JPR Advance Access published June 3, 2014

Journal of
Plankton Research

plankt.oxfordjournals.org

J. Plankton Res. (2014) 0(0): 1–9. doi:10.1093/plankt/fbu045

Re-assessing copepod growth using the Moult Rate method

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Received December 5, 2013; accepted April 22, 2014

Corresponding editor: Marja Koski

Estimating growth and production rates of mesozooplankton, and copepods in particular, is important in describing flows of material and energy though pelagic systems. Over the past 30 years, the Moult Rate (MR) method has been used to estimate juvenile copepod growth rates in ~40 papers. Yet the MR method has been shown to have serious flaws. Here we re-examine the results from the majority of published MR method studies and re-estimate growth rates using the modified Moult Rate (MMR) method, which ascribes changes in mass to the appropriate time period over which it was accrued. The MR method has typically over-estimated growth rates (on 80% of occasions) for life stages where the subsequent stage is actively moulting; the median and mean MR values are 138 and 164%, respectively, of the corrected MMR values. We were unable to correct the original data for life stages that are followed by a non-moulting stage, e.g. copepodite stage 5 to adult. We performed experiments with *Calanus pacificus* to estimate growth of stage C5 using an alternative method. We found that the error size and sign varied between mass type (i.e. DW, C and N). Recommendations for practical future assessments of growth in copepods are made.

KEYWORDS: copepod; error; growth; modified Moult Rate

INTRODUCTION

Copepods are a key component of the epipelagic realm, transmitting material to higher trophic levels, including many other crustaceans, gelatinous zooplankton, fish and fish larvae. They recycle nutrients through their excretion (Banse, 1995), and are important grazers on microplankton (Calbet and Saiz, 2005). Growth and production rates of marine copepods have been studied as a means

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of understanding population dynamics, physiological budgets and food web processes (e.g. Durbin *et al.*, 1983; Hopcroft and Roff, 1998; Hirst *et al.*, 1999; Campbell *et al.*, 2001). Growth can be divided into somatic (increase in body mass of individuals) and reproductive types. The latter is the output of reproductive mass, usually assessed as egg production in females [although see Escaravage and Soetaert (1995) for male spermatophore production estimates]. Growth in adults can take both forms (reviewed by Hirst and McKinnon, 2001), but clearly juvenile growth is limited to body mass accumulation (and exuviae loss). Production is determined as the product of mass-specific growth rate and the biomass of a population, and is dependent upon the accurate estimation of growth. Determining growth and production is important because these parameters are central to physiology, energetic budgets and population dynamics and enable determination of energy, carbon and nutrient fluxes.

Unfortunately, it is often the case that growth cannot be measured using natural cohorts; these either cannot be identified (e.g. reproduction in the tropics can be semi-continuous), or are too difficult to follow over time in advective environments. Cohorts have, however, been used in both laboratory settings (Rey-Rassat *et al.*, 2002) and mesocosm experiments (Hygum *et al.*, 2000). As a consequence of the difficulties in the cohort approach, alternate methods have been developed for field application. The two most commonly used to determine juvenile copepod growth are the Moult Rate (MR) and Artificial Cohort (AC) methods. The AC method was adopted by Kimmerer and McKinnon (Kimmerer and McKinnon, 1987) and has since been applied in ~15 studies. The MR method equation was first used in 1982 (published in the same year by both Burkill and Kendall, 1982 and Klein Breteler *et al.*, 1982), and has since been used in ~40 published studies. It has been the most commonly-applied field-based method.

In a major revision of copepod (and mesozooplankton) growth methodology, the MR method was shown to have errors in the equation formulation (Hirst *et al.*, 2005), while the AC method was shown to have important flaws in the equations used, and method of implementation (Kimmerer *et al.*, 2007). It is typically not possible to reconstruct correct growth rates from the data collected for the AC method (Kimmerer *et al.*, 2007), because in many cases the mass determination method is at fault. In contrast, it is possible to correct MR method values for the mass component of the error using the Modified Moult Rate (henceforth MMR) equations where the original mass values for consecutive stages and their durations are available. The original MR equation is typically given as

$$g_{i_\mathrm{MR}} = \ln\left(\frac{W_{i+1}}{W_i}\right) \times \mathrm{MR}_i, \quad (1)$$

where g_{i_MR} was described as the mass-specific growth rate of stage i, W_i is the mean mass of stage i and W_{i+1} the mean mass of stage $i+1$. MR_i is the moult rate (day^{-1}), i.e. the proportion of animals in stage i moulting per day. The inverse of development time (i.e. $1/D_i$) has in many cases been used rather than a measure of moult rate. In most practical circumstances, mean masses have been arithmetic rather than geometric, in part because direct mass measurements have typically required bulking many individuals for a single estimate.

The MMR method correctly ascribes the change in arithmetic mass between two consecutive stages to the correct time period (see Fig. 1). Assuming no mortality, this is given as (Equation 22 in Hirst *et al.*, 2005)

$$\ln\frac{(\mathrm{AW}_{i+1}/\mathrm{AW}_i)}{[(D_{i_\mathrm{actual}} + D_{i+1_\mathrm{actual}})/2]} = g_{i\rightarrow i+1} + \frac{[\ln h_0(g_{i\rightarrow i+1}, D_{i+1_\mathrm{actual}}) - \ln h_0(g_{i\rightarrow i+1}, D_{i_\mathrm{actual}})]}{[(D_i + D_{i+1_\mathrm{actual}})/2]}, \quad (2)$$

where AW is the arithmetic mean mass of the stage and $D_{_\mathrm{actual}}$ is the measured stage duration (not derived indirectly from the moult rate). The function $h_0(g, D_{\mathrm{actual}}) = [\exp(gD_{\mathrm{actual}}/2) - \exp(-gD_{\mathrm{actual}}/2)]/(gD_{\mathrm{actual}})$ measures the deviation from the mass mid-way through the stage.

