



Analysing individual growth curves for the copepod *Tigriopus brevicornis*, while considering changes in shape

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ABSTRACT

Understanding growth and development over ontogeny, and the effects of stressors on life history, requires bioenergetic analysis, for example using models based on dynamic energy budget theory. Such analyses require precise and accurate determination of the animal's biomass or biovolume over time. Automated imaging offers great advantages by allowing size measurements at a high temporal resolution and the possibility to follow individual animals, thus providing detailed growth trajectories as well as handles on inter-individual variation in growth. Here we report on a re-analysis of images from a life-cycle experiment with the coastal harpacticoid copepod *Tigriopus brevicornis*. Biovolume was estimated by approximating the organism's shape by a generalised ellipsoid. This analysis confirmed, rather unsurprisingly, that the moult from the last naupliar to the first copepodite stage is accompanied by a major change in shape. However, within the naupliar and copepodite stages, more gradual elongation was observed. These changes in shape imply that total body length is a poor proxy for body size throughout the developmental stages, and is therefore not suitable for bioenergetic analysis. Volumetric length (cubic root of estimated body volume) is far more appropriate. Interestingly, growth ceases for some 1.5 days around the moult to the first copepodite stage. Such a growth stop was not observed in earlier studies of several other copepod species. Furthermore, the growth rate of the copepodites exceeded that of the nauplii. These complexities in the life history of *T. brevicornis* pose challenges for bioenergetic analysis that will require determination of additional traits (e.g., feeding and respiration rates) to unravel.

1. Introduction

Copepods form an important link between primary producers and predators in freshwater and marine food webs. Understanding their growth and development over ontogeny, and the effects of stressors on the life history, is therefore of considerable interest. Bioenergetic analysis is particularly useful in this respect, since all organisms obey the conservation laws for mass and energy. Dynamic Energy Budget (DEB) theory (Jusup et al., 2017) is a prominent framework for bioenergetic analysis, which has also demonstrated its usefulness in understanding the mechanisms underlying the life history response to stressors (Ashauer and Jager, 2018; Jager and Zimmer, 2012). Copepods, however, offer specific challenges for DEB-based analysis (Jager et al., 2017). Specifically, the initial naupliar stages are morphologically very different from the subsequent copepodite stages. Furthermore, copepods exhibit true determinate growth (Maszczyk and Brzeziński, 2018), a feature they share with insects (see also Llandres et al., 2015). For other

crustaceans, such as daphnids, the somatic growth pattern is well-represented by the von Bertalanffy curve (e.g., Jager and Zimmer, 2012), which shows a smoothly decreasing growth rate over time with an asymptotic maximum size. In contrast, copepod somatic growth (with body size expressed on length basis) is close to linear up to the final moult to adulthood, after which growth ceases abruptly. Final size depends on environmental conditions such as food availability and temperature (Mauchline, 1998), but the underlying bioenergetic mechanisms triggering the moults, and thereby defining maximum size, are still poorly understood (Jager et al., 2017).

DEB-based models can improve our understanding of copepod life histories, and the effects of stressors on life-history traits. However, bioenergetic modelling requires accurate estimation of the animal's biomass over ontogeny. Measurement of dry weight, or quantification of elements like carbon and nitrogen (see e.g., Campbell et al., 2001) is extremely valuable as these metrics have a direct link to biomass. Unfortunately, such measurements are destructive, which often results in

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large uncertainty in the growth curves owing to inter-individual variation. In poorly synchronised cohorts, this variation can easily hide crucial patterns in the data. In contrast, automated imaging techniques can be used efficiently to follow growth of specific individuals over time at high temporal resolution (Heuschele et al., 2019). Even though length measures like total body length are easy to obtain from images, they are only a meaningful proxy for biomass or biovolume if the organism retains a constant shape over ontogeny (i.e., isomorphy) (Jusup et al., 2017). All copepods, however, have a major change in morphology at the metamorphosis from nauplius to copepodite. For some species, additionally, gradual changes in shape have been identified within the nauplius and copepodite phases (Koch and De Schampelaere, 2019). This implies that growth curves based on total body length do not reflect the underlying bioenergetics of copepods; the resulting growth curve will confound actual biomass growth with changes in body shape over ontogeny.

