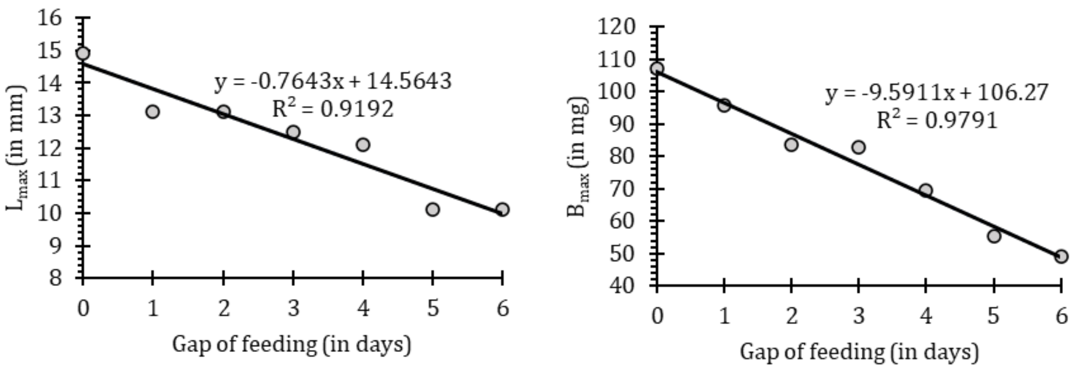


Figure 4. The compliance of the body weight (BW, in mg) increment with age (in weeks) with the von Bertalanffy growth equation for the snail *A. gracile* exposed to varying level of gap of feeding (seven levels of fasting, 0 through 6 day)

Table2 . The results of the t-test for the comparison of the expected and the observed values of the shell length and body weight of the snail *A. gracile* observed for 13 consecutive weeks. The non-significant differences of the shell length and body weight justify the fit to the von Bertalanffy growth equation by the concerned snail group. For the body weight data few weeks were discarded due to non-availability of the body weight of multiple individuals.						
Gap of feeding	For shell length			For body weight		
	t	df	P-value	t	df	P-value
0	-0.33	24	0.74	-0.01	23	0.98
1	-0.67	24	0.51	-0.29	22	0.77
2	-0.72	24	0.47	-0.34	23	0.74
3	-0.68	24	0.499	-0.47	24	0.65
4	-0.61	24	0.55	0.55	21	0.58
5	-0.89	24	0.38	-0.49	21	0.63
6	-0.87	24	0.39	-0.47	21	0.64

In Figure 5(a and b), we can see the linear decrease of maximum length and weight of individual found after 12 weeks, with increased level of starvation (R^2= 0.91 and 0.97 for length and weight, respectively).

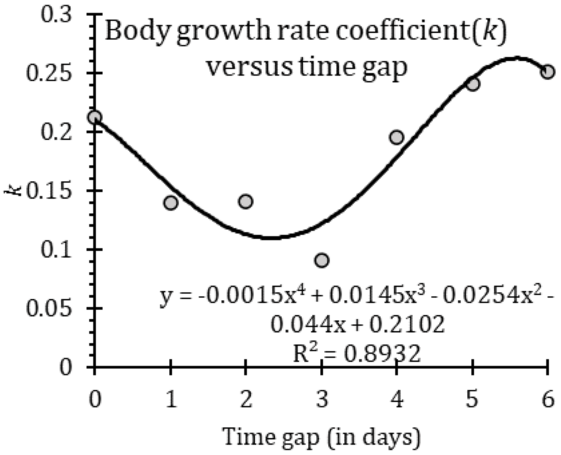


(a) (b)

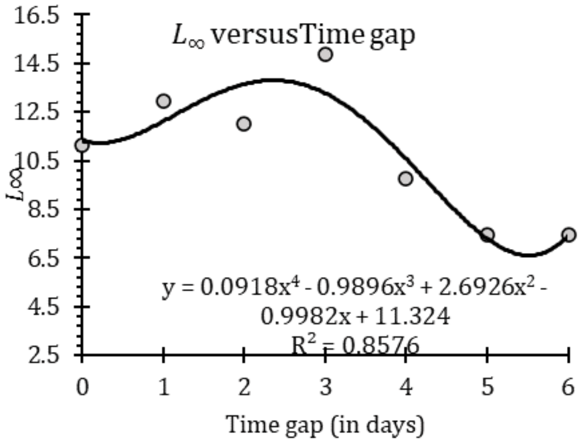
Figure 5. The maximum length and bodyweight after 12weeks of observation for the Control and six Treatments, were observed to decline with increasing feeding gap

Figure 6(a and b) shows that with increased feeding gap (in days), change in k and L_∞, follow a fourth order polynomial regression (with R^2= 0.89 and 0.86, respectively), of type $y = a_0 + a_1x + a_2x^2 + a_3x^3 + a_4x^4$, where, y represents k or L_∞, x is the feeding gap, and $a_0, a_1, a_2, a_3,$ and a_4 are the regression parameters.