A third equation, the stage-specific growth equation (Equation 4 in Hirst *et al.*, 2005), estimates growth within an individual stage (i) using the masses at entry to (W_{i_entry}) and exit from (W_{i_exit}) the stage:

$$g_{i_\mathrm{corr}} = \ln\left(\frac{W_{i_\mathrm{exit}}}{W_{i_\mathrm{entry}}}\right) \times \mathrm{MR}_i. \quad (3)$$

Equation (3) can be used in any stage, but is especially useful for the situation where the following stage ($i+1$) does not moult. Figure 1 is a simple representation of these three growth equations, highlighting the error in the original MR method.

Hirst *et al.* (Hirst *et al.*, 2005) approximated the size and sign of MR-derived growth errors in the literature, but as they did not have the original mass and stage durations, they did not attempt to correct historic data. Furthermore, Hirst *et al.* (Hirst *et al.*, 2005) were unable to tightly constrain the size of the error associated with the MR method in copepodite stage 5 (C5) growth estimates, or

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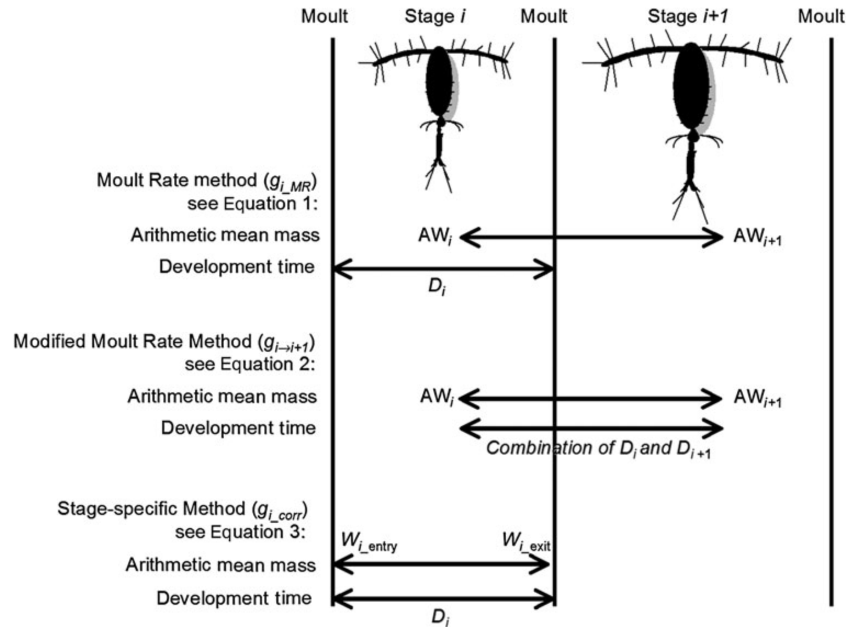

Fig. 1. Graphical representation of methods used to describe growth across moulting stages of copepods. The error in the MR method (Equation 1) is shown by the offset between the change in mass and the period to which the change is ascribed. The MMR method (Equation 2) measures change in mass across consecutive stages ascribed to the correct period. Finally, the stage-specific method determines growth from the change in mass at entry and exit of the stage (Equation 3). In all cases we assume $D_i = 1/\mathrm{MR}_i$.

indeed any stage which is followed by a non-moulting stage (e.g. where the following stage is entering diapause and hence not moulting), because of uncertainty as to the period over which the mass was accumulated post-moult. The MR method implicitly, but unjustifiably, assumes that this period of time is equal to half the duration of the preceding stage. Using a range of scenarios of the mass increase between the mass at moult-to-adult and the mean adult mass (from −50, 0 and +50% of the mass at point of entry), Hirst *et al.* (Hirst *et al.*, 2005) showed that the MR method commonly underestimates the correct growth value in C5. This is a significant drawback, as C5s contribute greatly to non-adult biomass and growth, and are critical to adult supply.

Given this context, the aims of this present paper are to:

(1) re-assess those studies that have used the MR method equation and determine MMR values from original stage durations and mass data;
(2) determine the likelihood that there has been a systematic error in the determination of juvenile copepod growth rates;
(3) experimentally determine the size of the error when applying the MR method to stage C5 copepods of *Calanus pacificus* and describe the main factors causing the error.

METHOD

Literature assessment

Using arithmetic mean masses of consecutive stages and stage durations (including both D_{actual} and D_{MR} stage durations), the MMR method was applied to estimate growth rates for a wide range of studies (see Table I). As field mortality rates were not obtained in these studies, we applied Equation (2) to correct the values. Although mortality can cause a bias when applying mean stage masses to derive growth, the MMR equation does not consider mortality. It is therefore much more practical to apply than alternates that do include this, but is not heavily biased under typical mortality rate scenarios (Hirst *et al.*, 2005). An exception is where stage durations are determined from moult rates of a random population of a stage; in this situation, the moulting rates are biased by mortality (age-within-stage of the sampled animals being skewed by mortality), confounding the overall bias. Stage durations have been reported using a variety of methods in the studies reported here; we have made no corrections or alterations to these.