Here, we focus on the coastal harpacticoid copepod *Tigriopus brevicornis*. In previous work, Heuschele et al. (2019) applied an automated imaging system to acquire individual growth trajectories for body length, at high temporal resolution, from nauplius stage N1 to adult copepodite stage C6. These data were used to investigate developmental effects of copper and predation risk in *T. brevicornis*. In this study, we reanalysed images from the control treatments of the previous study. The aim of this study is to provide a better understanding of the growth process and the inter-individual variation in growth patterns in these copepods, as a prelude to DEB-based studies on the dynamic effects of combined stressors. More specifically, we want to examine whether imaging can be used to construct meaningful growth curves, i.e., reflecting actual biomass. Since body length is not necessarily a good proxy for biomass, we set out to estimate biovolume of the copepods, which, if dry-weight density does not change much over ontogeny, is a good proxy for biomass. Furthermore, we also want to study how individuals differ in their growth patterns, and evaluate to what extent growth of this species follows the patterns established in DEB-based work for calanoid copepods (Jager et al., 2017; Jager et al., 2015). Deviation from these expectations would require modification of the mechanistic assumptions underlying the DEB model. These modifications will be needed to apply the DEB model in interpreting and predicting the effects of stressors on the *T. brevicornis* life history.

2. Methods

2.1. Image analysis

Images of individually kept copepods were acquired at 1-h intervals over their development from nauplii to adult using an automated imaging platform (Heuschele et al., 2019). For this analysis, we revisited 9 random individuals of the control treatment, including 4 females and 4 males. One individual died as a copepodite and did not reach maturity in the experiment. In images where animals were positioned in the focal plane and in ventral view, we used ImageJ (Schindelin et al., 2012) to manually measure the total length (tip of prosome to end of urosome), body width (maximum at prosome), and projected area. From lateral views, we measured (maximum) height of the animal at the prosome. The individuals are identified in the figures in the present paper by the same ID as used by Heuschele et al. (2019). Examples of the images and the measurements taken are provided in the supporting information (Fig. S1).

2.2. Geometric relationships

The biometrical results from the image analysis need to be translated into a biovolume, which requires an approximation of the animal by some standard 3-dimensional shape such as a spheroid or a cylinder. For copepods, several authors have used a combination of a general ellipsoid for the prosome and a cylinder for the urosome (Araoz, 1991; Napp

et al., 1999). The quality of our images, and the somewhat indistinct outline of the harpacticoids (often caused by overlapping swimming legs), did not allow measurements of the prosome and urosome separately. Therefore, we decided to approximate the entire volume of the copepods by a generalised ellipsoid as follows:

$$V = \frac{\pi}{6} L_t W H \quad (1)$$

Where V is biovolume (mm^3), L_t is total body length (mm), W is body width (mm), and H the body height (mm). We also have measurements of projected body area (mm^2 , from top view), which is likely a better measure for estimating volume of (non-perfectly ellipsoid) organisms than length and width measurements. The projected area (A_p) of a spheroid is an ellipse, and we can thus rewrite Eq. 1 to:

$$V = \frac{2}{3} A_p H \quad (2)$$

Unfortunately, only relatively few images allowed estimation of body height, since the animal would need to be positioned such that the image is taken from the side. Furthermore, width and height cannot be determined from the same image. Therefore, we first established general width:height ratios for *T. brevicornis*. To this end, we used the Matlab function 'smoothdata' (Savitzky-Golay filter, 2-day time window) to smooth width data over time, for each individual. More detail is provided in the supporting information (Section 2.3). Next, each height measurement was compared to the interpolated width at that measurement time point. To estimate biovolume with Eq. 2, height was estimated from the real width of each individual at each time point, using the mean width:height ratio for nauplius and copepodite stages. Volume is thus calculated at all observation time points where both width and projected area could be determined.

As a more practical measure of body size, we use the volumetric length (L): the cubic root of estimated biovolume:

$$L = V^{1/3} \quad (3)$$

Volumetric or structural length plays a central role in DEB theory (Jusup et al., 2017). Unlike physical length measures such as total body length, volumetric length is not affected by changes in shape and is often a good proxy for the bioenergetically meaningful body mass.

To quantify changes in shape over the life cycle, we calculate a shape correction coefficient as follows:

$$\delta_M = \frac{L}{L_t} \quad (4)$$

For an isomorphic organism, δ_M will be constant. Smaller values of δ_M imply more elongated or flattened individuals.

