EXPLORING THE MECHANISM OF HOW
ECTOTHERMS CHANGE SIZE WITH CHANGING
TEMPERATURE

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ABSTRACT

The “temperature-size rule” (TSR) describes the intraspecific, phenotypically plastic response of ectotherm body size to temperature: individuals take longer to mature at cooler temperatures but do so at a larger size. The TSR is ubiquitous, affecting >83% of organisms in which it has been studied. This suggests a fundamental physiological mechanism underpinning the TSR which requires explanation. Additionally, with increasing global temperatures it is vital that we understand how this will impact on body size. Currently, we lack a description of how size changes occur, and its ontogenetic basis. Using a simple conceptual model, it is shown that adult: progeny mass determines growth and development rates which drive the TSR. Adult size changes more than progeny size in acclimated metazoans. Conversely, due to the constraints of binary fission, these changes are equal in acclimated unicells. This suggests that how rates decouple is fundamentally different in uni- and multicellular organisms: the acclimated rates are not decoupled in unicellular organisms at different temperatures but are in multicellular organisms. This is supported with a multilevel analysis of data for over 30 copepod species across multiple life stages. Experimental data for the crustacean *Artemia franciscana* shows temperature-size changes to increase during ontogeny; data for multiple crustacean species supports this outcome, with the temperature-size response becoming more negative through ontogeny. Experimental examination of the TSR during thermal acclimation in the ciliate *Cyclidium glaucoma* supports the conceptual model: growth and development are temporarily decoupled for approximately one generation. Finally, I move from investigating proximate to ultimate mechanisms. I examine the primary hypotheses used to explain why the TSR is near-universal. This analysis highlights that environment type (i.e. aquatic or terrestrial) and organism size are major determinants of the size of the response, suggesting the TSR is adaptive and exists to maintain aerobic scope in ectotherms.
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CHAPTER 1

General Introduction

Changes in species’ body size have been described as the “third ecological rule” with regard to global warming and climate change, along with ecological changes in phenology and shifts in species ranges (Daufresne et al. 2009). One of the processes driving size changes with temperature has been termed the “temperature-size rule” (TSR, Atkinson 1994). The TSR is an intraspecific, phenotypic effect: individuals reared at cooler temperatures are larger at maturity than those reared at warmer temperatures (Atkinson 1994). These effects have been recorded in over 80% of those species in which it has been investigated, including bacteria, protists, insects, crustaceans and ectothermic vertebrates (Atkinson 1994, Atkinson et al. 2001, Atkinson et al. 2003). Furthermore, these effects can be substantial. For example, the crustacean Pseudocalanus newmani has been shown to be twice as large when reared at colder temperatures (3°C) than at warmer temperatures (15°C, Lee et al. 2003).

Body size is fundamental to the functioning of individuals, species, communities and ecosystems. Changes in adult size impacts on many other important traits, including growth, reproduction and mortality (Kingsolver and Huey 2008). Further, body size is an important determinant of food web dynamics (Woodward and Warren 2007, Barnes 2008). Given the increase in the duration and strength of short-term temperature fluctuations (e.g. heat waves, IPCC 2007), it is vital that we understand how species body size will change with changing temperature, and whether species from different taxa and environments have similar temperature-size responses.
Aside from being important within the context of climate change, the TSR also suggests important differences in fundamental life history rates. For size to change at maturity, rates controlling growth (mass increase) must differ from those controlling ontogeny (development). This important difference is often overlooked in ecological theory (Gillooly et al. 2002, Brown et al. 2004).

Despite its ubiquity, the TSR remains poorly understood. There are multiple independent theories (Fischer and Fiedler 2002, Angilletta and Dunham 2003, Atkinson et al. 2006) which attempt to explain why size changes with temperature, yet none of these have received universal support. These theories focus on finding an adaptive explanation for the TSR, i.e. why size changes increase species fitness. However, one of the main reasons why we still lack an adaptive explanation for the TSR is that there has been little focus on how size changes are actually brought about. We do not know how the rates underpinning the rule (growth and development) become decoupled and whether this mechanism is universal, when in the life cycle of organisms size changes occur, nor if there are similarities in the establishment of the TSR across different groups. Further, although a quantitative analysis of the strength of the TSR has been undertaken in protists (Atkinson et al. 2003), no quantitative study of the TSR exists for Metazoa. Without a fuller description of how size changes are brought about, it is not possible to confidently determine why the TSR occurs.

To understand how the TSR is brought about, we first need an appropriate conceptual model to describe how size changes. Adult mass \((m)\) is dependent upon progeny (e.g. egg) mass \((m_0)\), growth \((g, \text{ mass change day}^{-1})\), and development rate \(D \text{ (day}^{-1})\). Assuming linear growth (van der Have and de Jong 1996) this is described as:
This equation, or variants upon it (e.g. those which consider growth to be exponential or to have a von Bertalanffy form), are commonly used to describe species growth rates. Historically, the TSR has focused on size change in the adult stage of metazoans. However, Equation 1.1 makes it clear that we will only appreciate how adult changes are brought about by also considering progeny size.

Beyond examining changes in mass with temperature, we need to establish how growth and development impact adult size changes (Equation 1.1). The temperature dependence of these fundamental rates has been established for some individual ectothermic species (Smith-Gill and Berven 1979, Davidowitz and Nijhout 2004), but we lack a quantitative assessment of the temperature dependence of these rates across a range of species. Determining the differences between these rates will provide insight into how these rates decouple and drive size changes with temperature. Further, examining these rates through the life cycle is necessary to understand when in the life cycle these rates decouple.

Following on from establishing where rates decouple, we need to determine where in ontogeny size changes occur. Mortality rates are significantly higher during early stages of many species (McConaughha 1992, Cornell and Hawkins 1995, Hirst and Kiorboe 2002). This is, therefore, an intense period of selection with important fitness consequences for species. Further, common ontogenetic patterns of size change may help to identify the drivers of the TSR. Previous investigation of the proximate mechanism of the TSR assumes temperature-size responses to be of similar magnitude.
throughout ontogeny in acclimated species (van der Have and de Jong 1996). This thesis experimentally examines size changes through ontogeny to determine when the TSR is established, and whether this is common to all ectotherms.

The TSR is a phenotypic plastic response. Despite this, historical size constraints may prevent size changes being completed within a single generation. Without understanding the impact of multi-generational acclimation, we do not know whether size changes are completed rapidly or require multiple generations. Investigating the parameters outlined in Equation 1.1 over multiple generations would provide an insight into whether size changes are reduced, maintained or increased with multi-generational acclimation, and how this is driven by growth and development rates. This would provide important insight into longer term impacts of temperature on species size.

We are therefore currently lacking key information in understanding how size changes with temperature in ectothermic species. We do not know whether the mechanism of establishing size change is similar across different ectotherms, where in the life cycle size changes occur or whether acclimation is important in determining the strength of the TSR. By establishing the answers to these important questions we should be able to determine whether a universal proximate mechanism for the TSR exists. This will greatly further the work of the TSR, pointing towards an adaptive explanation, which can subsequently be the focus of further study. This thesis therefore aims to answer the following questions:

1. Are the mechanisms of temperature-size change in progeny and adult masses similar in uni- and multicellular organisms? (Chapter 2)
2. How do the rates underpinning the TSR, growth and development, change with changing temperature? (Chapter 3)

4. How are size changes established during ontogeny in uni vs. multicellular organisms, and how does acclimation over multiple generations affect the strength of the TSR in both groups? (Chapter 4 + 5)

Having addressed these questions in the thesis, Chapter 6 is dedicated to a quantitative analysis of the TSR, to determine whether an ultimate mechanism for the TSR can now be identified.
CHAPTER 2

How do organisms change size with changing temperature? The importance of reproductive method and ontogenetic timing

Introduction

Body size is fundamental to the functioning of all organisms, impacting on all aspects of life including growth, reproduction and mortality (Kingsolver and Huey 2008). Therefore understanding what drives species body size is a critical aspect of ecology. One widespread pattern of body size in ectothermic organisms is the ‘temperature-size rule’ (TSR). The TSR refers to how, within a species, lower rearing temperatures leads to increased size at a given developmental stage (Atkinson 1994). Changes in size have been described as the “third universal ecological response to global warming” (Daufresne et al. 2009). A feature of current climate change is the predicted increase in frequency and intensity of heat-waves (IPCC 2007); therefore, understanding how organisms will respond to increasing temperature in the short and longer-term, and the mechanisms underpinning these responses, is critical.

