Theoretical considerations in determining allometric growth within instars of crustaceans, with special reference to *Americamysis bahia*

Running head: Theoretical allometry in crustaceans

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HIGHLIGHTS

- We postulate four theoretical instar allometry models for Crustacea.

- We provide a theoretical framework for evaluating allometric growth in arthropods.

- *Americamysis bahia* experiences two-rate continuous allometry.

Abstract

Allometry in crustaceans is typically considered growth over several instars primarily because crustaceans are presumed to grow only during ecdysis (discontinuous growth). Using theoretical distributions of the sizes of two morphometric variables over several instars, four theoretical instar allometry models are postulated: continuous allometry (indiscrete and discrete); discontinuous allometry (indiscrete and discrete); mixed allometry (simple or complex); and two-rate continuous allometry. The estimates of proportions of allometry within the instars is determined using $Y = f(X)$ and $X = f(Y)$ for variables X and Y. The amount of allometry in each variable is estimated using the mean ±s.d. on the independent variable. Application of these theoretical instar allometry models using carapace and abdomen sizes in six instars indicates *Americamysis bahia* experiences two-rate continuous allometry, rather than "traditional" discontinuous allometry, with 85% or more of total growth occurring in the intermolt phase, and with the abdomen accounting for about 60% of the expansion.

1 | INTRODUCTION

During growth it is usual for increase in size of one body region of an organism to be proportional to increase in size of another body region (Diaz, Smith, Serafy, and Ault, 2001; Hartnoll, 1974; Ribeiro, Cascon, and Bezerra, 2013), with the size of each region bearing a specific relationship to overall body size (Diaz et al., 2001; Ewo-oboho and Abby-kalio, 2006; Stern and Emlen, 1999), a relationship usually described by power functions (Agutter andTuszynski, 2011; Hernandez-Llamas and Ratkowsky, 2004; Katsanevakis, Thessalou-Legaki, Karlou-Riga, Lefkaditou, Dimitriou, and Verriopoulos, 2007; Pasternack and Gianutsos, 1969; Stevens, 2009; West and Brown, 2005; Zeng and Wan, 2000). It is also common for the body regions under consideration to change during different growth phases of the organism. This proportional increase in two or more body regions is termed allometric, relative or, occasionally, heterogenic growth (Hartnoll, 1982). Although allometry or heterogony is actually the relative growth of a part of an organism in relation to the entire organism (Gayon, 2000), allometry is frequently used in describing the relative growth of two regions (e.g., abdomen, carapace, chela, etc.) of an organism. Reviews of crustacean allometry are provided by Teissier (1936), Hartnoll (1978, 1982) (and throughout this paper). Teissier's and Hartnoll's standard terms, particularly their use of allometry to mean the relative growth of any two body regions of an organism, will be used.

Crustacean growth typically involves transitions through multiple instars, thus complicating detailed mathematical descriptions of their morphological variation during development. Growth curves for crustaceans typically have been determined over several instars using an allometric equation that describes the proportional expansion of two

morphometric variables (e.g., carapace length and cheliped width) which are usually termed variables x and y (Clayton, 1990; Diaz et al., 2001; Hartnoll, 1974, 1978; Huxley, 1932; Teissier, 1960), allowing the use of allometric equations to describe changes in growth that occur as a result of sexual differentiations, puberty or differing environmental conditions during large portions of the organisms' life cycle (Hartnoll, 2001). Typically allometry of the two variables is constant over several instars, and then often, presumably as a result of changing physiological mechanisms, the allometric proportions are described by a different model over the next series of instars (Katsanevakis et al., 2007), a situation usually termed phase allometry (Teissier, 1960; Hartnoll, 1982). Chang, Sun, Chen, and Yeh, (2012) provide an extensive review of a great number of mathematical models that have been developed for modelling the growth of crustaceans.

Determinations of allometric growth models for crustaceans over several instars have been assisted by observations that crustaceans, like other arthropods, appear to exhibit discontinuous growth (e.g., Chang et al., 2012; Ewo-oboho and Abby-kalio, 2006; Franco, Ferreira and Nobre, 2000; Hoenig and Restrepo, 1989), resulting in distinct growth "stanzas," i.e., a stepwise growth over time (Klingenberg and Zimmermann, 1992), usually described with regard to the change in size at molt and the duration of the intermolt period (Botsford, 1985), with considerable research directed at quantifying the relationship between pre-molt and post-molt sizes of individual animals (Chang et al., 2012; Franco et al., 2000).

Because of their hard exoskeletons, crustaceans typically remain one size for a large portion of an instar (Benetti and Negreiros-Fransozo, 2004). At ecdysis, when the animal sheds its exoskeleton and before the new, soft exoskeleton has hardened, there is a

rapid uptake of water (or air in terrestrial arthropods) accompanied by a rapid expansion in size.

Using physiological and physical characteristics of individuals, instars have been subdivided into five or more stages (Passano, 1960) of which ecdysis (stage E of Drach's classification; Drach, 1939) *per se* occupies as little as 0.5% of the time spent in an instar. In this treatise, no distinction between the first four stages is made and will be termed the intermolt period, with ecdysis or molting the terminal period to any particular instar. As a result of discontinuous growth during each instar, allometric studies of crustaceans have considered proportional changes in body regions through several instars under the assumption that no increase in size is accommodated during the intermolt period (Hartnoll, 1978, 1982; Passano 1960; Teissier 1960), consequently, it is adequate to use mean values for the sizes of variables under consideration in individuals within the same instar or to use pre- and post-molt sizes for individuals in transition from one instar to the next.

However, recent work by Scarfe and Steele (2017) indicates no obvious discontinuities in the growth curve of *Americamysis bahia* (formerly *Mysidopsis bahia*), as usually associated with ecdysis in crustaceans. Apparently the majority of the expansion in standard length is accommodated during the intermolt period and not during ecdysis. To determine if the expansion is accommodated by abdominal arthroidal membrane stretching, as was originally suggested by Mauchline (1973) and Childress and Price (1978), or if the intermolt growth is due to continual stretching of the cuticle throughout the intermolt period, a series of allometric models within single instars are considered here.

In this study, we categorize all possible growth patterns of linear dimensions and propose a methodological approach to the analysis, which we test with the growth pattern of *A. bahia*, a species where instar and phase allometry differ. Our objective is to offer a valuable methodological approach to address crustacean growth and to further understand the principles underlying transformation during the crustacean molt. We recognize, however, that these details do not have major implications for estimating phase allometry, which ultimately controls the most important energetic and ecological aspects of crustaceans.