2.3. Model for growth

Standard DEB models for animals yield the von Bertalanffy growth curve, under constant conditions (Jusup et al., 2017). This curve is characterised by a smoothly decreasing growth towards an asymptotic maximum size. For copepods, however, growth is determinate and stops abruptly when reaching adulthood. Previous work suggests that this difference need not be caused by a major difference in metabolic organisation: the growth curve for copepods can be viewed as a truncated version of the von Bertalanffy curve (Jager et al., 2017). Copepods follow a rather standard DEB model, up to the final moult. After the final moult, they switch their metabolic organisation, such that resources are no longer allocated towards somatic growth. A very similar DEB adaptation was made for insects (Llandres et al., 2015). The initial part of a von Bertalanffy curve is almost linear, when expressed on suitable length basis (see supporting information, Section 5). A suitable length measure is a length measure that, when cubed, is proportional to body mass (volumetric length is such a measure, see Eq. 3). Earlier DEB-based work on calanoid copepods indeed confirmed that volumetric length

growth is close to linear (Jager et al., 2017; Jager et al., 2015).

In this study, we will not attempt to model growth in a fully mechanistic manner. This would also be impossible without information on other traits, such as reproduction and feeding rates. Instead, we choose a more descriptive model following from the expectation of linear growth. Deviations from linear growth would require adaptations in a DEB model for this species. Linear growth from early nauplius up to adulthood clearly fails to capture the growth curve of *T. brevicornis*. This species showed another stop of growth, temporarily, around the moult from nauplius to the first copepodite stage. The growth curve is thus better represented by two linear stages with plateaus (periods of no growth) at the end of the naupliar and copepodite phases, and different growth rates within the stages. We can thus specify a descriptive model as follows:

$$L(t \leq T_j + T_s) = \min(L_j, L_0 + r_n t) \quad (5)$$

$$T_j = \frac{L_j - L_0}{r_n} \quad (6)$$

$$L(t > T_j + T_s) = \min(L_m, L_j + r_c(t - T_j - T_s)) \quad (7)$$

For nauplii, volumetric length (L) thus increases from the initial length (L_0) to the length at metamorphosis (L_j) with a linear growth rate (r_n) over time (t). At age T_j the metamorphosis size is reached, and the growth stasis lasts for a duration T_s . After that, the copepodites will resume growth up to the adult size (L_m) with a linear growth rate (r_c). All length measures are in mm, all time measures in days, and the growth rates in mm/day. The following parameters will be fitted to the data for each individual: L_0 , L_j , L_m , r_n , r_c and T_s .

This simple model suffices to meet the aims of our study as specified in the introduction: to evaluate the effectiveness of imaging to construct meaningful growth curves, to see to what extent individuals differ in growth patterns, and to what extent growth of *T. brevicornis* deviates from a (truncated) von Bertalanffy curve.

2.4. Implementation

The growth model of Eqs. 5–7 was implemented into the BYOM v.5.2 platform for Matlab (<http://www.debttox.info/byom.html>) and fitted on the volumetric length data for each individual separately. Optimisation and construction of confidence intervals was performed using the parameter-space explorer algorithm (Jager, 2021), which is particularly well suited for models that contain switches. The likelihood function that is used for optimisation assumes independent observations that are normally distributed after square-root transformation. The observations on each individual are clearly not independent, although the residuals are likely dominated by measurement error (which will be approximately independent). Linear regressions on the shape-correction coefficient were performed with the Matlab function ‘regress’.

3. Results and discussion

3.1. Growth curves

Only relatively few images allowed determination of the individual's height, especially for the naupliar stages. The variation in the width:height ratio is considerable, also within the nauplius, copepodite and adult stages. This variation is likely dominated by errors in both biometrical measurements, rather than actual differences in shape between individuals (see individual-specific plots in supporting information, Fig. S5). Therefore, and because of the scarcity of height measurements, we decided to pool the data for all individuals to calculate width:height ratio. The width:height ratio is quite constant within the naupliar and copepodite stages, but clearly differs between nauplii and copepodites (Fig. 1). The adults do not deviate from the earlier copepodite stages and are therefore not considered separately.