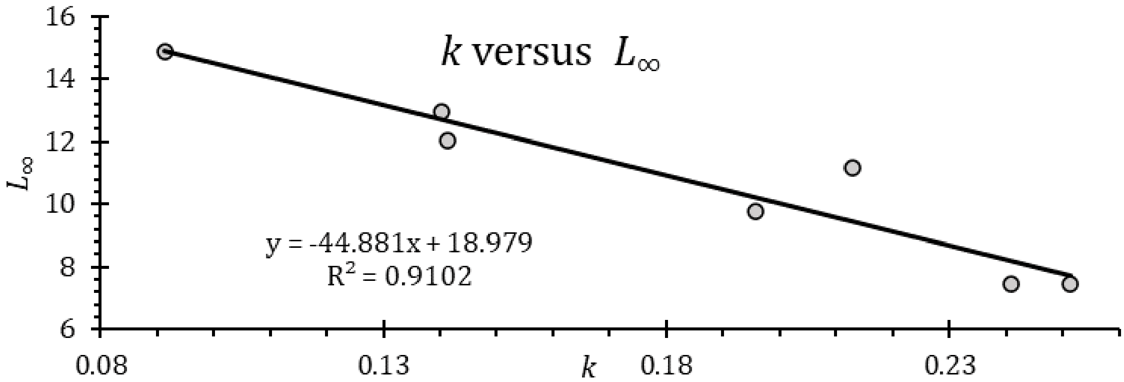
Figure 6c shows the linear regression between k and L_∞, i.e., with increase in k, L_∞ decreases proportionately, may be the reason being the increased rate to each reduced length-asymptote, due to short life span of the species. As here, with food scarcity, the snails lack required nutrients which leads to increased mortality, and reduced length-asymptote. But their inherent growth rate does not change, so the time required to reach L_∞ reduces, hence, implying a relative increase in *k*.



(a)



(b)



(c)

Figure 6. The growth rate (*k*) first decreased then increased for greater extent of starvation (a), whereas, the L_∞ showed the exactly opposite pattern (b), the *k* and L_∞ showed a negative correlation (r=-0.95) (c)

The value of *k* and L_∞, found from the polynomial regression equation, with varying number of days of starvation, were substituted in equation 4. The values got from this substitution, were superimposed with previous observed and expected values from equation 3 (Fig. 7), and were subjected to Kruskal-Wallis test (Table 3) stating no significant differences ($P > 0.05$) among three sets of values for all the six treatments. The P values were compared for the original and modified VBGF (Table 4), to predict a better fit, but as it was not enough to state the better-fit of the model, AIC (Akaike, 1974) was used later for conclusion.