2 | METHODS OF MODEL FORMULATION

We postulate four theoretical instar allometry models for determining intermolt growth patterns and for estimating proportional growth during the intermolt and molt periods of crustaceans, and possibly other arthropods. What we present is an approach that offers new thoughts and insights about "traditional" concepts regarding crustacean growth that may reveal more substantive information than the classical discontinuous growth model approach. In essence, our proposed allometry models free researchers from the limitations of considering only discontinuous growth in crustaceans. The application of these models is illustrated with allometric growth data for *A. bahia* (Scarfe and Steele 2017).

2.1 Calculations of allometry over several instars

Much of the early quantitative determinations of allometric growth in crustaceans was undertaken by Huxley (1924, 1927, 1932) and his colleagues (Huxley and Callow, 1933; Huxley and Richards, 1931; Huxley and Teissier, 1936). As a result, well-accepted

methodologies have been developed for estimating different forms of relative growth within different instar phases. Because overall growth is often best described by a non-linear power function of the relationship between two variables (Agutter and Tuszynski, 2011; Katsanevakis et al., 2007; Klingenberg and Zimmermann, 1992; Pasternack and Gianutsos, 1969; Stevens, 2009; West and Brown, 2005), a log-log relationship (log y = log a + b log x) of the two variables approximates what Huxley (1931) termed a constant differential growth ratio when plotted on log-log axes.

This linearized model assumes that allometry is constant as body size increases (Katsanevakis et al., 2007) and that all parameters are linear (Hernandez-Llamas and Ratkowsky, 2004). The relative growth rate or allometric constant can then be described by the slope (b) in the relationship (Hartnoll, 1978, 1982; Teissier 1960) and consequently four types of allometric growth covering several instars have been postulated: A power allometric function $Y=aX^b$ is defined as isometry when b=1, positive allometry when b>=1, negative allometry when 0=<b<1 and enantiometry when b<0 (Hartnoll, 1982; Teissier, 1960). Whatever the relationship between the two variables (log-log, log-linear or linear) linear transformation produces a constant relationship or slope between them. The magnitude, therefore, of the b terms can be used to describe the four types of allometric or proportional growth, irrespective of the magnitudes of x and y.

2.2 Alternative allometry within instars

If the overall development of an organism, or group, can be divided into identifiable, distinct stages, such as instars, several alternatives regarding the growth of two variables (x and y) within the instars are possible.

In biometry, x is considered the independent or reference variable and y the dependent variable. Over a series of measurements (within one or over many instars) the relationships of y as a function of x can be determined using regression modeling. While the true independence of x may be questioned (as below; also Hartnoll, 1982), the study of a particular variable (y) relative to another (x) has generally established the *a priori* independence of x. As Gould (1966) suggested: "a model, neither true nor false, whose criterion is utility" justifies its use.

Multiple measurements of the variables over the range of x under study must be obtained for one or many individuals and these data then subjected to regression analysis using the least squares method. These analytical relationships are valid under the assumptions that the individuals measured belong to the population, the selection of individuals is random, and the populations of measurements are normally distributed with a common variance (Sokal and Rohlf, 1995). Theoretically, these assumptions apply only to x. The relationships of y on x for each instar population can have several alternative allometric growth patterns, which may or may not coincide with the growth pattern over several instars.

For crustaceans and other arthropods, each instar can have up to five stages (Passano, 1960). The most easily identifiable stage is ecdysis, which also has the shortest duration ($< 0.5\%$ of the total instar time). The other stages are separated physiologically and are therefore sometimes difficult to distinguish without refined biochemical or histological techniques; these four stages together may be referred to as the intermolt period (anecdysis). Because the intermolt typically occupies 99.5% of the time spent in any instar, it is more likely that random sampling of individuals in any instar population

will produce 99% or more of the individuals in the intermolt period. In addition, because molting individuals are so readily identifiable, 100% intermolt observations can be achieved by an *a priori* decision to eliminate individuals that are molting.

2.3 Proposed theoretical allometry models

2.3.1 General considerations

The following theoretical allometric growth alternatives consider that, from the relative proportions of two variables measured from individuals in a specific stage or period of each instar (in these examples, the intermolt period), the relative allometry during the intermolt and molt (period or stage allometry) of each instar can be determined. Allometric growth over several instars (phase allometry) can be estimated from these instar populations. It is theoretically possible that these analyses could apply to all five stages of an instar provided that individuals can reliably be assigned to each stage. If so, allometric growth during all stages of an instar could be determined and may add substantially to the physiological understanding of the growth process in Crustacea.

Each of the theoretical allometry models discussed considers the relative growth of two variables within three instars in which individuals would have been randomly sampled and in the intermolt period of a specific instar, and the data normally distributed. Two alternative situations arise in each case. In one alternative, the sizes of one or both variables of one intermolt population are discrete and do not overlap with the sizes of the variables of the next population. In the other alternative, sizes do overlap and the instar populations are indiscrete.

Within each instar the size limits of expansion for each variable during the
intermolt period can be estimated from the minimum to the maximum values of each
variable for that particular intermolt population. The molt expansion in turn can be
estimated from the maximum values for that particular instar to the minimum intermolt
value of the succeeding instar. Estimations of the intermolt population limits will be
discussed later. Alternative allometric models are illustrated in Figures 1 through 5
considering the sizes of two variables (*x* and *y*) for 10 individuals within each intermolt
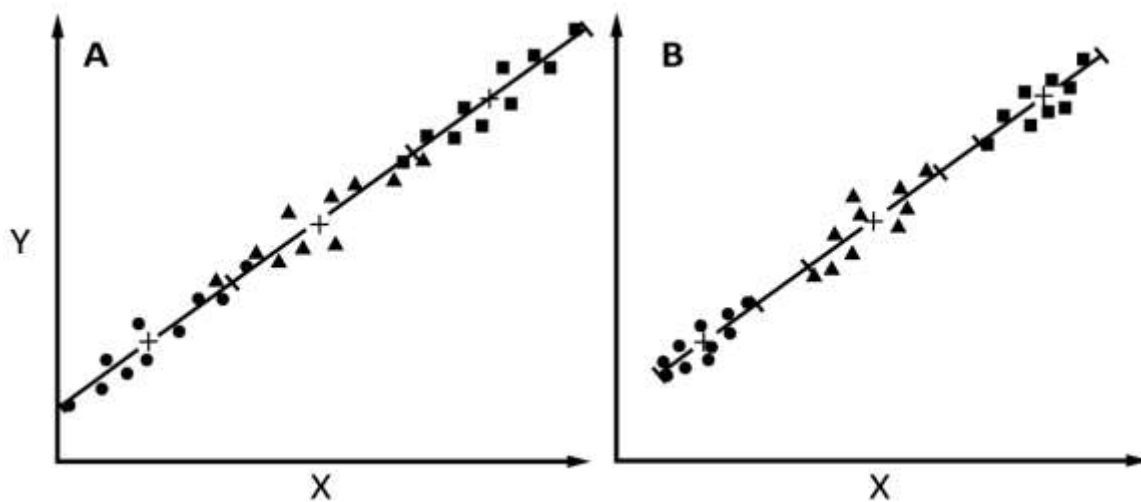population.