Although many of these original studies reported growth rates for C5s, the equations applied were incorrect, resulting in errors. The MMR method does not allow values to be retrospectively derived, as the times taken for adults to reach the observed mean mass are

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Table I: Summary of the studies in which MMR values could be obtained using the original data sets

Species	Stages	Condition	Publications
Acartia clausi	N1–C5	Laboratory	Klein Breteler *et al.* (1982)
Acartia clausi	N1–C5	Laboratory	Klein Breteler *et al.* (1994); Klein Breteler and Schogt (1994)
Acartia omorii	N2–C5	Laboratory	Liang and Uye (1996); Uye (1980)
Acartia steurei	N1–C5	Laboratory	Kang and Kang (1998)
Calanoides acutus	C1–C2	Field, South Georgia, Scotia Sea	Shreeve and Ward (1998); Shreeve *et al.* (2002)
Calanus chilensis	C1–C5	Field, Antofagasta, N. Chile	Escribano *et al.* (2001)
Calanus chilensis	C1–C5	Field, Mejillone, N. Chile	Escribano and McLaren (1999)
Calanus sinicus	C1–C5	Laboratory	Uye (1988)
Centropages abdominalis	N1–C5	Laboratory	Liang *et al.* (1996)
Centropages hamatus	N1–C5	Laboratory	Klein Breteler *et al.* (1982)
Eurytemora affinis	N6–C5	Field, Bristol Channel, UK	Burkill and Kendall (1982)
Paracalanus sp.	N2–C5	Laboratory	Uye (1991)
Pseudocalanus elongatus	N1–C5	Laboratory	Klein Breteler *et al.* (1982)
Pseudocalanus elongatus	N1–C5	Laboratory	Klein Breteler *et al.* (1994, 1995); Klein Breteler and Schogt (1994)
Pseudodiaptomus hessei	N2-C5	Laboratory	Jerling and Wooldridge (1991)
Pseudodiaptomus marinus	N2–C5	Laboratory	Uye *et al.* (1983)
Rhincalanus gigas	C2–C3	Field, South Georgia, Scotia Sea	Shreeve and Ward (1998); Shreeve *et al.* (2002)
Sinocalanus tenellus	N1–C5	Laboratory	Kimoto *et al.* (1986)
Temora longicornis	N1–C5	Laboratory	Klein Breteler *et al.* (1982)
Temora longicornis	N1–C5	Laboratory	Klein Breteler and Gonzalez (1986); Klein Breteler *et al.* (1994); Klein Breteler and Schogt (1994)
Calanus agulhensis	N6–C5	Field, Benguela, South Africa	Hutchings *et al.* (1995); Peterson and Hutchings (1995); Richardson and Verheye (1998, 1999)
Calanoides carinatus	N6–C5	Field, Benguela, South Africa	Richardson and Verheye (1998, 1999)
Calanus finmarchicus	C4–C5	Modified field	Diel and Klein Breteler (1986)

Growth of C5 stages were originally determined using the mass of the C5 and adult, and could not be corrected here.

unknown. As a consequence, we were able to obtain comparative values for all reported stages except C5s (and in any studies where the following stage was about to diapause, and hence was not moulting). In the case of the recent applications of the incorrect MR equation by Rey-Rassat *et al.* (Rey-Rassat *et al.*, 2004) and Yebra *et al.* (Yebra *et al.*, 2005), corrections are not possible as the methods were applied across the C5–C6 stages alone. A spreadsheet with the specific details of each study and the data used to make corrections are available online (JPR Supplementary data).

Calanus pacificus experiment

Because stage C5 growth is the most difficult to measure and few accurate measures have yet been made on this stage in the field, we present a practical method to estimate their growth based on Rey-Rassat *et al.* (Rey-Rassat *et al.*, 2002). Measurements require only the C5 development time, and the masses at entry and exit from the stage. To better appreciate errors associated with the MR method when applied to the C5 stage, we conducted an experiment to measure C5 growth and examined results when applying the MR compared with the MMR method.

Study location and sample collection

Late-stage *C. pacificus* were collected at a site with 150-m water depth from the Main Basin of Puget Sound, Washington, USA, during daylight hours in July 2012, using a slow vertical lift of a 335-μm mesh 1-m diameter ring net, equipped with a non-filtering cod end. After collection, contents of the cod end were immediately diluted with seawater and kept cool in large insulated containers for return to the laboratory within 2 h of collection. Forty litres of seawater were collected from 5-m depth at the same location and passed through a 60-μm filter to use in incubations.

Growth rate experiments

Upon return to the laboratory, animals were immediately sorted live by stage. Twenty individuals of each of the stages C5, C6 males and C6 females, and 10 stage C4 copepodites were randomly selected to measure mean dry mass and CHN content. Prosome lengths were also measured, then individuals were placed in small, pre-weighed tin capsules and frozen at −80°C until analysis.

To determine mass-at-entry to each stage, stage C4 and C5 animals were incubated separately in 150-μm filtered seawater in 0.5- or 1-L containers at a density of 2 individuals L^{-1}. A total of 78 stage C4 and 78 stage C5 were incubated in containers kept at 13°C on a 12-h light cycle. After 24 and 48 h, animals were gently poured into large beakers, then into small dishes to check for stage and condition. Those that had moulted to the next stage were recorded, measured and frozen for dry mass and CHN determination. In our study we made no correction for the exuviae lost upon moult. Those that had not moulted after 24 h were gently pipetted into refreshed

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60-μm screened water, which had been collected the previous day and stored at 13°C on a 12-h light cycle. Food concentrations were not measured. Less than 5% mortality occurred during incubations. The proportion of animals that had moulted by the end of the experiment was noted and used to determine stage duration as 1/MR. We note that we chose an incubation based around our knowledge of the species at hand, others applying these methods, for example in warmer waters, or with animals with much faster moult rates will need to modify the approach to suit their conditions (see Kimmerer *et al.*, 2007).