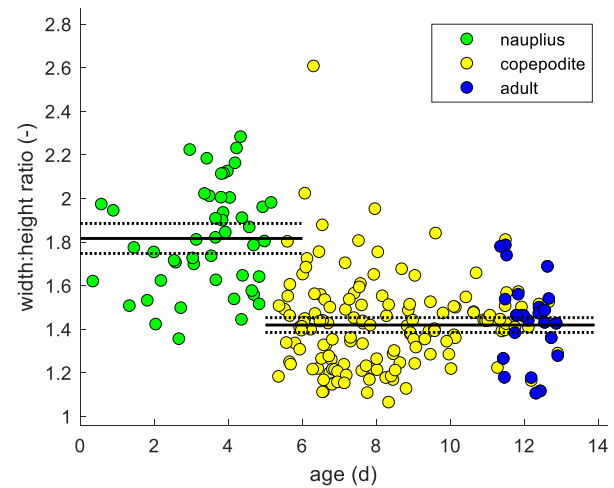


Fig. 1. Ratio of width:height, showing all data points, for all individuals, where measurement of height was possible. For this ratio, width was interpolated from smoothed observations over time, for each individual. Lines are separate means for nauplii and copepodites (incl. adults) with 95% confidence interval ($2 \times$ s.e.).

Despite the variation, the mean ratios are well defined. For nauplii, the width:height ratio is 1.82 (s.e. 0.034) and for copepodites 1.42 (s.e. 0.017). All stages are thus significantly flattened, but the nauplii more so than the copepodites. This contrasts the findings for other copepod species, which showed ratios closer to 1 (Araoz, 1991), implying an almost circular cross section. With the mean width:height ratios for nauplii and copepodites established, volumetric length was calculated (Eq. 2–3) and fitted with the simplified model (Eq. 5–7). The data for each individual was fitted separately (Fig. 2), and model parameters are compared across individuals in Fig. 3.

As shown in Fig. 2, the model provides an excellent fit to the data ($r^2 > 0.97$); growth expressed as volumetric length is very close to linear for a large part of the life cycle. Focussing on the copepodite stages, the volumetric-length growth of *T. brevicornis* corresponds to the expectation of linear growth up to a maximum at adulthood. However, there is a clear stop of growth in the final naupliar stage N6. Growth stops well before the moult to C1 and continues only after the moult. The initial copepodite sizes closely match the final naupliar sizes, which indicates that little biovolume is lost during the growth stasis and the metamorphosis. In the supporting information (Fig. S6), the same growth data and model fit are plotted on volume basis, rather than volumetric length.

3.2. Evaluation of parameter estimates

Fig. 3 compares the model parameters, with their confidence intervals, across the individuals (initial length L_0 not shown). In general, the parameters are well-identified from the data, as shown by the well-defined confidence intervals. Adult length (L_m) could not be properly identified for three individuals, as evidenced from the confidence intervals that extend all the way up to the maximum boundary set in the analysis (0.4 mm). For these individuals, there are insufficient measurements on the adult stage to fix the plateau size. Even though there will be no growth in the adult stage, the statistical parameter estimation would be served by an extension of the experimental duration to obtain a good estimate for maximum size. There are no obvious differences between males and females for any of the model parameters.

The linear growth rate of the copepodites is substantially larger than that of the nauplii (Fig. 3, confidence intervals do not overlap). The metamorphosis to a different morphology is therefore accompanied by an increased growth rate. Since growth is an energy-demanding process,