FIGURE 1 Theoretical distributions of individuals in three intermolt populations (● = instar 1; ▲ = instar 2; ■ = instar 3) showing two types of continuous allometric growth in two body regions (*X* and *Y*). In each population, a significant regression can be fitted and all have the same slope and intercept. **A**. Indiscrete continuous allometry with overlapping *X, Y* sizes in which no molt allometry is discernible. **B**. Discrete continuous allometry in which a line between maximum and minimum limits of sequential populations (having the same slope as intermolt populations) describes the molt allometry (+ indicates population means; ⎯⎯⎯ indicates intermolt allometric expansion; bars perpendicular to regression lines approximate maximum and minimum limits of each population)
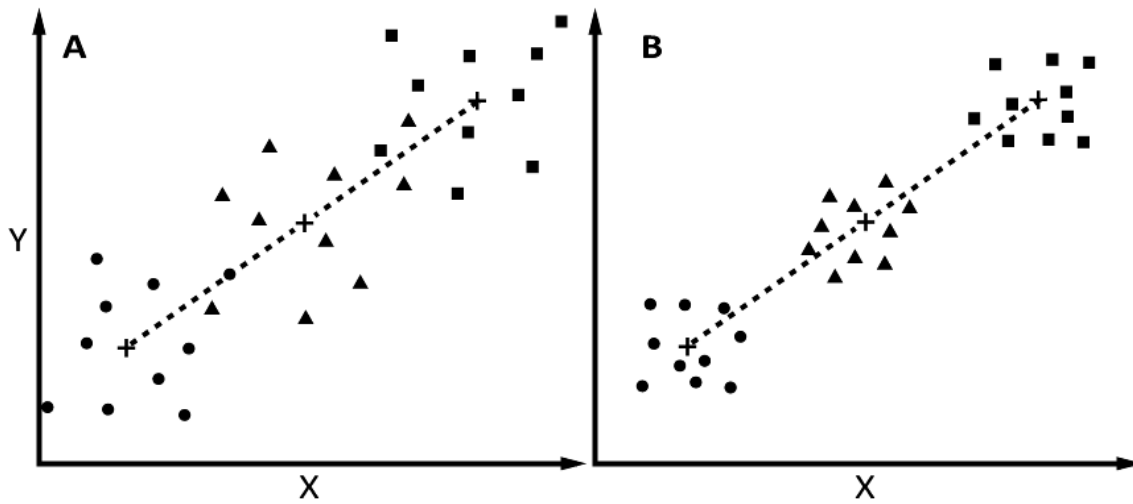
FIGURE 2 Theoretical distributions of individuals in three intermolt populations (● = instar 1; ▲ = instar 2; ■ = instar 3) showing two types of discontinuous allometry. Within each population no correlation exists between X and Y (i.e., r = 0; b = 0) and no intermolt expansion occurs. Molt expansion, best approximated by the change from the X, Y mean of the population to the X, Y mean of the next population accommodates all the expansion. **A**. Indiscrete discontinuous allometry. **B**. Discrete discontinuous allometry (+ indicates population means; ------- indicates molt allometric expansion)
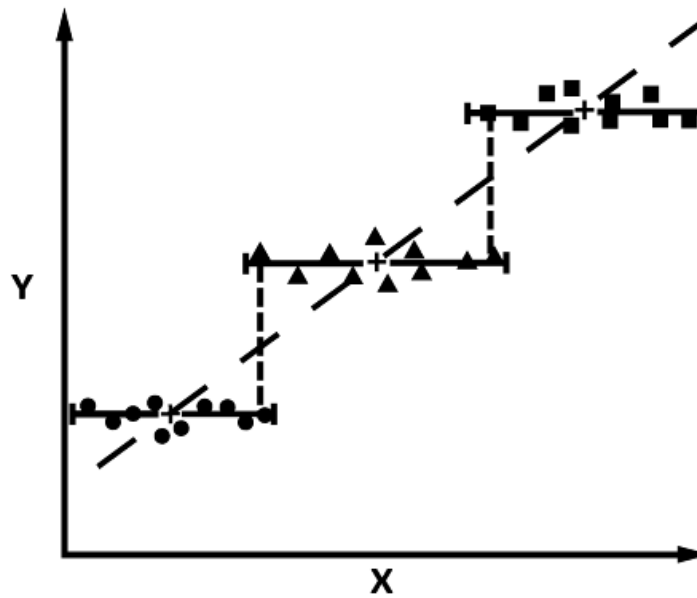
FIGURE 3 Theoretical distributions of individuals in three intermolt populations (● = instar 1; ▲ = instar 2; ■ = instar 3) showing simple mixed allometric growth. One variable (*X*) grows continuously, with the other variable (*Y*) having no growth, during the intermolt. The reverse situation occurs during the molt period, in which *Y* expands during molt and *X* remains the same size (+ indicates population means; ——— indicates intermolt allometric expansion; -------- indicates molt allometric expansion; — — — indicates phase allometry; bars perpendicular to regression lines approximate maximum and minimum limits of each population)
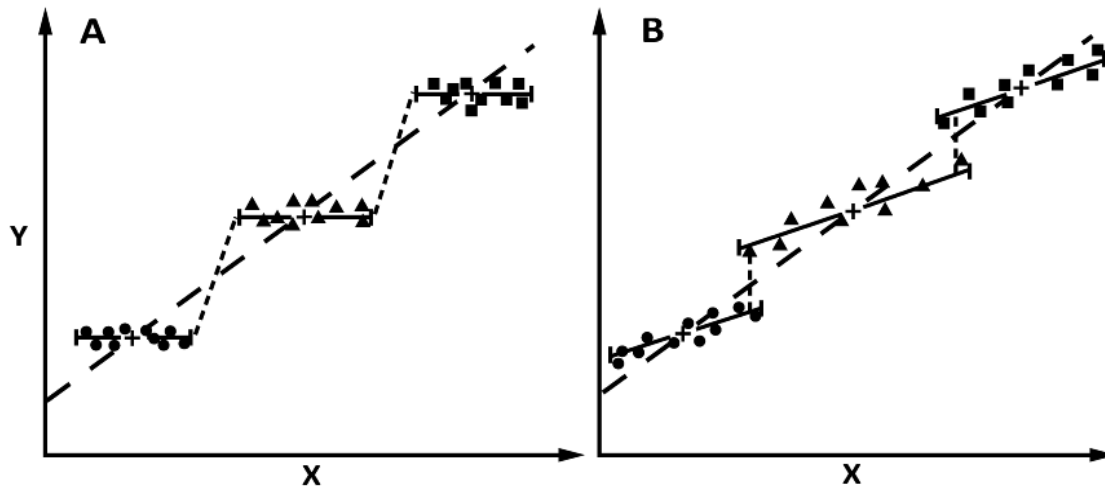
FIGURE 4 Theoretical distributions of individuals in three intermolt populations ($\bullet$ = instar 1; $\blacktriangle$ = instar 2; $\blacksquare$ = instar 3) showing complex mixed allometric growth in which only one variable expands during the intermolt and both expand during the molt period. **A**. Intermolt discontinuous, molt continuous or where both variables expand during the intermolt and only one variable expands during the molt period. **B**. Intermolt continuous, molt discontinuous (+ indicates population means; ———— indicates intermolt allometric expansion; -------- indicates molt allometric expansion; — — — indicates phase allometry; bars perpendicular to regression lines approximate maximum and minimum limits of each population)