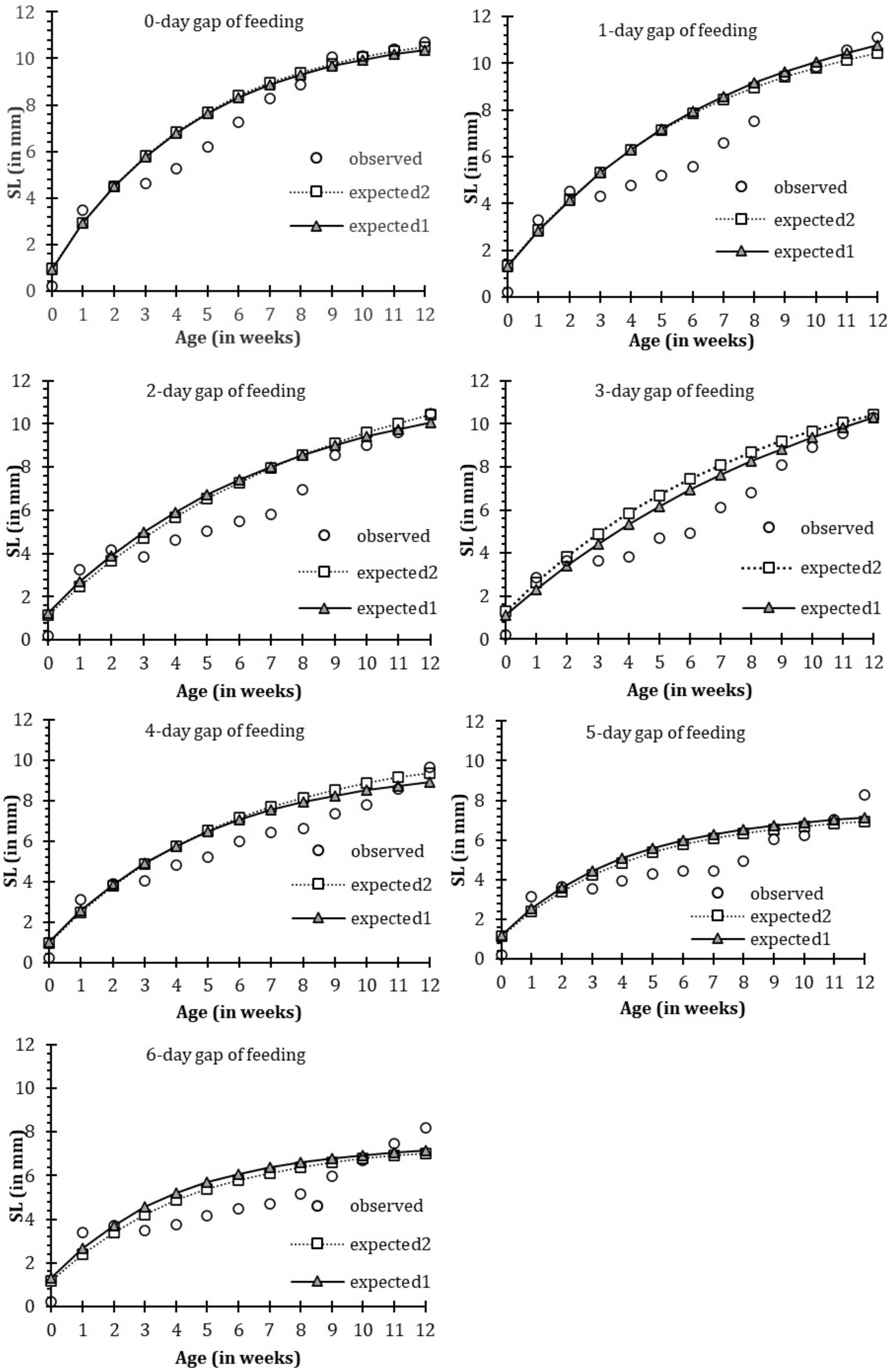


Figure 7. The *k* and L_∞ were expressed as function of starvation gap (T), substituted in VBGF to compare for presence of any significant difference, but the observed and expected values (expected1) showed no significant difference with the modified (expected2) VBGF (P<0.05) for all instances

Table 3. The results of the Kruskal-Wallis test for the comparison of the expected values from original and modified equations and the observed values of the shell length of the snail *A. gracile* observed in different weeks. The non-significant differences of the shell length justify the fit to the von Bertalanffy growth equation (both original and modified) by the concerned snail group (df for all comparisons is 2 and K (Expected value) is 5.991).

Gap of feeding	K (Observed value)	P value
0	0.164	0.921
1	0.385	0.825
2	0.701	0.704
3	1.073	0.585
4	0.828	0.661
5	1.431	0.489
6	1.303	0.521

Table 4. Comparing the p-value from original and modified VBGF through t-test with observed shell length (df for all comparison is 24).

Gap of feeding	P value from VBGF	P value from modified VBGF
0	0.74	0.70
1	0.51	0.56
2	0.47	0.51
3	0.499	0.33
4	0.55	0.48
5	0.38	0.52
6	0.39	0.57