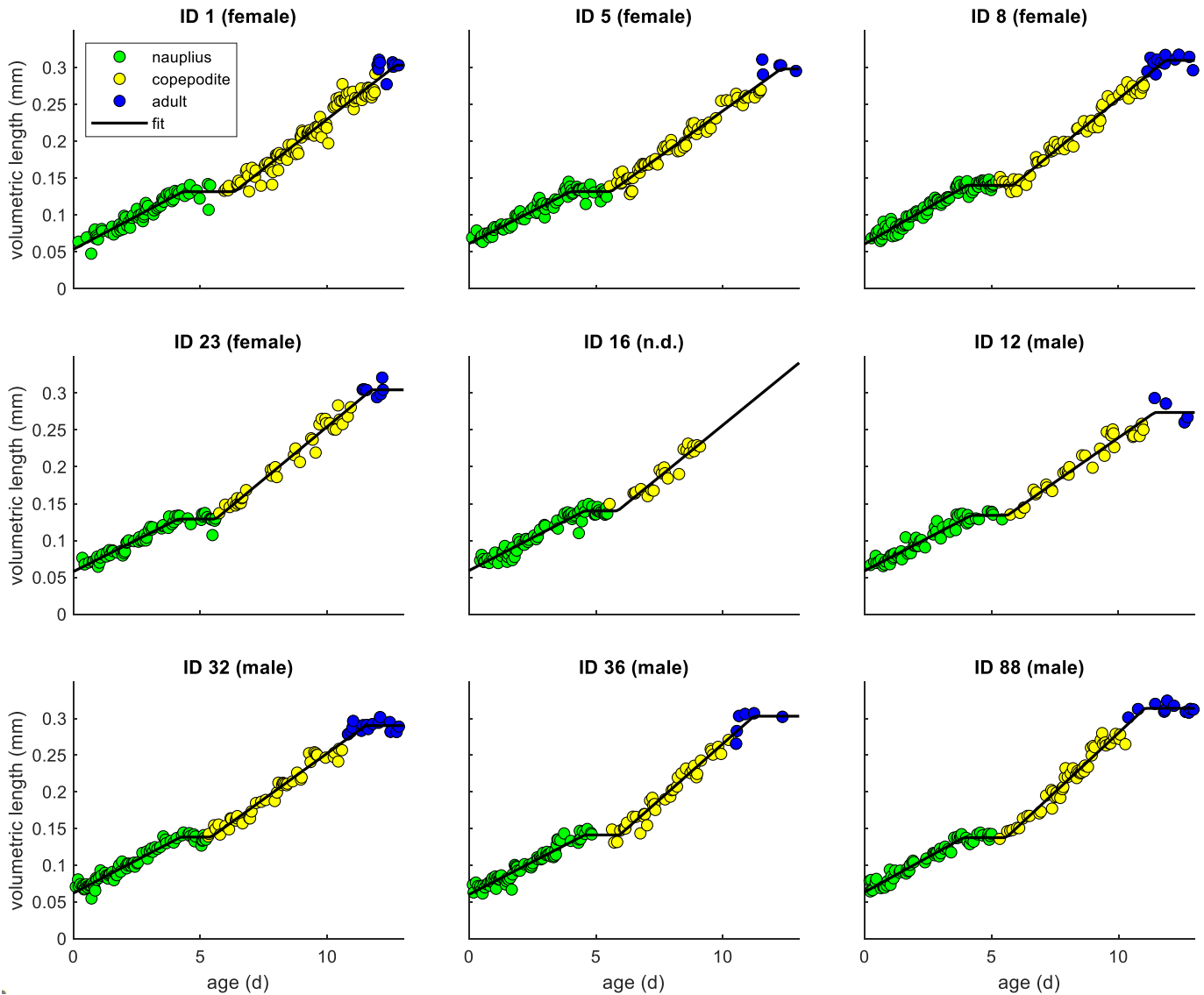


Fig. 2. Volumetric length (cubic root of estimated body volume) for each individual over time. Lines are model fits.