FIGURE 5 Theoretical distributions of individuals in three intermolt populations (● = instar 1; ▲= instar 2; ■ = instar 3) showing two-rate continuous allometric growth. Both variables are growing during both intermolt and molt periods, but at different proportional rates (+ indicates population means; ——— indicates intermolt allometric expansion; -------- indicates molt allometric expansion; — — — indicates phase allometry; bars perpendicular to regression lines approximate maximum and minimum limits of each population)
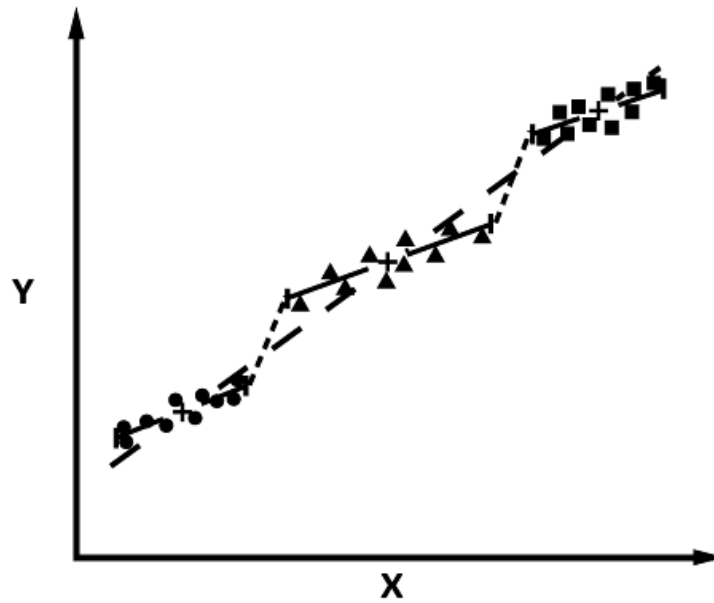
2.3.2 Continuous allometric growth

If, within each instar, the increase in the size of x is accompanied by a constant proportional increase in the size of y, such that the slope, b, and the intercept, a, are the same for more than one instar (Figure 1), then the relative growth of both variables can be described by a single regression model for all instars. Within each instar, and over all instars, statistical tests are available to determine if $b \neq 0$ and $a < 0 < a$, and if $b_i = b_j = b_n$ and $a_i = a_j = a_n$ (for regressions $i \ldots n$) (see Neter and Wasserman, 1974).

Two variations of continuous allometric growth are possible depending on the discreteness of each intermolt population. If the intermolt populations are indiscrete such

that the population limits overlap (Figure 1A) then no molt allometry (i.e., expansion during the molt period) occurs because no separation of sizes of individuals leaving one intermolt and entering the next intermolt is discernible. This pattern of growth is more typical of soft-bodied organisms in which any distinction between stages (instars) is artificial (e.g., age classes) and does not necessarily have a physiological basis. In this case, allometry is best described by a single regression over all slopes, periods or, in Crustacea, instars.

If the intermolt populations are discrete and have no overlap (Figure 1B) and the regressions of each are identical in terms of slopes and intercepts, it can be assumed that the slope of the molt allometry is identical to intermolt allometry. The intermolt allometry for each instar would then be described by the regression characteristics between the upper and lower intermolt population limits. A line fitted between the maximum population limits for the intermolt population of that instar and the minimum limits for the next intermolt population would describe molt allometry.

2.3.3 Discontinuous allometric growth

If no correlation exists between the variables within intermolt populations ($r = 0$) and $b = 0$, then the statistical inference is that there is no proportional change in one variable relative to the other during each instar. In this case no intermolt allometry exists, inferring wide variation of both variables about their means within each intermolt population and that an individual, having a proportion x_1, y_1 in intermolt 1, will maintain that proportion throughout that intermolt period. Similarly during intermolt 2, the same individual will be x_2, y_2. The best estimates of each intermolt population will then be $\overline{X}_1$, $\overline{Y}_1$; $\overline{X}_2$, $\overline{Y}_2$, etc.

With no intermolt expansion, the change from intermolt 1 to intermolt 2 must be accommodated by the molt. The molt allometry is calculated as $\overline{X}_2 - \overline{X}_1$, $\overline{Y}_2 - \overline{Y}_1$ for instar 1; $\overline{X}_3 - \overline{X}_2$, $\overline{Y}_3 - \overline{Y}_2$ for instar 2, etc., and intermolt allometry will be zero, meaning that all expansion during an instar is accomplished during the molt period and is the typical view of crustacean growth. Usually pre-molt (instar i) and post-molt (instar $i + 1$) individuals are sampled and the regression between the means of these populations, representing molt expansion, is considered the allometric growth of the population. If regressions through several such population comparisons are similar (in terms of slope and intercept) a single regression line through all points adequately describes overall allometry (phase allometry), the traditional approach to allometry in Crustacea. These considerations would apply equally to indiscrete (Figure 2A) or discrete intermolt populations (Figure 2B).

2.3.4 Mixed allometric growth

Depending on physiological processes working within different body regions under consideration, it is possible that during the different periods or stages within an instar, combinations of continuous and discontinuous growth may occur. Any combination of continuous and discontinuous growth within the intermolt and molt periods of an instar is here referred to as mixed allometry. Two types of mixed allometry are considered: simple mixed allometry in which only one variable grows discontinuously during each instar period; and complex mixed allometry in which one variable grows discontinuously in one period and both variables grow continuously in another period.