Growing to a smaller final size at warmer temperatures seems counterintuitive and has been termed a ‘life-history puzzle’ (Sevenster 1995). One might expect that as organisms have faster growth rates at higher temperatures, they should delay maturation to exploit the increase in fecundity, survival and mating success associated with larger size (Sibly and Atkinson 1994, Kingsolver and Huey 2008). Indeed, increased growth rate associated with improved food conditions results in larger adults, whereas the increased growth rate associated with higher temperature results in reduced adult size
(Kindlmann et al. 2001). Attempts to explain why temperature differences result in body size changes often consider the problem with respect to the maximization of fitness (specific growth rate, \( r \), and offspring production, \( R_0 \)), and include the interplay of multiple traits such as growth, fecundity, development and mortality (Sibly and Atkinson 1994, Kozłowski et al. 2004, Kiørboe and Hirst 2008). Rather than focus on why the TSR occurs, others have instead focused on the question of how body size changes. For example, Davidowitz and Nijhout (2004) formulated a physiological (endocrine-based) model for holometabolous insects. However, we still lack a general model to explain the TSR (Angilletta et al. 2004): such a general model would account for differences across taxa, changes in size during ontogeny and changes in size across generations. Here, we will explore how critical differences between methods of reproduction, and in growth and developmental responses to temperature between different ontogenetic stages, affect attempts to derive a universal mechanistic TSR model.

*How does body size change?*

The TSR indicates that when juveniles grow in cooler environments they develop into larger adults (Atkinson 1994); consequently, although the rate of development (passing through life stages) and growth (accumulation of mass) from embryo to adult both decrease with decreasing temperature, there must be a relatively larger decrease in the development rate. Although this seems obvious, previous general models of how size changes with temperature have often not explicitly indicated that these two rates are decoupled (e.g. explanations based on the von Bertalanffy growth equation (von Bertalanffy 1957, Perrin 1995)).
Van der Have and de Jong (1996) argued that the TSR must be a result of mismatch in the temperature dependence of growth rate and development rate (which they use synonymously with differentiation rate). They then built a biophysical model, assuming development and growth rates to have independent thermal reaction norms under non-limiting food conditions. Van der Have and de Jong (1996) state, “As a proximate model, the biophysical model applies to all ectotherms, including protists in which ‘differentiation’ consists only of cell divisions.” Van der Have and de Jong (1996) also suggest that progeny size may be impacted by different rates of differentiation and growth of oocytes (e.g. vitellogenin synthesis in insects; Ernsting and Isaaks (1997)). From this assumption, they argued that the same effect of temperature on oocyte size as on the size at metamorphosis could be predicted; that is, larger eggs will be produced at lower environmental temperature.

Van der Have and de Jong (1996) made an important advance in analysing the TSR by explicitly treating growth and development as separate rates. However, any general mechanistic model needs to explain fundamental differences in the establishment of the TSR in different organisms. In this study, we show how reproduction by binary division (i.e. cell dividing into two equally sized progeny) results in fundamental differences in the operation of the TSR between unicellular and multicellular organisms. Secondly, we perform a meta-analysis to measure the effects of temperature on adult vs. progeny size in Metazoa, and show that progeny size in multicellular organisms does not follow the same response as adult size. Thirdly, we explore size responses to temperature across ontogeny in multicellular organisms, to examine whether these organisms exhibit systematic changes in size throughout the whole life cycle or whether size responds mostly during specific stages after which these changes are maintained. Analysis of
shifts in size throughout the life cycle and across generations will yield a more complete quantitative description of how, and potentially provide clues to why, body size responds to temperature.

We specifically address the following questions:

1) How do constraints of a unicellular vs. multicellular life cycle affect the adjustments of size, and therefore growth and development associated with the TSR?

2) Does the TSR affect adult and progeny mass of multicellular organisms equally?

3) Does the TSR have consistent effects throughout ontogeny?

The Conceptual Model

We construct a model building on the linear equation used by van der Have and de Jong (1996) to link growth and development with adult and progeny size:

\[ g = \frac{(M_A)}{t} \]

Where \( g \) = mean juvenile mass-specific growth rate (day\(^{-1}\)), \( t \) = development time (days, e.g. egg hatch to maturation, or time between subsequent divisions in unicellular organisms), \( M_A \) = mass of adult, and \( M_P \) = mass of a single progeny. We use the term “progeny” to refer to young at the point of inception. This is the daughter cell just after binary division of the mother in a unicellular organism, or the newly produced egg, or the propagule at the point of budding in a multicellular organism. Using the inverse of development time \( t \) in Equation 2.1 converts this parameter to a mean rate of
development, \( D (D = 1/t) \). Individual growth from progeny to adult can then be expressed as:

\[
\frac{g}{D} = \frac{M_A}{M_P}
\]  

(Equation 2.2)

Thus, examining the ratio of adult to progeny mass provides a straightforward way to determine the effect of temperature on two fundamental biological rates (growth and development rate) and to test van der Have and de Jong’s (1996) hypothesis: that development and growth rates have independent thermal reaction norms under non-limiting food conditions. We consider the implications of this growth equation to the TSR in unicellular and multicellular organisms.

**Unicellular Organisms**

Most unicellular organisms reproduce by binary division (Adolph 1931), a term we use to encapsulate binary fission in prokaryotes, and mitosis in unicellular eukaryotes. In binary division an ‘adult’ cell (of mass \( M_A \)) divides into two ‘daughter’ cells (of mass \( M_P \)), each with a mass half that of the adult, i.e. \( M_A = 2M_P \). Thus at a fixed temperature across generations, and with other conditions constant, \( M_A/M_P = 2 \), Equation 2.2 then simplifies to:

\[
\frac{g}{D} = 2
\]  

(Equation 2.3)

At a fixed temperature unicellular organisms must have a fixed ratio of growth to development rate; thus, referring to Figure 2.1, \( g/D(\text{cold}) = g/D(\text{warm}) = 2 \). This is in clear disagreement with the assumption of the van der Have and de Jong model, that development and growth rates have independent thermal reaction norms under non-
Figure 2.1. Hypothetical example of the effect of temperature change on a unicellular organism that adheres to the TSR. At a cold temperature, the ratio of adult to progeny mass ($M_A/M_P$) is fixed thus the ratio of growth to development rate ($g/D$) is fixed. It is then displaced into a warmer environment (indicated by the dashed arrow), where $g/D$ is temporarily decoupled thus adult and progeny must change. However, $g/D$ is forced to return to a fixed state of 2 again due to the constraints of binary division, thus $g$ and $D$ are not independent.
Figure 2.2. Hypothetical example of the effect of temperature change on a multicellular organism which adheres to the TSR. The organism starts at a cold temperature, where growth and development rate \( g/D \) and thus the size ratio of adults to individual progeny \( M_A/M_P \) is a constant \( x_c \). The organism is then displaced into a warmer environment (indicated by the dashed arrow), to which it adjusts by modifying juvenile and progeny growth and development rate to a new constant ratio \( x_w \). The change in the ratio of adult to progeny mass between the states is exaggerated here to emphasize that this ratio can differ between temperatures, unlike in unicellular organisms (see Figure 2.1). In this example progeny mass changes proportionally less than that of the adult; consequently \( g/D \) in the warm is less than that in the cold \( x_c > x_w \). However, the opposite is also possible, producing \( x_c < x_w \), when progeny mass is more sensitive than adult mass to warming.
limiting food conditions. In fact, binary division imposes strict limits on adult and progeny size ratio and forces $g/D$ to return to a fixed ratio of 2. However, most unicellular organisms obey the TSR, becoming larger at cooler temperatures and smaller at warmer temperatures (Atkinson 1994, Montagnes and Franklin 2001, Atkinson et al. 2003). Therefore the size of unicells must change when exposed to a new temperature and $g/D$ must become temporarily decoupled (see Figure 2.1). After $g/D$ adjustment is complete, the rates must become coupled once more; these rates cannot be considered independent as binary division requires that total temperature compensation occurs (i.e. Equation 2.3 is restored) to prevent cells continuing to get smaller or larger ad infinitum.