4. Discussion

As shown in the results, the growth of *A. gracile* was dependent on food availability. With increasing starvation period, a decrease in increment in the shell length and biomass was observed. These suggest that the consistent availability of the food facilitates the growth of *A. gracile*. Food availability and subsequent nutrient assimilation enables the balance of anabolic (formation of biomolecules as building materials for cells) and catabolic (breaking these biomolecules to provide energy for physiological reactions, reproduction and survivability) pathways, which is ultimately reflected in snail growth. Such growth pattern can be portrayed through different models (Hernandez-Llamas & Ratkowsky, 2004; Helidoniotis et al., 2011; Noor et al., 2012) including the von Bertalanffy Growth Function (VBGF). Generally, abiotic factors (such as temperature, humidity, soil features, etc.,) affect growth rate (Pagalay et al., 2016; Vincenzi et al., 2016; Gündoğdu & Çevik, 2018; Nandy & Aditya, 2022a, b). In our study, the varying starvation levels with the pre-set food availability led to the differences in growth. Apparently, the growth pattern of *A. gracile* under different levels of starvation (Figs 3 and 4), followed the norms of the VBGF, but the change in the magnitudes of shell length and body weight were prominent, corresponding to the starvation level. The initial application of the VBGF to the data on the growth of the snails under different levels of starvation was followed by the use of the parameters, k and L_∞ to portray the effect of the starvation on the growth of *A. gracile*. The maximum length or weight observed after 12 weeks of experiment, reflects that resource abundance (food availability) positively affect growth, as both the variables reduced linearly with increased level of starvation (Figs 5a and b). The increase in shell length and body weight can be viewed as a result of two opposing physiological processes (anabolism and catabolism) contributing to organismal growth. For growth, the nutrients (to synthesize biomolecules by anabolism) and energy (to get energy by breaking biomolecules in catabolism) are received from food only. So, with depletion in food, the body cannot fulfil its requirements of metabolism and starts to breakdown the stored metabolites (Lavarías et al., 2023), thus resulting in a decrease in body-mass.

As the experimental results have shown, with increasing duration of starvation (starvation time), the values of the growth rate coefficient (k) tend to be higher, while L_∞ tends to decrease (Figs 6a and b), corresponding to a negative correlation (r=-0.95) (Fig. 6c). As seen in most long-lived species L_∞ tends to be larger, with smaller k, indicating the organism takes a great amount of time to reach the asymptotic length. For most instances, species tends to grow towards L_∞, but do not reach it (Hart & Chute, 2009). Comparing the equations 6 and 7, it appears that k is directly proportional to ln(m+1), whereas L_∞ is inversely proportional to m, which results in the negative correlation between k and L_∞ in VBGF. The increase in k reduces with the time to attain L_∞, but it can also be perceived as a way-out compensating food scarcity by sacrificing growth on reducing anabolism, so the organism can survive on minimum nutrient, increasing the catabolism processes resulting in individual growth. The observed data shows, both $L_{maximum}$ and $B_{maximum}$ (highest length and body weight of individual found after 12 weeks, respectively) tend to decrease (Figure 5a and b) indicating that, *A. gracile* are very susceptible to environment perturbation, as both $L_{maximum}$ and $B_{maximum}$, started to reduce drastically. The snails showed high mortality during rearing in laboratory condition, that increased with starvation period.

The change of k and L_∞ with increased level of starvation, show fourth order polynomial regression (with R^2= 0.89 and 0.86, respectively), of type $y = a_0 + a_1x + a_2x^2 + a_3x^3 +$

a_4x^4, where, y represents k or L_∞, x is the feeding gap, and a_0, a_1, a_2, a_3, a_4 are the regression parameters. From the observed values of k and L_∞, the function $k = f(T),\ L_\infty = g(T)$ best fitting to VBGF with constant coefficients of the above polynomial equation, are,

$k = -0.0015T^4 + 0.0145T^3 - 0.0254T^2 - 0.044T + 0.2102$ and

$L_\infty = 0.0918T^4 - 0.9896T^3 + 2.6926T^2 - 0.9982T + 11.324$ with $f(T)$ and $g(T)$ shown in figures 6a and 6b.

If the functions $f(T)$ and $g(T)$ ae substituted in VBGF (equation 4 and 5), they become (shown as equations 10 and 11 respectively):

$$L_{t=} (0.0918T^4 - 0.9896T^3 + 2.6926T^2 - 0.9982T + 11.324)(1 - e^{-(-0.0015T^4 + 0.0145T^3 - 0.0254T^2 - 0.044T + 0.2102)(t-t_0)}) \quad \text{.........10}$$

$$BW_t = (0.0918T^4 - 0.9896T^3 + 2.6926T^2 - 0.9982T + 11.324)(1 - (e^{-(-0.0015T^4 + 0.0145T^3 - 0.0254T^2 - 0.044T + 0.2102)(t-t_0)}))^b \quad \text{.......11}$$