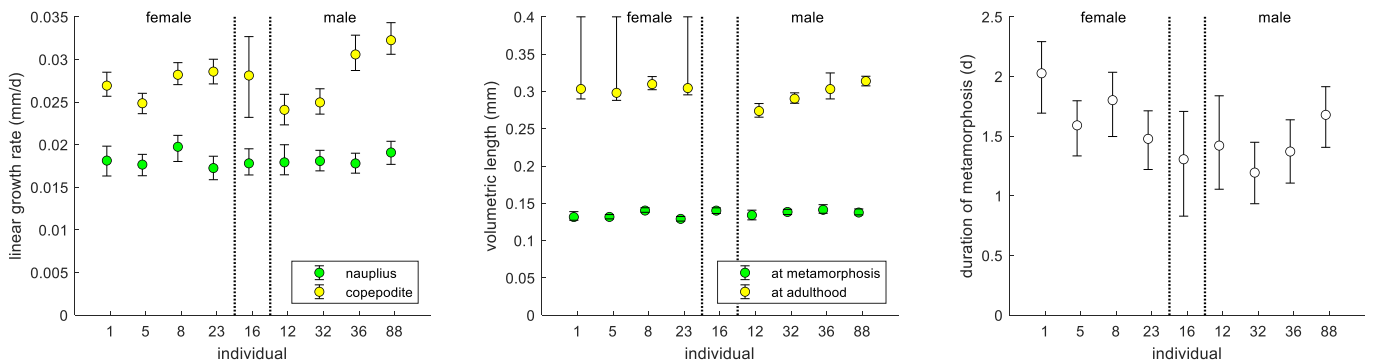


Fig. 3. Parameter estimates from the model fits on each individual. Maximum likelihood estimates with 95% likelihood-based confidence intervals. Parameter for maximum body size at adulthood was bounded at 0.4 mm. Individual 16 died before reaching adulthood, and hence its maximum size and sex could not be determined.

an increase in growth rate strongly suggests an increased feeding rate. Such metabolic accelerations are not uncommon, and the underlying mechanisms have been categorised under several options within DEB

theory (Kooijman, 2014). The most likely explanation for *T. brevicornis* is that the feeding rate increases after the moult to C1. This may relate to a change in morphology and efficiency of the feeding apparatus at

metamorphosis, allowing higher feeding rates. However, nauplii and copepodites of *T. brevicornis* also have a different mode of feeding and may prefer different food sources (Harris, 1973). There is a possibility that the experimental conditions, have inadvertently led to a food limited situation for the nauplii (see also Zimmer et al., 2012). Finally, it cannot be entirely excluded at this moment that the apparent metabolic acceleration is an artefact caused by bias in the volume estimation for the different stages. We approximated the animal's volume by an ellipsoid but their shape is obviously more complex and changes over ontogeny.

The individuals differ only little in the plateau sizes at metamorphosis and adulthood (L_j and L_m , Fig. 3). The duration of growth stasis around metamorphosis (T_g) seems to be more variable, although this parameter was somewhat difficult to establish from the data, as evidenced from the substantial confidence intervals. This analysis shows that the animals stop growing for some 1.5 days around the moult from final naupliar stage N6 to the first copepodite C1. From a bioenergetic viewpoint, a growth stop can only have two mechanistic causes: less resources are taken up (i.e., a reduction in feeding or in assimilation efficiency) or assimilated resources are used for other purposes than production of biomass (e.g., for fuelling metabolic work needed for metamorphosis). This matter may be settled by observations on feeding and respiration rates before, during and after the growth stasis.

3.3. Comparison to other species

Comparing our findings to those for other copepod species is complicated by the tradition of plotting body size versus stage, rather than versus age as needed for bioenergetic modelling. Data for a range of calanoid species, as collected by Mauchline (1998), show that the dry weight increment over the N6 stage is on average low, and even negative in some species. This indicates that a growth stasis in the N6 stage could be more common. In contrast, a growth stop around the N6-C1 moult was not observed in detailed growth analyses for the calanoid copepods *C. sinicus* (Jager et al., 2015) and *C. finmarchicus* (Jager et al., 2017), and also not for the harpacticoid copepod *Nitocra spinipes* (Koch and De Schampheleare, 2019). The lack of growth stasis in some studies makes it unlikely that the metamorphosis itself requires exceptional metabolic expenses in copepods; a reduction or stop in feeding in N6 of *T. brevicornis* is a more likely explanation. This hypothesis is to some extent supported by behavioural observations during the experiments: Heuschele et al. (2019) report that most nauplii remained motionless during the transition to C1 for several hours (but not for the entire no-growth duration). However, it remains unclear why *T. brevicornis*, in contrast to some other species, should show reduced feeding around metamorphosis. This example shows the potential for imaging

individuals at high temporal resolution to address behavioural questions alongside the bioenergetically relevant measurements.

A similar stepwise increase in the growth rate was also seen in *C. finmarchicus*, with a corresponding increase in filtration rates (Jager et al., 2017). Structural body length was estimated from nitrogen content, so uncertainties about shape changes could be excluded. In *C. finmarchicus*, however, the step-up occurred at a later stage (with the start of C2) than in *T. brevicornis*.