Dry mass and CHN analyses

Dry masses were measured on a Cahn C-31 microbalance after being dried for 24 h in a 55°C oven. C and N were analysed by the University of Washington Marine Chemistry Laboratory using a Leeman Labs Model CEC440 Elemental Analyzer. We present all masses as geometric means for specific stages and arithmetic standard deviations (SD).

RESULTS

Literature assessment

Growth rates for copepods determined using the MMR method should be much more accurate than those previously published using the MR method. Across all of the published values for growth rates we have corrected here, we compare the original MR values to the MMR values and determine the degree to which errors may have been systematic (Fig. 2). Figure 2a and b show that, typically, the corrected MMR-derived values are lower than those originally reported (i.e. the original MR-derived rates are overestimates). There are a few cases, however, where the revised rates are higher than the originals. The median and mean MR-derived values are 138 and 164%, respectively, of the MMR-derived values. There is a caveat to these findings. As noted above, we cannot revise original MR values for C5 stages because the time it takes to pass from the mean field mass of C5 to the mean mass of adult is unknown. In most cases, we anticipate that the original MR estimate will be too low, and hence the sign of the error is the opposite of that for the stages prior to C5.

MR-derived errors are largest in the earliest and the latest moulting stages (Fig. 3). It is clear that stage duration patterns lead to radical divergence in the size of error across taxonomic divisions in growth patterns. Genera which are typically smaller and have stage durations that are close to isochronal (*Acartia*, *Centropages*, *Eurytemora*, *Paracalanus*, *Pseudocalanus*, *Pseudodiaptomus*, *Sinocalanus* and *Temora*) (Fig. 3a) typically show smaller errors compared with *Calanus*, *Calanoides* and *Rhincalanus* (Fig. 3b). Errors are greater in the very earliest stages and the very latest stages. *Calanus*, *Calanoides* and *Rhincalanus*, typically larger genera which have progressively longer stage duration with increasing copepodite stage, show the largest errors. Although the direction of the error (over- or underestimation) is somewhat systematic by life-stage, there are both over and underestimates when considering the whole data set, especially bearing in mind the C5 stages which are missing from Figs 2 and 3.

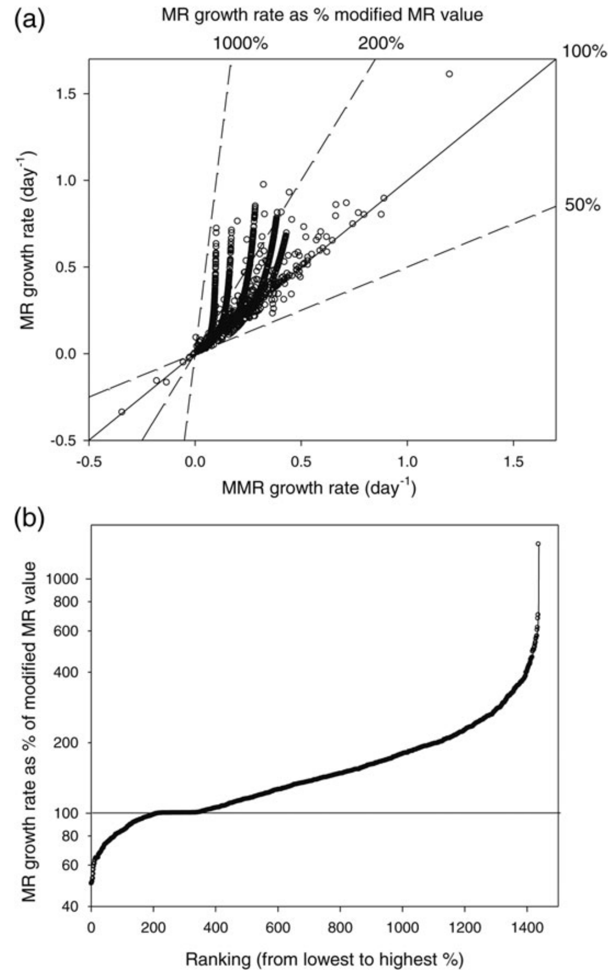

Fig. 2. (**a**) Comparison of original published values of growth (day^{-1}) derived from the MR method versus the MMR method (Equations 1 and 2, respectively). (**b**) MR method values as a percentage MMR values plotted against ranking (lowest to highest %). The horizontal line denotes where MR = MMR growth rates. Note the *y*-axis is logged. The sources of data are described in the Methods. Where the following stage is not moulting, errors have not been determined.