**Multicellular Organisms**

Application of Equation 2.2 is more complex for multicellular organisms. As they do not replicate by simple binary division of the adult, the progeny mass is not restricted to be a fixed proportion of adult mass and in many species individual organisms are able to produce progeny that can vary in size (Blanckenhorn 2000, Atkinson et al. 2001, Fischer et al. 2003b, Fischer et al. 2004). Therefore, unlike unicells, individual progeny are not so strictly constrained by maternal size, thus the ratio $M_A/M_P$ need not be fixed across different temperatures and consequently growth and development rates would not need to return to a fixed ratio (Equation 2.2). If this were the case, growth and development rates could change independently with temperature, which supports the assumption of the biophysical model applied by van der Have and de Jong (1996). There is much evidence supporting a temperature-size response in adults (see review in Atkinson 1994) but less evidence for eggs (see review in Atkinson et al. 2001). We show this potential temperature independence of $g$ and $D$ in Figure 2.2 in which adult
mass is assumed to change with temperature more than progeny mass, thus 
\[ \frac{M_A}{M_P} \text{(cold)} > \frac{M_A}{M_P} \text{(warm)}. \]

If adult and progeny mass show different temperature-size responses in metazoans, this would suggest a fundamental difference between the TSR in uni- and multicellular organisms: acclimated unicellular organisms living at different temperatures must have a constant ratio of \( g/D \) whereas multicellular organisms have a variable ratio of \( g/D \). Is this supported by experimental data in the literature? We conduct a meta-analysis on metazoan adult and progeny size data for a wide range of species, and test whether the thermal responses of these data sets differ. Where data were available, changes in mass were also examined separately throughout ontogeny, described in the Methods.

*Alternative growth equations*

We have constructed the conceptual model using a linear growth model with mass-specific growth rates. However, these conclusions are not qualitatively affected by altering the growth function from mass-specific linear increase in mass per unit time or using either an exponential or the von Bertalanffy growth functions:

1. *Linear growth rate*

In our conceptual model, we use mass-specific growth rates. However, using van der Have and de Jong’s (1996) original formulation:

\[ \frac{g}{D} = M_A - M_P \quad \text{Equation 2.4} \]
where $G$ is growth rate (mass day$^{-1}$) does not alter this outcome for differences between uni- and multicellular species. For unicells, the outcome is exactly that of Equation 2.3, except the constant becomes 0.5 rather than 2. Similarly to our initial model, the right hand side of this equation need not be constant, following the result of Equation 2.3.

2. Exponential growth model

Assuming that mass of an individual unicell increases exponentially from the point of division yields the following equation:

$$g = \frac{\ln \left( \frac{M_A}{M_P} \right)}{t}$$  \hspace{1cm} \text{(Equation 2.5)}

where $t = \text{development time}$. Converting development time to a rate ($1/t$) and rearranging this model results in a form similar to that of the linear model.

$$\frac{g}{D} = \ln \frac{M_A}{M_P} \Rightarrow \frac{g}{D} = \ln 2$$  \hspace{1cm} \text{(Equation 2.6)}

By incorporating the restriction of binary division on adult:progeny mass ratio into the model results in growth and development being fixed (ln2) thus if unicellular organisms which are size acclimated to their thermal environment did follow the exponential growth model, growth and development rates are still a fixed constant, whereas this can vary in multicellular organisms.

3. von Bertalanffy growth model

The von Bertalanffy growth curve (von Bertalanffy 1957, Perrin 1995) has often been
used to describe changes in mass in ectothermic groups and is based on an exponential
growth function, most commonly presented as:

$$M_t = A(1 - \lambda e^{-ht})^3$$  \hspace{1cm} (Equation 2.7)

where $M_t =$ mass at time $t$, $A =$ asymptotic mass, $\lambda = 1 - (A^{-1/3} - M_0^{1/3})$, $M_0$ is initial mass, $h$ is the growth coefficient related to time taken to reach the asymptotic mass (Perrin 1995) and $t =$ development time. In the case of unicellular organisms, we are interested in the time to reach adult mass from progeny mass so we can assign mass at time $t$ ($M_t$) as adult mass ($M_A$) and can consider initial mass ($M_0$) to be exactly the same as progeny mass ($M_p$). By rearranging Equation 2.7 the von Bertalanffy growth model can be examined in a similar way to a simple linear or exponential growth model:

$$t = \left[ \frac{\ln \left( \frac{A^{1/3} - M_A^{1/3}}{A^{1/3} - M_p^{1/3}} \right)}{-h} \right]$$ \hspace{1cm} (Equation 2.8)

In single celled organisms dividing by binary division, asymptotic mass $A$ is equal to adult mass $M_A$, the size of the adult just before complete division. Thus, by converting development time to a rate ($D = 1/t$), Equation 2.8 can be simplified to:

$$\frac{h}{D} = \ln(M_A^{1/3} - M_p^{1/3})$$ \hspace{1cm} (Equation 2.9)

Therefore when unicellular organisms are size acclimated to their thermal environment, $h/D$ is a fixed ratio, which need not be the case for multicellular organisms. This is exactly the outcome of our initial model in Equation 2.3.
**Methods**

*Data collection*

Initially, we synthesised data for adult and progeny mass that could be found in the literature. We started by compiling progeny data referenced in Atkinson et al. (2001), then compiled all further data by extensively searching the Web of Science (years 1970-2011) and Google Scholar (years 1930-2011) using the search terms: “(egg OR progeny OR young OR larva*) AND temperature AND (size OR mass OR weight)” and “(adult OR maternal OR pupa*) AND temperature AND (size OR mass OR weight)” for progeny and adult data respectively. Only laboratory studies were included in which sizes were measured at a range of constant temperatures, but food concentrations had been maintained at or above saturation (therefore removing the confounding impact of food limitation). In addition to published data we include our own unpublished data for the brine shrimp species *Artemia franciscana* (see Chapter 4 for details).

To ensure sizes were comparable across different species, data were recorded as fresh mass, dry mass, carbon content or volumes. Where data were only provided as lengths or areas, these were converted to mass or volumes using species-specific regression equations from the literature. To act as direct comparison with size-acclimated unicellular organisms where $g/D = 2$, body sizes were only compared from studies in which time had been allowed prior to the experiment for the completion of size acclimation. We assumed progeny were acclimated as long as they were produced at the experimental temperature, i.e. the parental generation were introduced to temperatures prior to copulation and kept at these temperatures until egg laying (e.g. oviposition). The minimum period of acclimation for the inclusion of adult mass data was set so that only individuals who had been raised from egg or first larval stages were included. The
data for progeny are displayed in Appendix 2.1 and data for adults in Appendix 2.2.

**Paired Data**

From the large data sets (Appendix 2.1, 2.2), we refined the data such that we could examine $M_d/M_P$ in individual species, and thus compare this ratio with the constant ratio found in unicellular organisms. To ensure the most accurate possible comparison with acclimated unicells, we used stricter terms for adult acclimation, such that only studies where individuals were grown at a constant temperature from egg formation until adulthood were included. An exception is the adult data for *Lycaena* spp., in which larvae spent several weeks at the acclimation temperature before a larval diapause at 4°C (5-6 months) and were then returned to their previous fixed acclimation temperatures. We accepted these data because the active growth and development periods were under the acclimating temperature. Although we maintained the same assumptions for the acclimation of progeny data as before, most of the paired studies used progeny data which were produced at the same temperature as the parental rearing temperature (10/15 species, Appendix 2.3).

For some species, more than one paired data set was available. Therefore three criteria used to select the most appropriate data set were ranked in order of importance, which first favoured the dataset with largest number of data points, then the largest temperature range, then with actual values provided (rather than digitised). For example, if data existed from two studies and both had six data points, then the response with the largest temperature range was chosen. When animal sizes were presented as lengths, these were converted to mass using length-weight regressions. The sources and a more detailed summary of paired data compiled for this study are described in detail.
Modelling adult and progeny mass data

An appropriate model for the response of adult and progeny size to temperature was required that could be applied across all species. There is conflicting opinion as to the form that the body-mass thermal reaction norm should take within a species, and many equations have been proposed (Karan et al. 1998, Atkinson et al. 2003, de Jong 2010). We, therefore, required a method to apply a range of equation forms (linear, exponential, Arrhenius, power) to the full data set to determine which best described the empirical data; this was achieved using a linear mixed effects model (O’Connor et al. 2007). Applying this type of mixed effects design allows models to be fitted to all adult or progeny mass data at once, as the large differences in mass across different species are accounted for by species-specific intercept terms. Further, interspecific differences between temperature-dependent slopes can be accounted for in species-specific slope terms. Initially to test for linearity, a power model was fitted to the data:

\[
\ln M = \beta_{0ij} + \beta_{1ij} (\ln T - \ln 15) + \epsilon_{ij} \tag{Equation 2.10a}
\]

\[
\beta_{0ij} = \beta_0 + u_{0ij} \tag{Equation 2.10b}
\]

\[
\beta_{1ij} = \beta_1 + u_{1ij} \tag{Equation 2.10c}
\]

where \( M \) = mass of progeny or adult of a single species, \( T \) is temperature (°C), \( i \) indexes the species and \( j \) the temperature. \( \beta_0 \) and \( \beta_1 \) are intercept and slope fixed effects respectively, \( u_0 \) and \( u_1 \) are species-specific random effects terms that allow for interspecific differences in the intercepts and slopes respectively, assumed to be normally distributed; \( \epsilon \) is the error, assumed to be normally distributed. Following the
methods of O’Connor et al. (2007), temperatures were centred to improve the interpretation of parameter terms and to reduce the correlation between slope and intercept terms. A centring temperature of 15°C was applied, as this temperature is within the boundaries used in most studies in the database and therefore required minimal extrapolation. Subtracting this centring temperature from each of the model types allowed each rate to be examined in terms of changes from that at 15°C.