3.4. Shape changes over ontogeny

The calculated shape-correction coefficient (Eq. 4) is shown in Fig. 4 as a function of age. The lines show linear regressions for nauplii and copepodites (including adults) separately. Regressions for the shape coefficient could, in principle, be used to translate total body length to volumetric body length, although using measurement of projected area and width to calculate volumetric length is expected to be more accurate (as there may be small shape differences between individuals). The results confirm that *T. brevicornis* changes shape dramatically at the moult from nauplius to copepodite. The shape-correction coefficient drops substantially, which implies more total length for the same amount of body volume in copepodites. This drop in shape coefficient is not caused by flattening, since copepodites were actually less flattened than nauplii (Fig. 1). Also, within the nauplius and copepodite phases, a clear, gradual, elongation over ontogeny was discernible. These changes were significant as the confidence intervals of the regression lines exclude zero. Similar patterns were observed for *N. spinipes* (Koch and De Schampheleare, 2019), but were not obvious for calanoid species (Jager et al., 2017; Jager et al., 2015).

Shape changes imply deviations from isomorphy, and require careful consideration within DEB theory (Kearney and White, 2012). Resource acquisition (i.e., assimilation) is assumed to scale with surface area and hence with L^2 for an isomorph (Jusup et al., 2017), which also underlies our expectation for linear growth. Fig. 4 clearly shows that *T. brevicornis* is not an isomorph, but should this affect our expectations for the scaling of assimilation? Firstly, it is important to realise that this scaling in DEB theory relates specifically to the surface area involved in resource uptake (Kearney and White, 2012), and clearly, copepods do not acquire nutrients through their external surface. It is unclear which specific area is limiting resource acquisition and how it scales with body mass. To shed some light on this matter, we made a rough estimation of external surface area of the copepods (supporting information, Section 4). Interestingly, this analysis showed that the external surface roughly scales with L^2 over ontogeny, despite changes in shape: nauplii are more flattened, while copepodites are more elongated. The observed metabolic acceleration at metamorphosis is thus not accompanied by an obvious

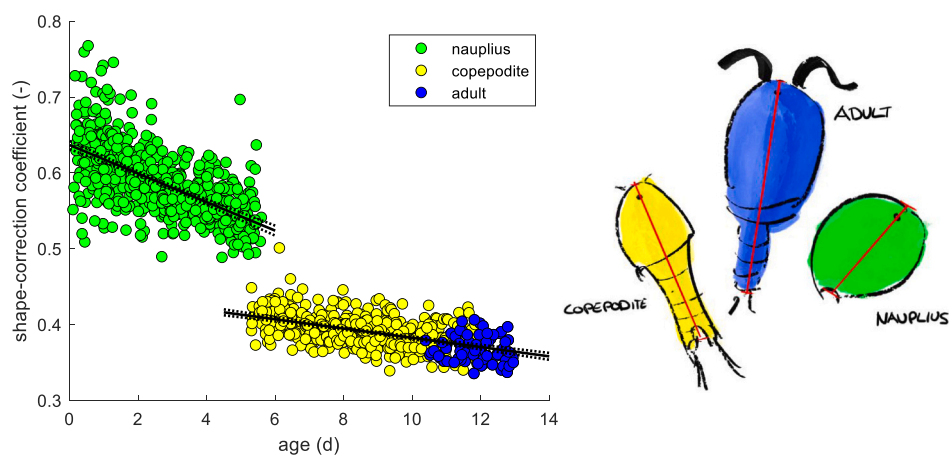


Fig. 4. Shape-correction coefficient (δ_M) over time (volumetric length divided by total body length). Lines are linear regressions with 95% confidence intervals. The drawings to the right illustrate the shape changes over ontogeny in *T. brevicornis*, and show how total body length was determined.

increase in external surface area. This should not come as a surprise; metabolic acceleration also occurs in species without clear shape changes (Kooijman, 2014).

3.5. Relationship between biovolume and biomass

Based on the results in this study, we are confident that we can establish reliable estimates for the biovolume of *T. brevicornis* from images, despite changes in shape over ontogeny. However, to what extent can this volume serve as a proxy for biomass? There is very little information in the literature on dry-weight density of copepods over ontogeny, which likely also relates to experimental difficulties of obtaining reliable wet weights. Yamaguchi and Ikeda (2002) showed that water content of three calanoid copepod species changed over ontogeny, but these changes were related to the utilisation and build-up of lipid stores. Since *T. brevicornis* does not rely on lipids to the same extent, we propose that our estimated biovolume can be used as a proxy for biomass as well.