Calanus pacificus experimentation

From the 78 stage C4 and 78 stage C5 *C. pacificus* copepodites incubated for growth, 22 moulted from C4 to C5

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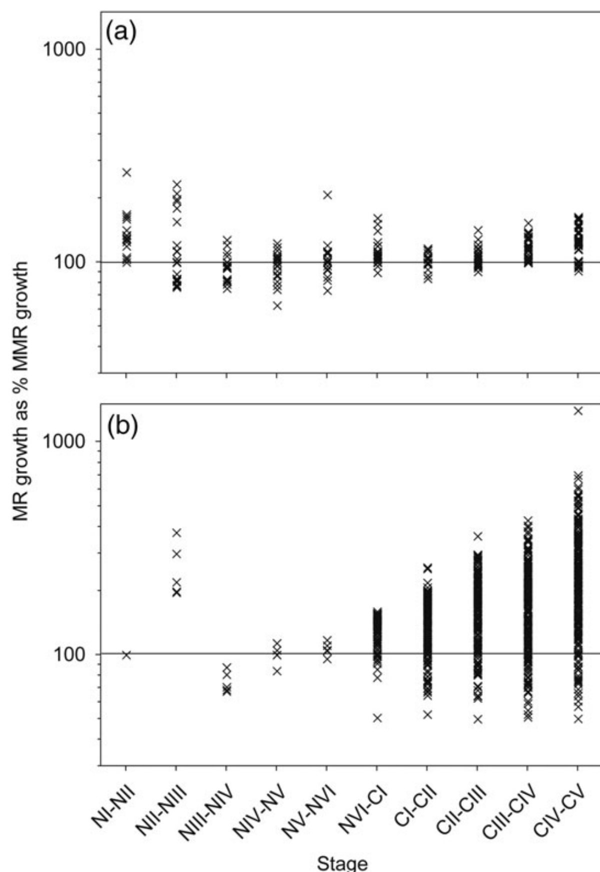

Fig. 3. (**a**) MR method growth values as a percentage of MMR values by stage, separated on the basis of genera with known different life-history trajectories. (**a**) *Acartia*, *Centropages*, *Eurytemora*, *Paracalanus*, *Pseudocalanus*, *Pseudodiaptomus*, *Sinocalanus* and *Temora*. (**b**) *Calanus*, *Calanoides* and *Rhincalanus*. The horizontal lines denote where MR = MMR growth rates. Note the *y*-axis is logged.

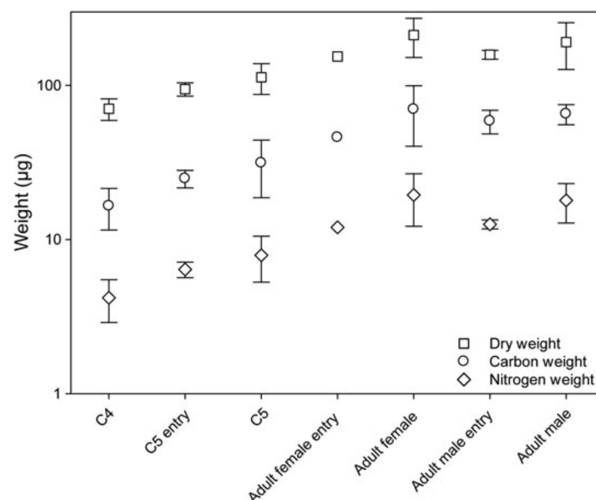

Fig. 4. Geometric mean (± 1 arithmetic SD) dry and carbon masses of *Calanus pacificus* from the experimental work conducted in this study. Stage masses (CIV, CV, female and male adults) are those determined from field-collected animals. The mass at 'entry' and 'exit' are those determined from copepodites which moulted into or from the stage during the course of the incubations. Note the *y*-axis is logged.

and 8 moulted from C5 to adult over the 48-h experiment. This gives C4 and C5 stage durations, estimated by 1/MR, of 7.1 and 19.5 days, respectively. The mean geometric dry mass (±1 SD) ranged from 70.5 µg (±11.27) for field-collected stage C4 copepodites and 112.9 µg (±25.53) for field-collected C5 copepodites, to 212.2 µg (±60.34) for field-collected adult females (Fig. 4). Moulters into stage C5 averaged 94.5 µg (±9.46). In units of carbon, the mean geometric mass for field-collected C5 copepodites was 31.4 µg C (±12.71); moulters into stage C5 were 24.8 µg C (±3.21), while those exiting C5 into C6 averaged 56.7 µg C (±10.61), the adult females from the field had a mass of 69.9 µg C (±29.65), while for adult males this was 65.26 µg C (±9.70).

Using dry mass, stage C5 growth rates from the experiments were estimated using the incorrect MR method to be 0.029 day^{-1}, while the correct value, calculated using the stage-specific method g_{i_corr} (see Fig. 1) was 0.026 day^{-1}. This corresponds to a 12% overestimate when applying the MR method. Using carbon mass, stage C5 growth rate using the MR was 0.038 day^{-1}, while the correct g_{i_corr} value was 0.042 day^{-1}, corresponding to a 10% underestimate by the MR method. Using nitrogen, stage C5 growth rate using the MR was 0.043 day^{-1}, while the correct g_{i_corr} value was 0.034 day^{-1}, corresponding to a 26% overestimate using the incorrect MR method. There is a marked difference in growth and error size between methods and depending upon the type of mass examined (i.e. DW, C and N).

DISCUSSION

We have revised many published copepod growth rates by correctly applying mass change between stages to the appropriate stage duration. Our results demonstrate that, in most cases, the $g_{i\rightarrow i+1}$ estimates from the MMR method are lower than g_i estimates from the MR method. This agrees with predictions by Hirst *et al.* (Hirst *et al.*, 2005) because later (older) stages tend to have a longer duration (see Hart, 1990; Peterson, 2001), where $Di/Di{+}1 < 1$, growth rates will be overestimated using the MR method. There is no absolute limit to the degree to which the MR method can overestimate. Indeed, these patterns are well shown by comparing the different groups of genera between Fig. 3a and b.

We are unable to assess the degree of error in previously published MR values for the C5 stages because the necessary information to correct these was not available.