To test for linearity, we wished to see whether the fixed-effects parameter $\beta_1$ was significantly different from 1. The slope parameter $\beta_1$ represents the exponent of the power model; thus if the best fit model had a slope of 1, this would indicate a linear relationship between temperature and mass. We, therefore, did not include a species-specific term ($u_1$) at this stage as we wished to calculate the mean parameter $\beta_1$ across all species at once. As the best-fit values for parameter $\beta_1$ were significantly different from 1 for both adult and progeny masses (-0.356 ±0.095 (95% CIs) and -0.003 ±0.080 (95% CIs) respectively, a simple linear model (as an alternative model type) could be rejected.

To determine the best fit model for both adult and progeny data, power, exponential and Arrhenius models were subsequently fit to the entire data set. Average species masses varied greatly for both adult and progeny masses; therefore in each model type intercepts were allowed to vary randomly to account for species-specific masses. These models were initially fitted assuming a fixed slope, assuming similar relative changes in mass with temperature. However, these models were also fitted allowing slopes to vary randomly, thus allowing species-specific changes in mass with temperature. The six
models applied to the data are shown in Table 2.1, following the notation of O’Connor et al. (2007).

Initially, the best equation was chosen for each model type (power, exponential, Arrhenius) using modified likelihood ratio tests to determine whether each model type required slopes with species-specific random effects to improve fit. Having selected the best equation, the model types were compared by using Akaike’s Information Criterion (AIC, Table 2.2). The best fit model was subsequently applied to the paired adult and progeny data. We next analysed both the full data set and the paired data to examine if the slopes of the temperature-size response in adults and progeny were significantly different, comparing the mean slopes for the full data set and conducting a paired t-test on individual species in the paired data set.

Mass change during ontogeny

Beyond examining progeny and adult masses, an appreciation of where in the development schedule changes in the mass to temperature relationship occur in metazoans will provide insight into the causes of these changes. Within the data, two studies had individual masses and times for multiple larval stages between egg and adult (including prior acclimation), which allowed us to examine how the response of mass to temperature varies throughout ontogeny. These were both for copepods, *Acartia tonsa* (Leandro et al. 2006), and *Calanus finmarchicus* (Campbell et al. 2001), and included egg, 6 naupliar stages, and 6 copepodite stages, the final stage being the adult. To determine the mass vs. temperature relationship for each stage, the best-fit model type, as shown from our analysis of all progeny and adult data, was applied to each species individually by allowing stage-specific parameters for both slope and intercept.
The stage-specific slopes provide an estimate of the temperature-size response associated with each individual stage. These slopes were subsequently compared across the duration of maturation to assess the strength of the temperature-size effect throughout ontogeny.

**Results**

*Adult to progeny size ratios of multicellular organisms*

For the larger unpaired dataset, we collected progeny data for 33 species and adult data for 100 species (Appendix 2.1, 2.2). Within this larger set, there were adult and progeny paired data for 15 (sub)species that fulfilled the more rigorous requirements (Appendix 2.3). Given the choice of models used here, we found that an exponential model with species-specific intercepts and slopes provided the best fit to both the adult and progeny mass vs. temperature responses (Table 2.2), with the basic form:

\[
\ln M = a + bT
\]  
(Equation 2.11)

where \( M = \) mass, \( T = \) temperature, \( a \) is the mean intercept and \( b \) is the mean slope term.

According to the fitted slopes for this best fit model, adult mass had a significantly more negative slope (\( b = -2.60 \times 10^{-2}, 95\% \) CIs = ±0.57 \( \times 10^{-2} \)) than progeny mass (\( b = -0.90 \times 10^{-2}, 95\% \) CIs = ±0.61 \( \times 10^{-2} \)) across the entire data set (t-test, \( t = 6.19, p < 0.001, \) Figure 2.3, Table 2.2). This is equivalent to a 0.9\% decrease in mass \( ^\circ \text{C}^{-1} \) in progeny, but a 2.5\% decrease in mass \( ^\circ \text{C}^{-1} \) in adults, with the magnitude of size change in adults being similar to that seen in protists (Atkinson et al. 2003). Similar results were found for paired data; the mean slope for progeny mass vs. temperature was \(-0.14 \times 10^{-2}\)
Table 2.1. Set of equations applied to adult and progeny mass data. $M =$ mass, $a =$ intercept term, $b =$ slope term, $M_{ij} =$ mass for species $i$ and temperature $j$, $T =$ temperature ($°C$), $k =$ the Boltzmann constant = $8.62 \times 10^{-5}$ eV K$^{-1}$, $\beta_0 =$ fixed intercept parameter, $\beta_1 =$ fixed slope parameter, $u_{0i} =$ species-specific intercept term with normally distributed variance, $u_{1i} =$ species-specific slope term with normally distributed variance, $\varepsilon_{ij} =$ error term with normally distributed variance.

<table>
<thead>
<tr>
<th>Model</th>
<th>Equation</th>
<th>Random parameters</th>
<th>Linear mixed effects model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power</td>
<td>$\ln M = a + b \ln T$</td>
<td>$u_{0i}$</td>
<td>$\ln M_{ij} = (\beta_0 + u_{0i}) + \beta_1 (\ln T_j - \ln(15)) + \varepsilon_{ij}$</td>
</tr>
<tr>
<td>Power</td>
<td>$\ln M = a + b \ln T$</td>
<td>$u_{0i}, u_{1i}$</td>
<td>$\ln M_{ij} = (\beta_0 + u_{0i}) + (\beta_1 + u_{1i})(\ln T_j - \ln(15)) + \varepsilon_{ij}$</td>
</tr>
<tr>
<td>Exponential</td>
<td>$\ln M = a + b T$</td>
<td>$u_{0i}$</td>
<td>$\ln M_{ij} = (\beta_0 + u_{0i}) + \beta_1 (T_j - 15) + \varepsilon_{ij}$</td>
</tr>
<tr>
<td>Exponential</td>
<td>$\ln M = a + b T$</td>
<td>$u_{0i}, u_{1i}$</td>
<td>$\ln M_{ij} = (\beta_0 + u_{0i}) + (\beta_1 + u_{1i})(T_j - 15) + \varepsilon_{ij}$</td>
</tr>
<tr>
<td>Arrhenius</td>
<td>$\ln R = \ln a - b \frac{1}{kT_1(k)}$</td>
<td>$u_{0i}$</td>
<td>$\ln M_{ij} = (\beta_0 + u_{0i}) + \beta_1 \left(\frac{1}{k(T_{ij}+273)} - \frac{1}{k(288)}\right) + \varepsilon_{ij}$</td>
</tr>
<tr>
<td>Arrhenius</td>
<td>$\ln R = \ln a - b \frac{1}{kT_1(k)}$</td>
<td>$u_{0i}, u_{1i}$</td>
<td>$\ln M_{ij} = (\beta_0 + u_{0i}) + (\beta_1 + u_{1i})\left(\frac{1}{k(T_{ij}+273)} - \frac{1}{k(288)}\right) + \varepsilon_{ij}$</td>
</tr>
</tbody>
</table>
Table 2.2. Comparison of model types to determine best model. AIC is the Akaike Information Criterion, $\Delta_i$ is the AIC differences, $\omega_i$ is the Akaike weight. Values for fixed effect parameters are shown, along with standard errors (subscript values in brackets). The overall best fit model is shown in farthest right column (Best Model), and is defined as that with the highest Akaike weight.