4. Conclusions and outlook

Bioenergetic analysis of animal life histories requires accurate and precise estimation of biovolume over ontogeny. Automated imaging offers many advantages over destructive size measurements, especially once the time-consuming part of manually taking measures from the images can be replaced by machine learning approaches. Non-destructive imaging allows following individuals over time, with high temporal resolution. This provides a much clearer picture of individual growth trajectories, avoiding potential bias when using the mean response from a variable population (Jager, 2013). Furthermore, following the growth trajectories of multiple individuals provides handles on the nature and extent of inter-individual variation in growth (and thereby variation in underlying energy-budget parameters). A disadvantage of using imaging is that it does not provide a direct estimate of biovolume. This task is specifically non-trivial for animals that drastically change in shape over ontogeny. Here, we approximated copepods by a simple geometric shape, namely the generalised ellipsoid, and biovolume is calculated from the projected area and the animal's height. This is a somewhat crude approximation; images at a higher resolution, and including more height measurements, would be needed to improve this estimation. This could allow, for example, approximation by more complex shapes, such as the combination of an ellipsoid and a cylinder (Araoz, 1991; Napp et al., 1999). However, we show that even with less detailed images we can obtain convincing growth curves; the high temporal resolution tends to average out the measurement errors.

The current analysis showed that the harpacticoid copepod *T. brevicornis* changes shape over ontogeny, most dramatically at the moult from nauplius to copepodite. However, also within the nauplius and copepodite phases, a clear, gradual, change in shape over ontogeny was discernible. This implies that body length is a poor proxy for biomass and should not be used for bioenergetic analyses. Accounting for these changes in shape, *T. brevicornis* grows almost linearly over time on volumetric length basis, which is consistent with previous DEB-based data analyses for copepods (Jager et al., 2017; Jager et al., 2015). However, in contrast with earlier findings, *T. brevicornis* stops growing in the final naupliar stage before metamorphosis to the first copepodite stage. This growth stasis lasts for approximately 1.5 days, after which linear growth resumes, at a higher rate. We are not aware of previous reports on this phenomenon for other copepod species.

The growth cessation at metamorphosis, as well as the different growth rates for nauplii and copepodites, are challenging for DEB-based analysis; the underlying mechanisms would need to be unravelled, and included into a DEB model, before we can fully understand the effects of stressors on life history. If these deviating growth patterns indeed relate to changes in feeding rate, they have the potential to interact with

stressors such as toxicants; DEB models predict synergistic interactions of feeding limitation with the effects of stressors (Zimmer et al., 2012). The transition from N6 to C1 may thus prove to be a specifically sensitive time window in the life cycle of *T. brevicornis*, which we hope to test in future work on multi-stress by combined predation risk and copper exposure. Clarifying the mechanisms underlying the unexpected growth patterns for the pre-adult stages requires determination of additional traits (e.g., respiration and feeding rates). The cessation of growth at adulthood also requires further study since the final moult determines maximum body size, and thereby the feeding and reproduction potential of adult copepods. This second growth stop is unlikely to relate to energetic constraints; adults do feed substantially and fuel egg production from food. It is likely that understanding adult size requires a closer look at the details of the moulting process (see e.g., Talbot et al., 2019; van den Bosch and Gabriel, 1994).

In this study, the individuals do not differ much in their parameter values, and no obvious differences between the sexes were observed. In fact, lumping the data for all individuals yielded a good fit, with parameter estimates that lie within the range of the individual values (see supporting information, Fig. S7 and S8). This is an important result for when we extend the analysis to stressed animals; quantification of stress effects while accounting for inter-individual differences in basic life history is by no means trivial (Jager, 2013). Even though we can use a single set of model parameters for all individuals under control conditions, we may need to consider inter-individual differences for the parameters governing stress responses: previous work showed increased inter-individual variability for development time under stress, and especially under combined stress (Lode et al., 2018).

Our findings will help us refine automated imaging and to design the experimental work needed to develop a fully DEB-based model for the species. This study should also act as a general warning against assuming simple metrics such as body length as unbiased proxies for body size. If we were to plot growth as physical length, we would observe a sudden jump in size at the start of C1, and predict a larger difference in absolute growth rate between nauplii and copepodites (see supporting information, Fig. S2). Such biases have the potential to distort bioenergetic analyses.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.seares.2021.102075>.

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