2.3.4.1 Simple mixed allometric growth

As with the examples of discontinuous growth, an intermolt population can have both b

and r = 0, but at the same time can have a distinctively different scatter population of x, y

sizes (Figure 3). For each intermolt population, r = 0, $b = 0$, $Y = a \neq 0$ and, unlike that for

pure discontinuous allometry, the variance of x_i's about $\overline{X}$ would be greater than the

variance of y_i's about $\overline{Y}$ (i.e., $\sigma_x^2 > \sigma_y^2$). The inference is that within the intermolt

population, variable X is growing continuously while Y is not expanding. (The reciprocal

situation in which the x, y scatter of the degree of association created a vertical

distribution, i.e., Y was continuous and X discontinuous, is possible, but in this case "Y"

would become the independent variable plotted on the abscissa.) The final qualifying

criterion for simple mixed allometry would be that the intermolt populations for two

sequential instars were not discrete and overlapped to a greater or lesser degree on the

abscissa only (Figure 3). Within an instar, therefore, the expansion during the intermolt

would be accommodated by continuous growth in variable X and no growth in Y, while

during the molt, Y would account for the remainder of the instar growth.

2.3.4.2 Complex mixed allometric growth

Two forms of complex mixed allometry are possible and depend upon the discreteness of

intermolt populations and the statistical significance of regression of each intermolt

population: intermolt discontinuous-molt continuous allometry (Figure 4A) and intermolt

continuous-molt discontinuous allometry (Figure 4B).

Intermolt discontinuous-molt continuous allometry produces intermolt

populations similar to simple mixed allometry, except that the sequential intermolt

populations are discrete and do not overlap along the abscissa. Intermolt expansion within each instar would only be accommodated by the X variable, none by the Y, and the slope of a line between the maximum values of one intermolt population to the minimum values of the next sequential intermolt population would describe the proportional increases of X and Y during the molt. The slope of molt allometry would not be the same as that described over all instars.

Intermolt continuous-molt discontinuous allometry would be produced by intermolt populations that had positive correlations, significant regressions (i.e., $b \neq 0$, $Y \neq a$), had indiscrete populations, and the slopes of each population differed significantly from the slope described by a regression over all populations (Figure 4B). Intermolt expansion would be continuous for both variables, but during the molt, expansion would occur only within the body region described by Y. The molt expansion would be calculated by the difference between the minimum Y-value described by the next sequential intermolt regression and the maximum Y-value for the intermolt regression belonging to the same instar as the molt under consideration.

2.3.5 Two-rate continuous allometry

This instar growth pattern combines aspects of the discrete continuous allometry and the intermolt continuous-molt discontinuous allometry, complex mixed allometry cases. Intermolt regressions would be significant (i.e., $b \neq 0$; $a \neq 0$) and intermolt populations would be discrete, but the intermolt regressions would have slopes and intercepts different from the regression through all instars (Figure 5). Both variables would grow continuously during both the intermolt and molt periods but at different rates. The slope

of the intermolt regression would describe the intermolt allometry and the slope of a line between the maximum and minimum sizes of two sequential intermolt molt periods would describe the molt allometry.

2.4 Overall allometric growth rate through several instars

In all cases described above, the allometry over all instars (phase allometry) considered is identical. Irrespective of the type of instar (or instar period) allometry, the phase allometry can adequately be calculated from the mean X, Y values of each intermolt population. This situation is not dissimilar to the allometry described for other crustaceans (e.g., Hartnoll, 1978; Teissier, 1960;). The most frequently used method to calculate the sizes of each instar is to measure variables in pre- and post-molt individuals (i.e., non-molt individuals) through several instars. Assuming these organisms do not change size during the intermolt, the post-molt sizes will be the same as the pre-molt sizes for individuals in the same instar, and the best estimate of the size of any variable during any particular instar is represented by the mean of that variable for each instar population. Using the methods described previously to examine instar allometry, there would be no inconsistencies with methods of previous studies.

The concept of instar allometry is not new. Mayrat (1965, 1967), for example, in developing his growth increment hypothesis considers growth-allometry as the rate of relative growth of each individual (within the same population) over several instars within a phase, which corresponds to the slope of the regression over the entire phase. Thus, the proportion exhibited by the entire population within the phase is termed the phase allometry. Because often not all individuals shift during identical instars from one

phase of growth to another, Mayrat suggested another parameter of growth: stage allometry.

Stage allometry, which is the allometry exhibited by each instar within a particular phase (and which may or may not coincide with the phase allometry), was used by Mayrat to describe variation in instar phase shifts. The stage allometry suggested by Mayrat is somewhat similar to what is termed instar allometry in this paper, in that the allometry within periods of an instar may or may not coincide with phase allometry (allometry through the means of several instars).

Hartnoll (1978) discussed Mayrat's growth-increment and Huxley's (1932) size-equilibrium hypotheses in relation to phase shifts. As Hartnoll (1978) was more concerned with the determination of proportional growth than the amount of growth during any instar or phase, he concluded that "there is no reason to postulate a series of stage allometries corresponding to each instar within a phase and differing from the overall phase-allometry" (Hartnoll, 1978, p. 283). The fact that intermolt growth, which previously has been assumed to not be present, has been observed in crustaceans (Childress and Price, 1978; Mauchline, 1973; and this treatise) negate this assumption, and any of the theoretical instar allometries proposed earlier which have intermolt growth (realizing that instar allometry does not require growth within a particular instar, but may merely express the relationship between different sized specimens within an instar) are possible. In these proposals, both the determinations of the proportional growth and the amount of growth during each instar and instar periods are important.

2.5 Determination of proportions and amounts of allometry within instars

If within an instar two or more periods or stages are present, in which physiologically distinct actions occur, the amount of growth during that instar is represented by the sum of the growth of all periods or stages. In other words, instar growth equals intermolt plus molt growth. It is important, therefore, to estimate the minimum size limits of individuals entering and the maximum size of individuals leaving that instar and the periods within the instar. Proportional growth of two body regions under consideration during these periods is estimated by the correlation and regression between the two variables which in turn determines the type of allometry, making it necessary to estimate not only the regression relationship between the two variables but also the sizes of animals entering and leaving each substage (Figure 6).