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We can, however, demonstrate the potential error from our own experiments on *C. pacificus*. Had we determined growth using the original MR method, values would have been 112% of the g_{i_corr} rates by dry mass, 90% by carbon mass and 126% using nitrogen mass, i.e. both under- and overestimation would have occurred. The actual size of these errors is in the low range of predictions of Hirst *et al*. (Hirst *et al*., 2005). This is because C5 growth rates were slow and stage durations long, even for *Calanus* (e.g. Vidal, 1980a,b), and the field adult masses were only slightly higher than at entry, indicating a possible dominance by young members in the stage. The only other direct measurements of errors in the C5 stage are presented by Rey-Rassat *et al*. (Rey-Rassat *et al*., 2002). Using laboratory-reared cohorts of *Calanus finmarchicus*, Rey-Rassat *et al*. (Rey-Rassat *et al*., 2002) estimated growth through application of both the MR method (what they term Method I), and mass at entry and exit and duration of C5 [their Method II, Equation 3 herein]. MR growth values for C5 as a percentage of the correct growth value ranged between 30 and 65% for two cohorts when growth was measured in units of carbon mass and from 102 to 149% in nitrogen mass. In our study and that of Rey-Rassat *et al*. (Rey-Rassat *et al*., 2002), therefore, the growth rate calculated using carbon led to an underestimate when applying the MR method, and an overestimation when based on nitrogen. The difference between these likely arises because of differences in the rate of accumulation of structural (e.g. protein) and non-structural (lipid) mass. This suggests that because of the variety of issues at stake, and especially the amount (and type) of mass that may be accumulated in the adult stage, the size of the errors associated with the historic use of the MR method for the C5 stage cannot be easily constrained. These studies both used a large lipid-storing species within the genus *Calanus*, clearly other species and genera may differ. Future work on genera with very different C5 growth processes and mass trajectories, including genera such as *Acartia*, are needed.

Most growth rate methods ultimately measure population averages, changes in mean weight across stages or through stage duration from moult rates of a group of individuals. One clear limitation is that these allow no expression of individual variability in growth or development rates. Achieving this still seems impractical in the majority of field studies, and this is a shortfall. Such methods can however be used in the laboratory under controlled conditions, and some insights will be gained by developing approaches in the laboratory, with the hope that they can eventually be taken to the field.

Our results have important implications: results using the Moult Rate method dominate field-based estimates of copepod growth and so have largely contributed to our understanding of growth and production of mesozooplankton. Yet there are sizeable errors in these values. The MR method has typically over-estimated growth rates, often by >2-fold, and at times by >4-fold, for stages prior to C5. For the C5 stage we demonstrate that the sign and size of the error varies by mass type, of course the case may also be true of other stages. Given the errors associated with the method that we have highlighted here, results of computer and empirical models dependent upon these growth rates will be less exact (e.g. Hirst and Bunker, 2003). More measurements of copepod growth are needed to quantify their role in the ecosystem and to parameterize such terms in ecosystem models. We must also look to develop methods further to consider individual variability, mortality and the biases these can cause with some methods where we only measure rates on surviving animals.

Researchers wishing to determine growth rates of copepods in nature are recommended to apply the two alternate methods, either stage-specific or across stage pairs (Hirst *et al*., 2005; see Fig. 1). These methods present a practical approach and need only a little extra data and effort than was needed for the MR method. One unfortunate aspect is the typical inability to include mortality in the calculations. This can be overcome when using weight at entry, weight and exit, and stage duration estimated by following individuals through the entire stage rather than from moult rates. We have applied such weight change methods here to *C. pacificus*, although still found the need to rely upon moult rates to assess stage duration. For species with stage durations greater than 1 day, it is very difficult to obtain field-based estimates by following individuals through full stages to assess durations. The degree to which the ideal set of measurements can be achieved in the field will vary from species to species, area to area and the sheer amount of effort that can be applied. Our use of moult rates to determine stage duration is far from ideal (see Hirst *et al*., 2005 for a description of the error associated with this), but we have a practical limitation here, longer incubations, even with replenishment of food resulted in clear change in moult frequency. Our approach is certainly possible on-board ship, but was only conducted for one stage at one site. Any experimental protocol requires consideration of the balance between effort and accuracy; researchers must bear in mind the shortfalls present in some growth methodologies when considering the required accuracy of the estimates they desire. New approaches for measuring zooplankton production using biochemical materials and enzyme activity (i.e. biochemical-based approaches) have been developed and explored (e.g. Oosterhuis *et al*., 2000; Sastri and Roff, 2000; Wagner *et al*., 2001; Yebra and Hernández-León, 2004; Gusmão and McKinnon, 2011).

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The development of biochemical or other proxies to growth are progressing, and may eventually become a more efficient way to determine growth across large suits of copepods and other zooplankton species and to increase spatio-temporal coverage or resolution. Nonetheless, we are still likely to need direct mass-based approaches in order to calibrate and compare such methods against. As such it is imperative that these direct methods are also improved and corrected where possible, including the improvements suggested here, as well as those described for the Artificial Cohort method (see Kimmerer *et al*., 2007).

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

ACKNOWLEDGEMENTS

We wish to thank the great many authors who donated their hard-earned data to this study. We thank Wim Klein-Breteler, Shin-Ichi Uye and the anonymous reviewers, whose comments greatly helped improve this work.

FUNDING

A.G.H. was funded by the School of Biological and Chemical Sciences, Queen Mary University of London. J.E.K. was funded by the School of Oceanography, University of Washington, Seattle.

REFERENCES

Banse, K. (1995) Zooplankton: pivotal role in the control of ocean productivity. *ICES J. Mar. Sci.*, **52**, 265–277.