<table>
<thead>
<tr>
<th>Mass</th>
<th>Model</th>
<th>Fixed Effects</th>
<th>AIC</th>
<th>$\Delta_i$</th>
<th>$\omega_i$</th>
<th>Best Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\beta_0$</td>
<td>$\beta_1$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progeny</td>
<td>Exponential</td>
<td>-.052(.422)</td>
<td>-.009(.003)</td>
<td>80.4</td>
<td>0.00</td>
<td>0.97</td>
</tr>
<tr>
<td>Mass</td>
<td>Power</td>
<td>-.052(.420)</td>
<td>-.115(.032)</td>
<td>104</td>
<td>24.0</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Arrhenius</td>
<td>-.035(.420)</td>
<td>.056(.015)</td>
<td>104</td>
<td>23.5</td>
<td>0.00</td>
</tr>
<tr>
<td>Adult</td>
<td>Exponential</td>
<td>2.11(.270)</td>
<td>-.026(.003)</td>
<td>201</td>
<td>0.00</td>
<td>0.96</td>
</tr>
<tr>
<td>Mass</td>
<td>Power</td>
<td>2.11(.197)</td>
<td>-.460(.057)</td>
<td>244</td>
<td>42.9</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Arrhenius</td>
<td>2.11(.270)</td>
<td>.192(.002)</td>
<td>208</td>
<td>6.53</td>
<td>0.04</td>
</tr>
</tbody>
</table>
(95% CIs = ±0.71 x10^{-2}, Figure 2.3, 2.4A), but was -2.32 x10^{-2} for their adult mass (95% CIs = ±1.21 x10^{-2}, Figure 2.3, 2.4B). The ratio of $M_A/M_P$ was calculated for the paired data using species-specific slope terms (Figure 2.4C). The species-specific slope parameters for the paired data were used to test whether these slopes were significantly different, by conducting a pair-wise t-test for adult vs. progeny slopes. This indicated that adult mass has a stronger temperature dependence than progeny mass, (paired t-test, $t = 4.34$, $p = 0.001$), and consequently the ratio of $M_A/M_P$ is not fixed within single species at different temperatures. Comparison of the slopes of ln mass vs. temperature for the paired data show the mean slope for progeny mass is not significantly different from zero (mean = -0.14 x10^{-2}, 95% CIs = -0.86 x10^{-2}, 0.57 x10^{-2}) whereas the mean slope for adult mass is significantly negative (mean = -2.32 x10^{-2}, 95% CIs = -3.53 x10^{-2}, -1.10 x10^{-2}). Thus after allowing time for size acclimation, the ratio $M_A/M_P$ does not return to a fixed temperature-independent constant, but this ratio is generally larger at low temperatures and smaller at high temperatures, as shown in Figure 2.3. These changes in $M_A/M_P$ were extremely large in some cases; the ratio of $M_A/M_P$ at the highest experimental temperature was half of that at the lowest experimental temperature in Pseudocalanus newmani (Figure 2.4C). Further, more than half of the species in the paired data (8/15) showed changes in the ratio of $M_A/M_P$ of >30% over their thermal range (Figure 2.4C). A consequence of this is that multicellular organisms must have a $g/D$ ratio which varies substantially across temperatures, i.e. growth and development rates have a different temperature dependence. By contrast in unicells reproducing by binary division we know that $g/D$ must be fixed (Equation 2.3).

**Timing of size adjustment during ontogeny**

The effect of temperature on the size of specific larval stages in the copepod species
Figure 2.3. Mean slopes of the best fit exponential model for multicellular organism data. “Progeny” and “Adult” data represent the entire dataset (Appendix 2.1, 2.2). “Paired Progeny” and “Paired Adult” represent a subset of high quality data, where progeny and adult data were measured by the same study group (Appendix 2.3). Error bars indicate 95% confidence intervals.
Figure 2.4.
Figure 2.4. Change in mass of ectotherms as a function of temperature for: (A) progeny, (B) adult, and (C) adult to progeny mass ratio. Symbols give individual data points in A and B, whilst in C the symbols do not give individual values but rather indicate which species the line is for. Progeny and adult data fitted with exponential best-fit models, adult to progeny mass ratio determined for each species by dividing results from the best fit equation for adults at a specific temperature by the best fit equation for progeny at the same temperature. To improve visualisation, data for (C) were converted to % change in mass with temperature.
Acartia tonsa and Calanus finmarchicus shows some variation between these two species (Figure 2.5); however there are general patterns in these responses. There is no discernible effect of temperature on size of progeny (represented by early larval stages) in either species. The mass vs. temperature relationships exhibit a generally increasing negative trend throughout ontogeny when examined in relation to time (Figure 2.5A, B). When examined with respect to mass, the majority of the temperature-dependence of size has been completed by ~20% of the adult mass (Figure 2.5C, D). The majority of the temperature-size effect has been completed before the last 3-4 larval stages, despite these stages accounting for the majority of mass accrual (~80% of total mass) due to the exponential nature of mass accrual with time exhibited in copepod species (Escribano and McLaren 1992).

**Discussion**

Using a conceptual model, we have shown that unicellular organisms acclimated to different temperatures must have a ratio of growth to development rate which is a constant; this is due to the constraints of binary division. When a unicellular organism that follows the TSR is exposed to a new thermal environment, any decoupling of growth and development rate is constrained within a period of acclimation, and $g/D$ must return to a fixed value of 2. If $g/D$ did not return to this value, at increased temperature cells would get progressively smaller with each division. Although this specifically only applies to those organisms that divide by binary division, this is the major reproductive strategy in prokaryotes (Angert 2005), and in many unicellular eukaryotic cells (Sleigh 1991, Reynolds 2006). Therefore, the temperature dependence of growth and development rates are not independent in the majority of unicellular organisms.
Figure 2.5. Slopes of the relative changes in mass with temperature (ln Mass vs. Temperature) for consecutive larval developmental stages of the copepods: (A) *Acartia tonsa* (Leandro et al. 2006), and (B) *Calanus finmarchicus* (Campbell et al. 2001) size changes as a proportion of time to adult. (C) *Acartia tonsa* (Leandro et al. 2006), and (D) *Calanus finmarchicus* (Campbell et al. 2001) size changes as a proportion of adult mass. Larval stages comprise six nauplii stages (NI-NVI) and six copepodite stages (CI-CVI), the CVI stage is the adult. Error bars indicate 95% confidence intervals.
Adult to progeny mass ratios

Our synthesis of adult and progeny mass in multicellular organisms shows the ratio of these is not constant at different temperatures. Combining this evidence with our conceptual model shows that, unlike in unicells, multicellular organisms can maintain different temperature dependence for growth rate relative to development rate. Our meta-analysis demonstrates that progeny mass shows a reduced response to temperature compared to adult mass. Although both show a negative response, adult mass has a significantly more negative slope than that of progeny. Furthermore, when the paired data were compared, the ratio of $M_A/M_P$ was consistently negative across the 15 (sub)species, with the average progeny temperature-size response not being significantly different from zero. This novel finding suggests that it is incorrect to assume a temperature-size effect on progeny size in multicellular organisms that is of similar magnitude to adults. Van der Have and de Jong (1996) proposed that “the same effect of temperature on oocyte production as on size at metamorphosis could be predicted, that is, smaller eggs will be produced at higher environmental temperature”. This statement requires clarification: we find that the magnitude of this change is consistently larger in adults than in progeny. This is the case even after allowing for acclimation of both adult and progeny size. Referring to Equation 2.2, this would lead us to predict that growth and development rates have a different temperature dependence in multicellular organisms, with development being more temperature-sensitive than growth.

Timing of size adjustment during ontogeny

There is evidence from two copepod species that early larval stages (i.e. beyond egg stage) show no size response to temperature, whereas later stages show strong negative
relationships. The data suggest that thermal selective pressures act increasingly during the maturation of the two copepods (Figure 2.5), and the unequal effect of temperature on growth and development rates only begins acting on size during post-embryonic growth. Although temperature-size effects are cumulative during ontogeny, the majority of the temperature-size response is established by the point at which ~0.2 of the adult weight has been achieved. This is in contrast to larval development in the butterfly *Lycaena tityrus*, where the TSR is only established during the final larval stage associated with the largest (~80%) increase in mass (Karl and Fischer 2008). Further, ontogenetic size changes in Figure 2.5 indicate that these copepod species may be seen as adjusting size in every generation: changes in size are effectively being reset or considerably muted at egg/progeny stage. This, again, is not the case in butterfly species, which show marked changes in egg size at different temperatures (Fischer et al. 2003b, Fischer et al. 2006). This suggests that although the TSR applies to the majority of metazoa (Atkinson 1994) there may be taxon-specific changes in size with temperature, that impact on different life stages to different extents.

Outcomes from the conceptual model

How do these differences between unicellular and multicellular organisms impact on the potential causes of the TSR? The results of our conceptual model, combined with the meta-analysis reveal that unicellular organisms are restricted in the adjustment of their rates of growth and development. The ratio of $g/D$ must return to a constant of 2 in a species living at a fixed temperature, thus any temperature-induced changes in this ratio is limited to a temporary acclimation phase. In multicellular organisms, the ratio of $g/D$ need never be a fixed constant when comparing across different temperatures, because these organisms alter their adult:progeny size ratio (see Equation 2.2 and
Although there must be limits imposed on size changes set by physical constraints, such as maternal ovipositor/birth canal diameter (Atkinson et al. 2001), this does not impose strict limits on the ratio of $M_A/M_P$, and therefore $g/D$, as it does in unicells. Indeed, the results from the paired meta-analysis show the ratio of $M_A/M_P$ can change substantially over a species’ thermal range in multicellular organisms. Consequently, there can be large alterations in the ratio of $g/D$ (Equation 2.2). For example, $M_A/M_P$ data for *Pseudocalanus newmani* show that development rate must increase by more than twice the rate of growth over this copepod’s thermal range.