The simplest and most direct way to determine both the proportion and amount of allometric growth is by measuring the appropriate body regions of the same individuals at regular intervals during an instar, knowing when the individual enters and leaves each period, which has apparently not as yet been attempted, although continuous measurements of total and standard lengths of individuals has been done for a few crustaceans (Meixner, 1969). It is most common for populations of organisms within known instar periods to be sampled and estimates made from population regression relationships and minimum and maximum sizes of individuals within each period.
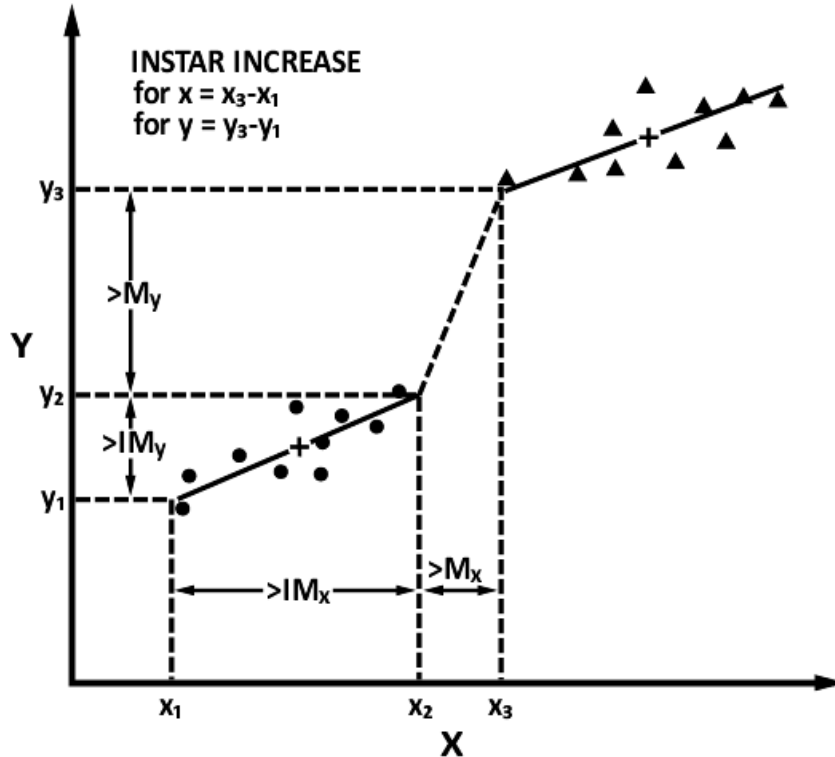
FIGURE 6 Proportion and amount of allometry calculated for two periods (IM = intermolt; M = molt) of

an instar. Proportional allometry in each period is represented, respectively, by the regressions between X_1,

Y_1 and X_2, Y_2 and X_2, Y_2 and X_3, Y_3. The amount of growth is represented by a shift in each variable from the

minimum to the maximum sizes during each period (> IM_x, > IM_y for the intermolt and > IM_x, > M_y for the

molt) (● = instar 1; ▲ = instar 2; + indicates population means; ——— indicates intermolt allometric

expansion; -------- indicates molt allometric expansion)

In establishing regression models that quantitatively describe the proportional

relationship between two variables, in which a functional relationship is represented by a

statistical relationship ("best fit model"), establishment of the independence of one

variable is paramount (the selection of independent variables is discussed by Neter and

Wasserman, 1974). This functional relationship does not, however, necessarily imply a

causal relationship. For example, there is no implication that Y casually depends on X in any given instance. A statistical requirement for a regression, whether it is used for descriptive, control or prediction purposes, is that X is chosen *a priori* and the x_i's are measured without error. In practice these requirements are not always met (Lovett and Felder, 1989) because as Katsanevakis et al. (2007) point out: "…with morphological measurements … the functional relationship between two … morphological variables … are subject to natural variability and measurement errors."

In studies of crustacean allometry, it is not always mentioned why one or another variable was designated as the independent variable. Hartnoll (1982) criticized this failing by suggesting that "X can seldom be considered as a genuine independent variable." He concluded that if Y is a measure of a relatively small body region and X is a measure of a relatively large one, designating X as the independent variable is satisfactory. He insists, however, that if the two body regions are of "essentially the same order" (e.g., length and width of carapace), neither is strictly dependent or independent, and "it may be more theoretically appropriate to calculate the major axis or the reduced major axis," i.e., a Model II regression (see Katsanevakis et al., 2007).

In cases where intermolt allometry has been examined (Childress and Price 1978; Mauchline 1973), the carapace has been considered as independent, presumably under the assumption that it did not expand during the intermolt. Consequently these authors neglected several alternative overall functional relationships between abdomen and carapace allometry and concluded that all expansion occurred in the abdomen, with no carapace expansion.

In dealing with unknown functional dependency of two variables and because allometric relationships are based on population estimates, it may be more appropriate, therefore, to use both $Y = f(X)$ and $X = f(Y)$ to estimate the limits of proportional allometry in any instar population. This consideration is particularly true if the criteria of errorless measurements of x_i's or multiple measures of Y around set values of X cannot be made (Eisenhart, 1939; Winsor, 1946). As with all population estimates, the "real" relationship usually lies within these limits. When a calculated regression of Y on X is fitted it will, except in the case of an isometric relationship, produce a model with different values (Figure 7). A case of positive allometry between two variables can, therefore, become one of negative allometry depending upon which variable is considered as statistically independent. The descriptive terms of positive and negative allometry may, in some cases, lose functional meaning unless the independence of one variable is either functionally established or defined.

Determination of the amount of allometry within an intermolt population is also necessary to estimate how much expansion in both variables occurs during each period of an instar. Several estimates of population limits for one-variable populations exist (e.g., confidence intervals or limits) and these have been adapted for bivariate populations to produce confidence belts around a regression (Sokal and Rohlf, 1995). These bivariate estimates neither satisfy the determination of maximum or minimum limits of the population nor do they meet the criterion of individual (x, y) values of one variable, say X, and subsequently measuring the corresponding values of Y. These requirements are necessary for variance estimates of Y on specified X values (Winsor, 1946).
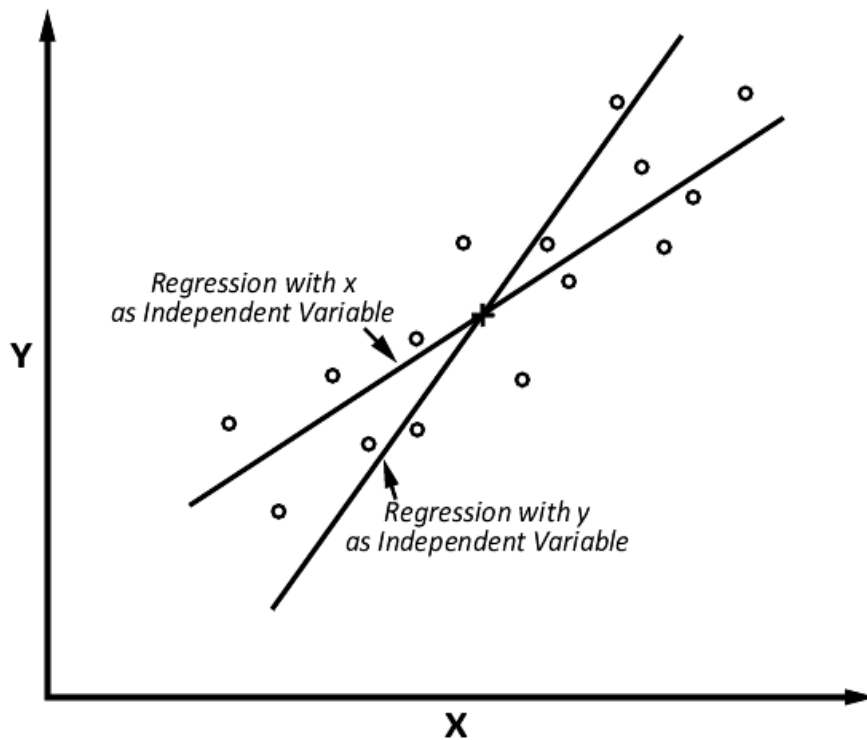
FIGURE 7 Fitted linear regressions of two variables (*x* and *y*), using *X* and *Y* separately as the independent variable (+ indicates population means)