Burkill, P. H. and Kendall, T. F. (1982) Production of the copepod *Eurytemora affinis* in the Bristol Channel. *Mar. Ecol. Prog. Ser.*, **7**, 21–31.

Calbet, A. and Saiz, E. (2005) The ciliate-copepod link in marine ecosystems. *Aquat. Microb. Ecol.*, **38**, 157–167.

Campbell, R. G., Wagner, M. M., Teegarden, T. J. *et al*. (2001) Growth and development rates of the copepod *Calanus finmarchicus* reared in the laboratory. *Mar. Ecol. Prog. Ser.*, **221**, 161–183.

Diel, S. and Klein Breteler, W. C. M. (1986) Growth and development of *Calanus* spp. (Copepoda) during spring phytoplankton succession in the North Sea. *Mar. Biol.*, **91**, 85–92.

Durbin, E. G., Durbin, A. G., Smayda, T. J. *et al*. (1983) Food limitation of production by adult *Acartia tonsa* in Narragansett Bay, Rhode Island. *Limnol. Oceanogr.*, **28**, 1199–1213.

Escaravage, V. and Soetaert, K. (1995) Secondary production of the brackish copepod communities and their contribution to the carbon fluxes in the Westerschelde Estuary (the Netherlands). *Hydrobiologia.*, **311**, 103–114.

Escribano, R., Marin, V. H. and Hidalgo, P. (2001) The influence of coastal upwelling on the distribution of *Calanus chilensis* in the Mejillones Peninsula (northern Chile): implications for its population dynamics. *Hydrobiologia.*, **453/454**, 143–151.

Escribano, R. and McLaren, I. (1999) Production of *Calanus chilensis* in the upwelling area of Antofagasta, northern Chile. *Mar. Ecol. Prog. Ser.*, **177**, 147–156.

Gusmão, L. F. M. and McKinnon, A. D. (2011) Nucleic acid indices of egg production in the tropical copepod *Acartia sinjiensis*. *J. Exp. Mar. Biol. Ecol.*, **396**, 122–137.

Hart, R. (1990) Copepod post-embryonic durations: patterns, conformity, and predictability. The realities of isochronal and equiproportional development, and trends in the copepodid-naupliar duration ratio. *Hydrobiologia*, **206**, 175–206.

Hirst, A. G. and Bunker, A. J. (2003) Growth of marine planktonic copepods: Global rates and patterns in relation to chlorophyll a, temperature, and body weight. *Limnol. Oceanogr.*, **48**, 1988–2010.

Hirst, A. G. and McKinnon, A. D. (2001) Does egg production represent adult female copepod growth? A call to account for body weight changes. *Mar. Ecol. Prog. Ser.*, **223**, 179–199.

Hirst, A. G., Peterson, W. T. and Rothery, P. (2005) Errors in juvenile copepod growth rate estimates are widespread: problems with the Moult Rate method. *Mar. Ecol. Prog. Ser.*, **296**, 263–279.

Hirst, A. G., Sheader, M. and Williams, J. A. (1999) Annual pattern of calanoid copepod abundance, prosome length and minor role in pelagic carbon flux in the Solent, UK. *Mar. Ecol. Prog. Ser.*, **177**, 133–146.

Hopcroft, R. R. and Roff, J. C. (1998) Zooplankton growth rates: the influence of female size and resources on egg production of tropical marine copepods. *Mar. Biol.*, **132**, 79–86.

Hutchings, L., Verheye, H. M., Mitchell-Innes, B. A. *et al*. (1995) Copepod production in the southern Benguela system. *ICES J. Mar. Sci.*, **52**, 439–455.

Hygum, B. H., Rey, C., Hansen, B. W. *et al*. (2000) Rearing cohorts of *Calanus finmarchicus* (Gunnerus) in mesocosms. *ICES J. Mar. Sci.*, **57**, 1740–1751.

Jerling, H. L. and Wooldridge, T. H. (1991) Population dynamics and estimates of production for the calanoid copepod *Pseudodiaptomus hessei* in a warm temperate estuary. *Estuarine, Coastal and Shelf Sci.*, **33**, 121–135.

Kang, H. K. and Kang, Y. J. (1998) Growth and development of *Acartia steueri* (Copepoda: Calanoida) in the laboratory. *J. Korean Fish. Soc.*, **31**, 842–851.

Kimmerer, W. J., Hirst, A. G., Hopcroft, R. R. *et al*. (2007) Estimating juvenile copepod growth rates: corrections, inter-comparisons and recommendations. *Mar. Ecol. Prog. Ser.*, **336**, 187–202.

Kimmerer, W. J. and McKinnon, A. D. (1987) Growth, mortality, and secondary production of the copepod *Acartia tranteri* in Westernport Bay, Australia. *Limnol. Oceanogr.*, **32**, 14–28.

Kimoto, K., Uye, S.-I. and Onbé, T. (1986) Growth characteristics of a brackish-water calanoid copepod *Sinocalanus tenellus* in relation to temperature and salinity. *Bull. Plankton Soc. Japan*, **33**, 43–57.

Klein Breteler, W. C. M., Fransz, H. G. and Gonzalez, S. R. (1982) Growth and development of four calanoid copepod species under experimental and natural conditions. *Neth. J. Sea Res.*, **16**, 195–207.

Klein Breteler, W. C. M. and Gonzalez, S. R. (1986) Culture and development of *Temora longicornis* (Copepoda, Calanoida) at different conditions of temperature and food. *Syllogeus*, **58**, 71–84.