It is important to note that despite the different restrictions imposed by reproductive method in uni- and multicellular organisms, both follow the TSR. Therefore, despite the limitations of binary division, rates of $g/D$ must temporarily decouple in unicellular species to facilitate size change, even if they must eventually return to a fixed ratio. This suggests that there must be significant fitness benefits to this thermal plasticity, as it occurs in different groups through different means. Thus, although the proximate mechanism for the TSR differs between these two groups, the ultimate explanation for the TSR may still be the same. Despite many hypotheses having been proposed (Angilletta et al. 2004, Atkinson et al. 2006, Walters and Hassall 2006, Kingsolver and Huey 2008), we are yet to find a general, ultimate cause for the phenomenon of the TSR. To understand the variation in size responses to temperature, we propose that more attention be directed to fuller quantitative descriptions of responses throughout the period of population growth in unicells, and ontogeny in multicellular organisms. In unicells for example, by identifying the number of cell generations until $g/D$ adjustment is complete, and the amount of $g/D$ adjustment per cell cycle per °C, we can partition variation in size responses among species to the different mechanisms (average thermal
sensitivity of size per cell cycle per °C, number of cell divisions to complete acclimation), and seek patterns in these among taxa and ecological niches. Likewise, in multicellular organisms, differences between species in the period of $g/D$ adjustment, as shown in Figure 2.5 for two species of copepod, can help identify variation, or indeed similarities, between species and taxa. Another potential benefit from quantifying trends in TSR across ontogeny is to identify particular stages or size ranges when selection for size response to temperature may be particularly intense. Berven & Gill (1983) suggest that temperature-dependent variation in adult size in *Rana sylvatica* may be a consequence/correlate of temperature-dependent selection on offspring size plasticity. By quantifying which developmental phases actually show a size response to temperature (Figure 2.5), particular parts of the life cycle may be examined to see whether or not there are particular temperature-dependent selection pressures that affect those developmental phases or size classes.

Any proximate mechanism explaining how the TSR occurs must be applicable to all ectothermic groups. We have shown fundamental differences exist between unicellular and multicellular organisms in the way size changes are established. This suggests that there is no universal mechanism to explain how size changes are brought about.
CHAPTER 3

Growth and development rates have different thermal responses

Introduction

Development (passing through life stages) and growth (increase in mass) are fundamental to all living organisms. The rate at which individuals mature, along with their size, determines higher-level properties, such as population abundance (Di Cola et al. 1999), dispersal distance (O'Connor et al. 2007) and energy flow (Silvert and Platt 1978). It is, therefore, vital that we understand how these rates respond to variation in temperature, especially in the context of global warming. Average global air temperatures are expected to increase by between 1.1 and 6.4 °C this century, whilst shorter term fluctuations in temperature due to climate change are becoming increasingly common (IPCC 2007). Further, climate models predict that average sea surface temperature will have increased by 2–3.5°C by the end of this century, with the Arctic showing even greater increases (up to 8°C warmer, Richardson (2008)). More than 99% of species are ectotherms (Pincheira-Donoso 2008), in which metabolism is driven primarily by body size and environmental temperature (Brown et al. 2004). Changes in the latter will impact on organisms’ body temperature and in turn drive changes in development and growth rates. We therefore need to be able to make broad predictions on how these rates change with temperature, and the impacts of these changes on individual organisms.

Modelling growth and development
There is currently neither a consensus on how growth and development rates respond to temperature, nor on the most efficient way of modelling these relationships. Within species, models based on linear (Montagnes et al. 2003), power (Belehradek 1926, McLaren 1969, Corkett and McLaren 1970, Hart 1990, Peterson 2001), and exponential (Escribano and McLaren 1992, Escribano et al. 1997, Campbell et al. 2001) functions have often been applied to describe how these rates change with temperature. More complex relationships with a mechanistic basis, for example Arrhenius (Gillooly et al. 2002, Brown et al. 2004) and Sharpe-Schoolfield (van der Have and de Jong 1996, de Jong 2010) equations are also commonly used. When attempting to make broad predictions about the response of fundamental rates to temperature in individual species, it is important to balance accuracy and parsimony. We aimed to find a model that accurately describes the data, without over-parameterisation, and without requiring large quantities of additional information which is difficult and time-consuming to collect. We used an information theoretic approach to find out which of these models was best supported by data and hence best described the relationship between both growth and developmental rates to temperature.

Accurately describing the thermal response of growth and development rates across the life cycle of an organism (e.g. egg to adult) will provide valuable information on how these fundamental rates are likely to respond to climate change. Simply examining them as averages across the entire life cycle, however, fails to acknowledge important changes in rates through ontogeny (Forster et al. 2011a). We addressed this by comparing growth and development rates using data from egg, early and late larval stages. We focused our efforts on quantifying these processes using marine pelagic Copepoda; these are the dominant mesozooplankton in the world’s oceans and a key
component of the ocean food web (Mauchline 1998, Richardson 2008). Studying this group offers many advantages, as they have a fixed number of moults and exhibit determinate growth. Growth and development rates can therefore be determined across easily identifiable and distinct stages throughout ontogeny. Furthermore, detailed laboratory studies of these rates in marine copepods have been conducted for many years (see Hart 1990, Peterson 2001), and a large amount of data is available for many species.

*Growth, development and the temperature-size rule*

Examining the thermal response of growth and development rates separately has important implications for the understanding of the temperature-size rule (TSR, Atkinson 1994). The TSR is demonstrated in over 83% of the ectothermic species investigated, including our target group, marine Copepoda (Kimoto et al. 1986, Uye 1988, Uye 1991, Campbell et al. 2001, Hansen et al. 2011). It has been suggested that the TSR is driven by growth and development rates having differing temperature dependence within a species (Atkinson 1994, van der Have and de Jong 1996, Davidowitz and Nijhout 2004, Forster et al. 2011a): growth rate (accumulation of mass) increases with temperature, but is outpaced by the relative increase in development rate (passing through life stages), resulting in smaller adult size at warmer temperatures. A recent analysis considering marine, freshwater and terrestrial metazoans revealed that intraspecific changes in adult size with temperature are significantly greater than in progeny (Chapter 2, Forster et al. 2011a), further implying that growth and development rates must be decoupled through the egg to adult period across a wide range of taxa.

*The impact of decoupling on ecological theory*
Although the decoupling of growth and development rates has been supported experimentally in a handful of species (e.g. *Drosophila melanogaster* (van der Have and de Jong 1996), *Rana pipiens* (Smith-Gill and Berven 1979), *Chorthippus brunneus* (Walters and Hassall 2006) and *Manduca sexta* (Davidowitz and Nijhout 2004)), we need to test whether this result holds more generally for a range of species, as the thermal responses of these rates has wider importance with respect to the general ecological theory of metabolism. The metabolic theory of ecology (MTE, *sensu* Brown et al. 2004) assumes that biological rates are intimately linked with metabolism, such that rates as varied as heart rate, growth, development and mortality (when mass-corrected) follow a thermal response modelled by the Arrhenius function (Brown et al. 2004). Decoupling of growth and development for a range of species would suggest fundamental differences in the mechanistic processes underlying these rates (van der Have and de Jong 1996). This would suggest inadequacies in theories built upon the assumption that life-history rates share a common temperature dependence (Gillooly et al. 2001, Gillooly et al. 2002, Brown et al. 2004). Further, if we find that these rates do not follow an Arrhenius-type function, it would suggest fundamental flaws in the assumptions associated with this model type. Systematically and comprehensively determining the thermal response of growth and development rates allows us to test both of these points.

Changes in size with temperature have been described as the “third universal ecological response to global warming” (Daufresne et al. 2009). Because body size is a key determinant of food web structure and dynamics (Woodward et al. 2005, Barnes 2008, Woodward et al. 2010a, Woodward et al. 2010b, Yvon-Durocher et al. 2010), any temperature-mediated changes in organism size could have important impacts at the
ecosystem level. Accurately determining the general thermal response of growth and development rates to temperature across a range of copepod species will allow us to estimate the thermal reaction norm (the pattern of phenotypic expression of a single genotype) of individual body mass to temperature. These insights could ultimately be used to scale up to the higher levels of biological organisation, such as communities and food webs (Woodward et al. 2010a).