In most allometric studies, the (*x, y*) values of body sizes of individuals are obtained by random sampling from a large population of individuals in the same instar. A simple way to estimate *X, Y* maximum/minimum limits of a randomly selected instar population would involve measures of dispersion around the mean of the independent variable, assuming the independent variable values are normally distributed about this mean, and using the regression model as a predictor for the corresponding values of the dependent variable. A measure of dispersion frequently used is the standard deviation (σ) with $\overline{X} \pm 2\sigma = 95\%$ of the population. Using the mean of the independent variable $\pm 2\sigma$ and the regression model, therefore, the *X, Y* minimum and maximum values for an intermolt population can be estimated (Figure 8). Using this measure of dispersion also

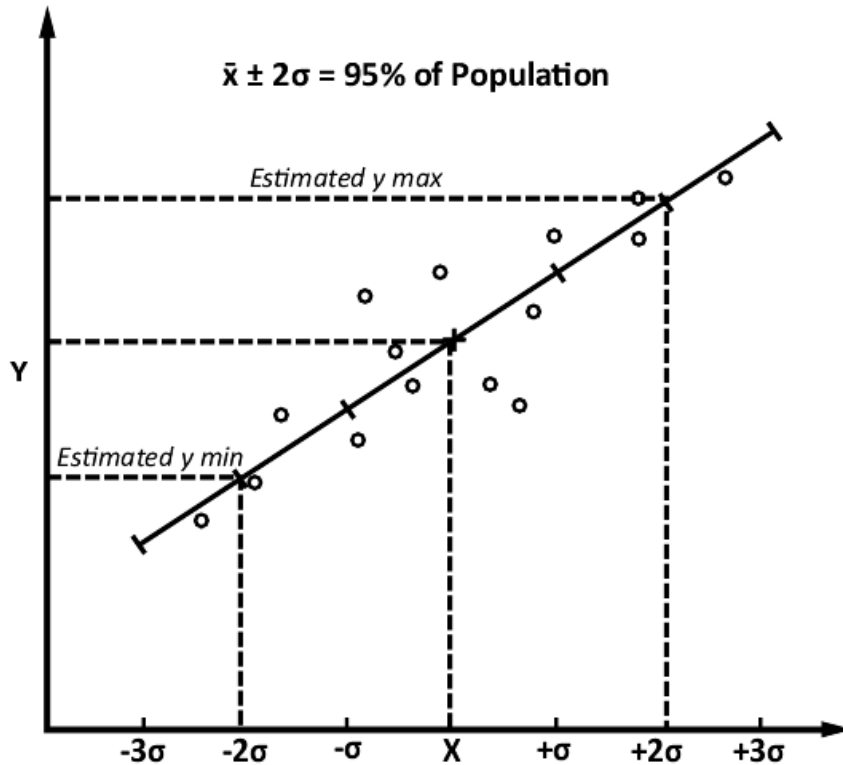allows ready comparison with many other studies that use standard deviations for describing populations.

FIGURE 8 Population estimates of the size limits of two variables based on the regression of Y on X and the mean (indicated by +) ± standard deviation (σ) of the independent variable population (bars perpendicular to regression lines approximate maximum and minimum limits of each population)

By using reciprocal regressions (Y on X and X on Y) and simultaneously using measures of dispersion on the independent variables the estimates of the limits of allometric proportions and the amounts of allometry, within an intermolt population, can be determined. Similar estimates can be made of the molt period within an instar (Figure 6) using the difference between the maximum intermolt size estimate for one instar and the minimum intermolt size estimate for the next sequential instar.

The case of discrete intermolt populations (Figure 6) presents less problem than indiscrete populations which may have an overlap in the values of either variable. In this case, providing the slopes of the two populations were not similar (see the case of Indiscrete Continuous Allometry, Figure 1A), no molt expansion can be adequately estimated on the axis that overlaps (increase of the variable on this axis is ≤ 0), but the molt expansion of the other variable can be estimated (Figure 9).
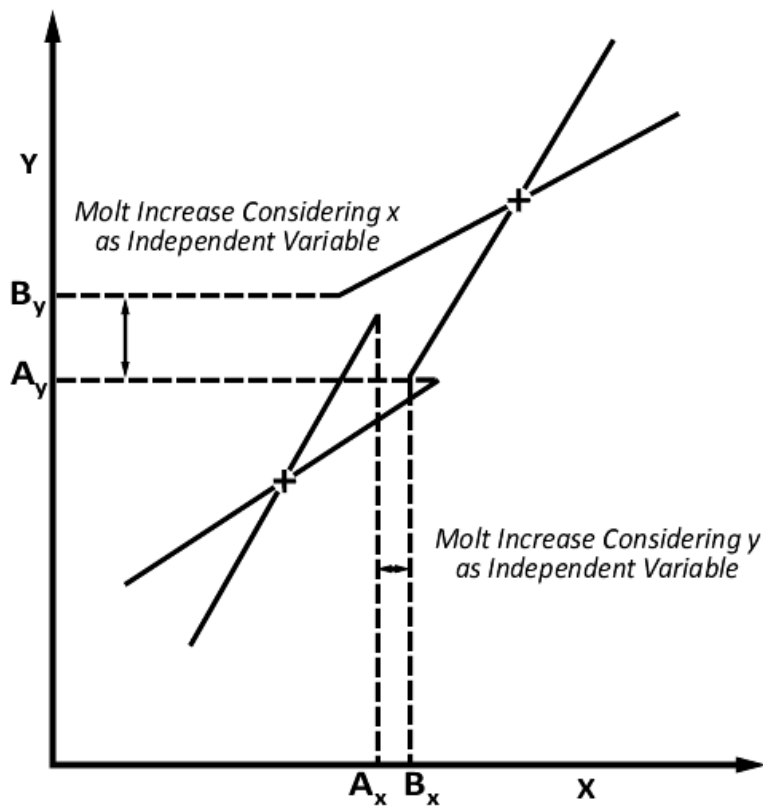