Klein Breteler, W. C. M., Gonzalez, S. R. and Schogt, N. (1995) Development of *Pseudocalanus elongatus* (Copepoda, Calanoida)

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cultured at different temperature and food conditions. *Mar. Ecol. Prog. Ser.*, **119**, 99–110.

Klein Breteler, W. C. M. and Schogt, N. (1994) Development of *Acartia clausi* cultured at different conditions of temperature and food. *Hydrobiol.*, **292/293**, 469–479.

Klein Breteler, W. C. M., Schogt, N. and van der Meer, J. (1994) The duration of copepod life stages estimated from stage frequency data. *J. Plankton Res.*, **16**, 1039–1057.

Liang, D. and Uye, S. (1996) Population dynamics and production of the planktonic copepods in a eutrophic inlet of the Inland Sea of Japan. II. *Acartia omorii*. *Mar. Biol.*, **125**, 109–117.

Liang, D., Uye, S. and Onbe, T. (1996) Population dynamics and production of the planktonic copepods in a eutrophic inlet of the Inland Sea of Japan. I. *Centropages abdominalis*. *Mar. Biol.*, **124**, 527–536.

Oosterhuis, S. S., Baars, M. A. and Klein-Breteler, W. C. M. (2000) Release of the enzyme chitobiase by the copepod *Temora longicornis*: characteristics and potential tool for estimating crustacean biomass production in the sea. *Mar. Ecol. Prog. Ser.*, **196**, 195–206.

Peterson, W. T. (2001) Patterns in stage duration and development among marine and freshwater calanoid and cyclopoid copepods: a review of rules, physiological constraints, and evolutionary significance. *Hydrobiologia*, **453/454**, 91–105.

Peterson, W. T. and Hutchings, L. (1995) Distribution, abundance and production of the copepod *Calanus agulhensis* on the Agulhas Bank in relation to spatial variation in hydrography and chlorophyll concentration. *J. Plankton Res.*, **17**, 2275–2294.

Rey-Rassat, C., Bonnet, D., Irigoien, X. *et al*. (2004) Secondary production of *Calanus helgolandicus* in the western English Channel. *J. Exp. Mar. Biol. Ecol.*, **313**, 29–46.

Rey-Rassat, C., Irigoien, X., Harris, R. *et al*. (2002) Growth and development of *Calanus helgolandicus* reared in the laboratory. *Mar. Ecol. Prog. Ser.*, **238**, 125–138.

Richardson, A. J. and Verheye, H. M. (1999) Growth rates of copepods in the southern Benguela upwelling system: The interplay between body size and food. *Limnol. Oceanogr.*, **44**, 382–392.

Richardson, A. J. and Verheye, H. M. (1998) The relative importance of food and temperature to copepod egg production and somatic growth in the southern Benguela upwelling system. *J. Plankton Res.*, **20**, 2379–2399.

Sastri, A. R. and Roff, J. C. (2000) Rate of chitobiase degradation as a measure of development rate in planktonic Crustacea. *Can. J. Fish. Aquat. Sci.*, **57**, 1965–1968.

Shreeve, R. S. and Ward, P. (1998) Moulting and growth of the early stages of two species of Antarctic calanoid copepod in relation to differences in food supply. *Mar. Ecol. Prog. Ser.*, **175**, 109–119.

Shreeve, R. S., Ward, P. and Whitehouse, M. J. (2002) Copepod growth and development around South Georgia: relationships with temperature, food and krill. *Mar. Ecol. Prog. Ser.*, **233**, 169–183.

Uye, S.-I. (1980) Development of neritic copepods *Acartia clausi* and *A. steueri*. *Bull. Plankton Soc. Japan*, **27**, 11–18.

Uye, S.-I. (1988) Temperature-dependent development and growth of *Calanus sinicus* (Copepoda: Calanoida) in the laboratory. *Hydrobiol.*, **167/168**, 285–293.

Uye, S.-I. (1991) Temperature-dependent development and growth of the planktonic copepod *Paracalanus* sp. in the laboratory. *Proc Fourth Int Conference Copepoda Bull Plankton Soc Jap, Spec Vol*, pp. 627–636.

Uye, S., Iwai, Y. and Kasahara, S. (1983) Growth and production of the inshore marine copepod *Pseudodiaptomus marinus* in the central part of the Inland Sea of Japan. *Mar. Biol.*, **73**, 91–98.

Vidal, J. (1980a) Physioecology of zooplankton. I. Effects of phytoplankton concentration, temperature, and body size on the growth rate of *Calanus pacificus* and *Pseudocalanus* sp. *Mar. Biol.*, **56**, 111–134.

Vidal, J. (1980b) Physioecology of zooplankton. II. Effects of phytoplankton concentration, temperature, and body size on the development and molting rates of *Calanus pacificus* and *Pseudocalanus* sp. *Mar. Biol.*, **56**, 135–146.

Wagner, M. M., Campbell, R. G., Boudreau, C. A. *et al*. (2001) Nucleic acids and growth of *Calanus finmarchicus* in the laboratory under different food and temperature conditions. *Mar. Ecol. Prog. Ser.*, **221**, 185–197.

Yebra, L., Harris, R. P. and Smith, T. (2005) Comparison of five methods for estimating growth of *Calanus helgolandicus* later development stages (CV-CVI). *Mar. Biol.*, **147**, 1367–1375.

Yebra, L. and Hernández-León, S. (2004) Aminoacyl-tRNA synthetases activity as a growth index in zooplankton. *J. Plankton Res.*, **26**, 351–356.

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