The aims of this study are therefore to address the following questions: 1. Which equation(s) best describe the response of growth and development rates to temperature, and is there evidence to suggest different responses to temperature for these rates? 2. Are the shapes of these responses maintained across different developmental stages? 3. Based on the relationships determined, how do we predict organism size will vary with temperature?

Methods

Data collection

Growth and development rate data for marine planktonic Copepoda were collected from the literature. The ISI Web of Science online database was searched for data on growth rates and development times of marine copepod species using the search terms copepod* AND temperature AND (growth OR development OR mass), with cited references being used to identify further articles. Further marine journals were searched through their own online journal content, and previous meta-analyses of marine copepod data were also searched (Hart 1990, Peterson 2001). This data set was cross-referenced with a large collection of data previously collated on copepod life history rates (by A.G. Hirst), to ensure this analysis included the maximum amount of data.
possible. Only laboratory studies were included, where temperature was altered, but food concentration was high (considered in the study to be maintained at or above saturation) to remove the potentially confounding effect of food limitation. Details of food concentrations are provided as part of the raw data (Appendix 3.1, 3.2). Studies were only included when ≥ 2 temperatures were tested. Development time data were divided into separate larval stages. Within a study, data for any single temperature were only included when the tested temperature was constant throughout the study period. Data were extracted from tables or digitised from figures. Where figures were of insufficiently high resolution, authors were contacted directly. The raw data collected for the analysis are available in Appendices 3.1-3.3. Data are summarised in Table 3.1.

Growth rates were compiled from data sets as mass-specific growth rates \((g, \text{day}^{-1})\), calculated as the slope of ln mass against time, or calculated using the Moult Rate Method (see Hirst et al. 2005), typically by applying the formula \(\ln(M_{i+1}/M_{i})/SD_{i}\), where \(M_{i}\) is the stage mass (carbon content or dry mass), \(i\) = life stage and \(SD_{i}\) = stage duration of stage \(i\). Growth rates calculated using the Moult Rate Method are subject to errors and have all been corrected following the revised methods of Hirst et al. (2005). Finite growth rates \((G, \text{day}^{-1})\) based on production to biomass ratios were converted to mass-specific growth rates (using the equation \(g = \ln(G+1)\)). Growth rates were included as across-stage rates (e.g. NI-NII, CI-CII), across-naupliar rates (NI-NVI), across-copepodite rates (CI-CVI) and growth rate of the entire larval development (NI-CVI, which we term “Total” growth). We included data for 15 species of marine copepod that fulfilled our selection criteria (Appendix 3.1), with 312 data points. Development data are typically presented in the literature as development times, either as stage durations or median development times (MDT). MDTs represent the total time
to a stage, rather than a stage-specific duration; thus we converted these to stage
durations. Development data were collected for eggs, each of the six nauplius stages
(NI-NVI) and the five juvenile copepodite stages (CI-CV). Stage durations were then
converted to development rates as 1/stage duration (in days). Development rates were
included as stage-specific rates, across-naupliar rates (NI-NVI), across-copepodite rates
(CI-CVI) or rate across the entire larval development (NI-CVI, which we term “Total”
development). Egg development rates were examined separately. We compiled larval
development rate data for 24 species (1059 data points, Appendix 3.2) and egg data for
34 species (345 data points, Appendix 3.3).

Data screening
We focused on examining the effect of temperature on rates under non-extreme
situations: i.e. we excluded growth or development data at low temperatures where
individuals did not attain maturity, and we removed those data at high temperatures
from the point that rates start to decline with increasing temperature (as judged by a
decline in rates from one temperature to the next highest). These criteria enabled us to
account for the different thermal tolerances between species, whilst avoiding
temperatures at which severe resource limitation might occur (Atkinson et al. 2003).
This approach also maximised parsimony, as fewer parameters were required to model
the species-specific rate changes. This screening process excluded relatively few data
from the analysis (~5% of data, Appendices 3.1-3.3). We searched the literature to
identify appropriate equation forms to apply to development and growth rates (Table
3.2). Linear models could not be applied as residuals were homoscedastic on an
arithmetic scale. Sharpe-Schoolfield equation forms required unavailable species-
specific data (viability) thus were not included.
Modelling growth and development data

One inevitable limitation of the use of data for from different studies is that experimental designs vary. Although one can minimise these differences by ensuring, for example, excess food concentrations, there will be a degree of heterogeneity between data sets due to variation between different species and stages of copepods used, as well as differences between studies. As highlighted by O’Connor et al. (2007), these different conditions can be statistically considered as blocks with differing random effects, making the reasonable assumption that the studies are a random sample from a larger population of normally distributed studies. We use a linear mixed effects model to fit different statistical models to growth and development rate data. This allows species-specific effects to be incorporated within the model and thus fit different statistical models to multiple species. As described by Bates (2005), covariates can be separated into “fixed effects” and “random effects” by considering their repeatability. Covariates that can be considered conceptually repeatable, such as temperature in this case, can be considered as a fixed, whereas species, stage and study cannot be regarded as repeatable and are thus random. The aim of modelling these covariates differ: with fixed effects we wish to examine the typical mean effect of a fixed covariate on the response (effect of temperature on development or growth rate), whereas with random effects we wish to characterise the variation in the response caused by the different covariates (species, stage, sex and study). A linear mixed effects model provides a simple way of incorporating both fixed and random effects terms into a statistical model. We demonstrate this approach, with the incorporation of different group-level effects, for a power model:
Table 3.1. The number of species and data points considered in the analysis of growth and development rates of marine planktonic copepods.

<table>
<thead>
<tr>
<th>Data Type</th>
<th>Number of Species</th>
<th>Number of Data Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nauplii Growth Rates</td>
<td>8</td>
<td>126</td>
</tr>
<tr>
<td>Copepodite Growth Rates</td>
<td>12</td>
<td>162</td>
</tr>
<tr>
<td>Total Growth Rates (NI-CVI)</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>Nauplii Development Rates</td>
<td>19</td>
<td>509</td>
</tr>
<tr>
<td>Copepodite Development Rates</td>
<td>21</td>
<td>505</td>
</tr>
<tr>
<td>Total Development Rates (NI-CVI)</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>Egg Development Rates</td>
<td>34</td>
<td>345</td>
</tr>
</tbody>
</table>
Table 3.2. Growth and development rate models identified from the literature, including centring temperature. Centring temperature was 15°C for all data. \( R \) = rate (day\(^{-1}\), growth or development), \( a, b \) and \( c \) are constants, \( T \) is temperature (°C), \( T \) (K) is temperature (degrees Kelvin), \( k \) is Boltzmann’s constant (8.617x10\(^{-5}\) eV K\(^{-1}\)), \( E_a \) is average activation energy for the rate-limiting enzyme-catalyzed biochemical reactions of metabolism. In the multilevel model, \( \beta_0 \) is the intercept term, \( \beta_1 \) the slope term, and \( \beta_2 \) the exponential-quadratic curvature term, \( \varepsilon \) is the residual error.

<table>
<thead>
<tr>
<th>Model</th>
<th>Equation</th>
<th>Statistical model</th>
<th>Multilevel Centred Model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power</td>
<td>( R = aT^b + \varepsilon )</td>
<td>( \ln R = \ln a + b \ln T + \varepsilon )</td>
<td>( \ln R = \beta_0 + \beta_1 (\ln T - \ln 15) + \varepsilon )</td>
<td>(Belehradek 1926)</td>
</tr>
<tr>
<td>Exponential-Quadratic</td>
<td>( R = aT^{(b+c\ln T)} + \varepsilon )</td>
<td>( \ln R = \ln a + b \ln T + c (\ln T)^2 + \varepsilon )</td>
<td>( \ln R = \beta_0 + \beta_1 (\ln T - \ln 15) + \beta_2 (\ln T - \ln 15)^2 + \varepsilon )</td>
<td>(O’Connor et al. 2007)</td>
</tr>
<tr>
<td>Exponential</td>
<td>( R = ae^{bT} + \varepsilon )</td>
<td>( \ln R = \ln a + bT + \varepsilon )</td>
<td>( \ln R = \beta_0 + \beta_1 (T - \ln(15)) + \varepsilon )</td>
<td>(Campbell et al. 2001)</td>
</tr>
<tr>
<td>Arrhenius</td>
<td>( R = ae^{-E_a/(kT(\infty))} + \varepsilon )</td>
<td>( \ln R = \ln a - b \left( \frac{1}{kT(\infty)} \right) + \varepsilon )</td>
<td>( \ln R = \beta_0 - \beta_1 \left( \frac{1}{kT(\infty)} \right) - \frac{1}{k(288)} + \varepsilon )</td>
<td>(Cossins and Bowler 1987)</td>
</tr>
</tbody>
</table>
\[ \ln R = \beta_0 + \beta_1 (\ln T - \ln 15) + \varepsilon \]  \hspace{1cm} \text{Equation 3.1}