FIGURE 9 Estimation of molt increase between two sequential intermolt populations (A and B) that have indiscrete (overlapping) distributions. Figure utilizes the regressions of Y on X and X on Y, as well as the mean (indicated by +) $\pm$ 2 standard deviations of the independent variable to estimate the molt expansion in the dependent variable (i.e., molt increase of Y, using Y on $X = B_y - A_y$; molt increase of X, using X on $Y = B_x - A_x$)

3 | RESULTS OF MODEL APPLICATION

3.1 Determining instar allometry

The general requirements for determining the type of instar allometry, as well as the

allometric proportions and amounts of growth within periods of instar populations are: 1)

Individuals must be sampled from known instar periods (or stages) of two or more

sequential instars; 2) Individuals must belong to the same genetic and environmental

population exposed to the same environmental conditions; 3) Each period (stage)

population is randomly sampled to include a full range of proportional sizes for each

instar; and 4) Expansion (growth) is known to occur in one or more periods (stages) of

the instars. To establish one or more forms of continuous or mixed (continuous-

discontinuous) allometry, as earlier defined, an additional criterion should be met: 5) All,

or part, of the organism expands during all or part of the intermolt (anecdysis) period/s.

3.2 Instar allometry growth data for *Americamysis bahia*

All of the above criteria for determining type of instar allometry, allometric proportions

and amounts of growth within periods of instar populations are met in a study by Scarfe

and Steele (2017) that examined the theoretical allometric approaches discussed earlier

and determined the proportion and amount of allometry through several post-larval

instars of *A. bahia* by sampling individuals in the intermolt stage of instars 1 through 6

and measuring several body regions of each individual. Of primary importance here are

abdomen and carapace lengths (mm) because of an observed substantial intermolt

expansion in the standard lengths, SL (mm), and because it was necessary to determine

whether intermolt expansion occurred in either the abdomen or carapace or both. Scarfe

and Steele (2017) also determined the type of instar allometry exhibited by *A. bahia* and an estimation of the proportion and amounts of period allometry (molt/intermolt allometry) in the abdomen and carapace.

The overall growth pattern of *A. bahia* in the first few post-larval instars is one in which about 85% of the expansion occurs in the intermolt period, with the remainder at ecdysis. Of the intermolt growth, about 60% is accomplished in the abdomen and about 40% in the carapace, while ecdysis accounts for the remaining 15% of the total expansion, in which either abdomen or carapace can account for 40% to 100% of this expansion. The slopes of the intermolt regressions have intercepts different from the regression through all instars (Scarfe and Steele, 2017). Average molt:intermolt ratio in the carapace expansion is 1:3.16, and in the abdomen, 1:3.71, indicating that proportional growth between the carapace and the abdomen is continuous during both the intermolt and molt phases, but occur at different rates and proportions during the two periods (Scarfe and Steele, 2017), thus indicating two-rate continuous allometry (see Figure 5).

4 | DISCUSSION

Discontinuous growth at ecdysis in which either of the body regions expanded far more rapidly than the other, perhaps to the extent of no growth occurring in the other variable, would also allow for synchrony in the overall sizes of individuals moving from instar to instar. This situation has been examined by Hartnoll and Dalley (1981) in several crustaceans by comparing the variation in the size of individuals in several sequential instars. They suggest "the reduction of the coefficient of variation implies a negative feedback at the molts concerned, whereby specimens which are large for that instar

experience a smaller than normal molt increment and vice versa" (Hartnoll and Dalley. 1981, p. 237).

The observed trend in the reduction of the coefficient of variation of abdomen, carapace and SL is similar in *A. bahia* (Scarfe and Steele 2017). If Hartnoll and Dalley's (1981) explanation is correct, then for *A. bahia* a negative feedback mechanism (of extrinsic or intrinsic nature) is functioning through the instars under consideration and the molt at the end of each instar tends to synchronize the size at which individuals enter the subsequent instar. The synchronization of overall body size (SL) is more-greatly-negatively correlated with instar than either abdomen or carapace lengths (Scarfe and Steele, 2017). Apparently, different body regions differentially accommodate synchronization of overall body size at different stages (instars) of growth, again indicating two-rate continuous allometry (Figure 5). Knowing that intermolt expansion occurs in *A. bahia,* apparently in variable amounts among individuals, differential molting between individuals and between body regions would permit resynchronization of the sizes of individuals to enter the subsequent instar. Consequently, when individuals in a similar population reach the same stage in development, most will be similar in size.

Instar allometry, as discussed herein, does not negate the overall growth allometry over several instars, such as has been more traditionally examined (Hartnoll, 1978, 1982; Teissier 1960). *Americamysis bahia* has two distinctive phases of allometry (Scarfe and Steele, 2017). The first, which would be represented by a straight line plotted through the mean sizes of instars 1, 2 and 3, is different from the second allometric phase during instars 4, 5 and, possibly, 6. This disjunction or phase discontinuity (as opposed to instar or intermolt discontinuities) occurs at molt 3, and it is during instar 4 when the first

physical signs of sexual differentiation appear. Allometry during instars 1, 2 and 3 can, therefore, be considered as pre-pubital allometric growth, and that during instars 4, 5 and, possibly, 6 as pubital allometry.

In addition, something of a discontinuity in the variation of the carapace-abdomen sizes of individuals is seen between instars 3 and 4 (Scarfe and Steele, 2017). In this case the coefficients of variation drop dramatically in instar 4, suggesting a distinct resynchronization of sizes of individuals entering the pubertal phase (pubertal molt; see Hartnell, 1985; Hartnoll and Dalley, 1981). If correct, this observation infers that not only is the amount of growth regulated during each phase but that there is also a regulation of the proportional growth within these body regions over each phase. These results somewhat combine Mayrat's growth increment hypothesis (Mayrat, 1965, 1967) and Huxley's size-equilibrium hypothesis (Huxley, 1932). Hartnoll (1978, 1982) provides extensive discussion of both hypotheses. While proportional allometry within instars and over phases of growth covering several instars may assist in determining the physiological mechanisms controlling allometric growth, the amount of growth within the instars and phases can also assist in these aims.

AUTHOR CONTRIBUTIONS
Both A.D.S. and C.W.S. contributed to all aspects of this study.

COMPETING INTERESTS
The authors have no competing interests to declare.

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