Considering the values of the L_t and BW_t deduced in equations 4 and 10, along with the observed values in the laboratory (Figure 7), no significant differences were found through Kruskal-Wallis test (Table 3). Using Akaike information criterion (Akaike, 1974) [deduced through RStudio version 4.2.2; R Core Team (2022)], it was found that equation 10 is a better fit than equation 4, varying the value for t from zero to twelve. The Akaike (AIC) formula being $AIC = 2K - 2\ln L$, where, K is numbers of estimated parameters in the model and L is the maximum value of the likelihood function for the model. Here the AIC values for the equations 4 and 10 were: AIC_{eq10}=444.13 and AIC_{eq4}=444.70; keeping all the other parameters (t_0) constant. The t_0 did not show any significant correlation with starvation gap (R^2=0.14), but both k and L_∞ showed significant correlation (R^2= 0.89 and 0.86, respectively), justifying that k and L_∞ vary with different degrees of starvation. In the experiment, the time of birth was considered as basis of cohort formation to eliminate the categorical effect of age inducing growth, so, age was not included as a parameter/predictor in model-fitting. When we are including k and L_∞ as functions of starvation in VBGF, it is actually increasing model complexity by taking random effects of other biotic and abiotic factors into account. As a consequence, the model accuracy increases, implying that the higher the complexity (increasing number of parameters by including all possible factors) the higher is the model-accuracy (Vincenzi et al., 2016).

The substitution of k and L_∞ in equation 4 was an approach to predict the shell length and body weight, as responses to the levels of starvation. As shown in Figs 3 and 4, the values of k and L_∞ change with the levels of starvation. Using this substitution (eqn. 4), the modified VBGF (eqns. 10 and 11) now enables prediction of the changes in the shell length and body weight of the snail *A. gracile* under varying levels of starvation, using the parameter *T*.

The variations in the growth due to starvation was evident in the snail *A. gracile*, similar to those observed in other molluscs like *Helix aspersa* (Porcel et al., 1996), *Biomphalaria glabrata* (Vianey-Liaud & Dussart, 1994), *Pila globosa* (Haniffa, 1987), *Potamopyrgus antipodarum* (Jokela et al., 1999) and *Monacha cartusiana* (Sharaf, 2009). In all instances, the biomass and shell features were affected by the starvation or nonavailability of the food resources. Considering the habitat conditions preferred by *A. gracile*, resource availability is not strictly ensured. Assuming two distinct habitat types, the agricultural fields (where the snails are pestiferous) and in non-agricultural fields (where they are naturally occurring), the possibility of resource depletion and thus, starvation is quite obvious. In the agricultural fields, where *A. gracile* are pestiferous and grow prolifically, the seasonality of vegetations and available foraging areas may hinder the continuous feeding. Until the vegetations are palatable, the snails are obviously subjected to varying degree of starvation. In non-agricultural habitats also, the seasonal effects and the pattern of the rainfall and temperature leads to varying levels of food availability and the snails are vulnerable to starvation. The intermittent feeding may also affect the reproduction, which in turn may affect the population increment of *A. gracile*. In comparison to the growth and fecundity of *A. gracile* observed in laboratory (Capinera, 2017; Nandy & Aditya, 2022a, b) the present observation was made for restricted time period. On an average the cohorts of *A. gracile* lives for a period of more than a year (Nandy & Aditya, 2022a) that varies with the temperature (Nandy & Aditya, 2022b). In the present instance, the growth trajectories for the first twelve weeks of life were found to be similar to the earlier findings (Nandy & Aditya, 2022a, b). Although the deductions cannot be made for the reproduction and population increment, the individual growth patterns appear to be significantly influenced by the starvation in the initial phases of growth. The effects of the starvation on the growth of *A. gracile* was aptly portrayed through the VBGF, justifying its use in modelling the snail growth. Further studies may be required to highlight the physiological factors contributing to the differences in the growth of *A. gracile* subjected to starvation.

5. Conclusion

The growth of *A. gracile* under varying levels of starvation was assessed using von Bertalanffy growth function. The shell length and the body weight, used as markers of growth, changed in compliance with the VBGF model but varied with the levels of starvation. Parameters of the VBGF, k and L_∞, expressed as function of starvation gap (T), proved a better fit to the model depicting the complex relation of starvation and growth. The k and L_∞ decayed with the increasing level of starvation complying with a linear regression equation, indicating the impact on the shell length and the body weight and thus growth of *A. gracile* due to starvation. As an extension, the present model can be used to assess the effects of the biotic and abiotic factors on the growth pattern of the different land snails and slugs exhibiting similar biology to *A. gracile*.

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Author’s contribution

Conceptualized by GA; Experiment execution and data collection by AS, GN, AC, SM, TG, SC; data analysis and model assessment by SC, GN; manuscript preparation; SC, GN, GA.

Conflict of Interest

As authors we declare no conflict of interests.

Data disclosure

The deduction of the equations is provided in the Supplementary files. The calculations, if required may be provided upon authentic request.

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