where \( R \) = rate (day\(^{-1}\)), \( \beta_0 \) is the intercept parameter, \( \beta_1 \) is the slope parameter and \( \varepsilon \) is the residual error term. In all cases, incorporating the random effects of stage \((a)\), sex \((b)\) and study \((c)\) improved the fit of the models and thus had to be included to account for the differences between these factors. However, when terms \(a-c\) were incorporated into both intercept and slope parameter terms they were highly correlated in all statistical models for growth and development rates (i.e. correlation between \( a_0 \) and \( a_1 \), \( b_0 \) and \( b_1 \), \( c_0 \) and \( c_1 \)). To avoid over-parameterisation of models, we allowed random variation in intercepts only for sex, stage and study for all mixed effects models. Reproductive method, i.e. carrying eggs in sacs (“sac-spawners”) or releasing eggs into the water column (“broadcasters”), was initially included as random parameter, but this was not found to improve the fit of any model and was therefore excluded. It is likely that any differences in rates driven by reproductive method were already accounted for within the “species” term. We incorporated the important random effects into the intercept term, so our starting point (before considering differences in slopes) becomes:

\[ \ln R = \beta_{0ij} + \beta_1 (\ln T - \ln 15) + \varepsilon \]  \hspace{1cm} \text{Equation 3.2a}

\[ \beta_{0ij} = \beta_0 + u_{0i(c/b/a)j} \]  \hspace{1cm} \text{Equation 3.2b}

where the intercept term includes a fixed term \( (\beta_0) \) along with a species-specific intercept term \( (u_i) \) within which study \((c)\) is nested; sex \((b)\) is nested within study and stage \((a)\) is nested within sex.
Centring data about a fixed temperature when using mixed effects models improves the interpretability of each model and reduces the correlation between parameters (O'Connor et al. 2007). We adopted the centring method as applied by O'Connor et al. (2007), see our Table 3.2. A centring temperature of 15°C was applied, as this temperature is within the boundaries used in most studies in the database and therefore required minimal extrapolation. Subtracting this centring temperature from each of the models allowed each rate to be examined in terms of changes from that at 15°C.

The logic behind the nesting hierarchy is that separate studies on a single species may have absolute differences in rates due to sourcing the same species from different geographical locations or at different times of the year (e.g. see Lonsdale and Levinton (1985)). In copepods there is a trend for males to develop faster than females; thus sex is nested within study to account for the absolute differences between the sexes in those larval stages where sex can be identified (e.g. stage CIII to adult). Within each sex, and at earlier stages where sex is not identifiable (NI-CII), there are absolute differences between rates of development and growth within each stage; thus stage is nested within sex. In cases where sex is not identified, this level of nesting is not applied by the model, and the nesting simply reverts to stage within study. Similar reversions apply when a species is represented by a single study alone. This nested design becomes the basis for all the statistical models, where the random effects of sex, stage and study are incorporated within the intercept term. Unlike sex, stage and study, the intercept and slope term for different species \( (u_i) \) were not highly correlated; i.e. if a species had a low base rate at 15°C, this did not relate to a low or high rate of increase with temperature. It may be that slope and intercepts show low correlation because one is species-specific and one is relatively invariable and does not improve model fit.
appreciably by including it. Therefore, for each statistical model this gave three potential outcomes: a) species-specific intercepts only (as shown in Equation 3.2a); b) species-specific slopes only or c) species specific slopes and intercepts (Table 3.2). These potential outcomes for any particular statistical model are represented in Figure 3.1. Equations 3.3 and 3.4 represent the power model with the inclusion of species-specific slopes or both species-specific slopes and intercepts:

\[
\ln R = \beta_{0ij} + \beta_{1ij} (\ln T - \ln 15) + \varepsilon \quad \text{Equation 3.3a}
\]

\[
\beta_{0ij} = \beta_0 + u_{0(c/b/a)j} \quad \text{Equation 3.3b}
\]

\[
\beta_{1ij} = \beta_1 + u_{1ij} \quad \text{Equation 3.3c}
\]

for random slopes (Figure 3.1 B) and:

\[
\ln R = \beta_{0ij} + \beta_{1ij} (\ln T - \ln 15) + \varepsilon \quad \text{Equation 3.4a}
\]

\[
\beta_{0ij} = \beta_0 + u_{0i(c/b/a)j} \quad \text{Equation 3.4b}
\]

\[
\beta_{1ij} = \beta_1 + u_{1ij} \quad \text{Equation 3.4c}
\]

for random slopes and intercepts (Figure 3.1 C). This resulted in 11 different species-level models being fitted to each dataset (Table 3.3). As the exponential-quadratic model is a modification of the power model, there were only two mixed effects models for this set, with a fixed and random quadratic term, \(\beta_2\). Having applied all 11 models in Table 3.3 to each data set, the best mixed effects model for each statistical model (i.e. power, exponential-quadratic, exponential, Arrhenius) was chosen by conducting a likelihood ratio test a likelihood ratio test using ANOVA in the lme4 package in R 2.10.1, that calculates a conservative p-value (upper bound on the true p-value). The
mixed effects model which gave the best fit for each type of statistical model was subsequently used for between-statistical model comparisons.

Using the best fit models for growth and development rates as a function of temperature, it is possible to predict the effect of temperature on organism size. We assume exponential increase in mass with time, as this describes growth well for marine copepods (Huntley and Lopez 1992, Hirst and Bunker 2003):

\[
g = \frac{(\ln M_i - \ln M_0)}{t}
\]

Equation 3.5

where \(g\) = growth rate (day\(^{-1}\)), \(M_i\) is the mass at stage \(i\), \(M_0\) is mass in the previous stage, and \(t\) = development time (days). From the best fit models of stage-specific development and growth rates, the relative increase in mass (between \(M_0\) and \(M_i\)) can be calculated from rearranging Equation 3.5, as:

\[
\frac{M_i}{M_0} = \exp\left(\frac{g}{D}\right)
\]

Equation 3.6

where \(D\) = development rate (day\(^{-1}\), i.e. the reciprocal of time to develop (\(t\)) from \(M_0\) to \(M_i\)). We compare this size change with temperature as a percentage change from the maximum size ratio.

**Results**

*Best fit models for growth and development*

The best fit mixed effects model for growth rates was a power model (see Table 3.4), which was subsequently fitted to the data, both as a general best fit model and as group-specific models (i.e. including specific parameter values for species, stage, sex and
study, Figure 3.1 A). Larval development rates were best modelled using an exponential-quadratic model with species-specific random effects for intercepts and slopes; the exponential-quadratic model was a much better fit than all other model types (Table 3.4). Figure 3.1 B shows this best fit model, along with the group-specific models, applied to the data. Similarly, egg development rates were best modelled using an exponential-quadratic model, but with the additional species-specific random effects term for parameter $\beta_2$ (Table 3.4, Figure 3.1 C).

Examining these thermal responses throughout ontogeny (i.e. for naupliar and copepodite growth separately), we found that the power model was best supported by growth rate data for both naupliar and copepodite stages. Although the slope for the naupliar growth rates was steeper than that for copepodite stages, the difference between these slopes was not statistically significant (2 sample $t$-test, $t = 1.94$, $p=0.057$, Figure 3.2 A). Development rates for egg, naupliar and copepodite stages were best described by an exponential-quadratic response. The estimated parameters for the slopes and curvature were not significantly different for eggs, nauplii or copepodites (Figure 3.2 B).

Although the power model was the best fit model for growth, the exponential-quadratic model also provided a good fit to the data (Table 3.4). The evidence ratio $(\omega_{\text{power}}/\omega_{\text{exponential-quadratic}})$ for these two models was 2.0, in this case a value <2.7 suggests relatively weak support for one model over another (Burnham and Anderson 2002). We have therefore also compared the best exponential-quadratic model for growth rate and development rate in Figure 3.3. This indicates a difference in the direction of the curvature for growth rate against temperature than development against
Figure 3.1. Simplified illustration of the effect of including species-specific random effects into a power model. (A) Power model including species-specific random effects in the intercept term. (B) Power model including species-specific random effects in the slope term. (C) Power model including species-specific random effects in both the slope and intercept term. Different lines indicate different species in all three panels.

Table 3.3. Different models used in the multilevel analysis including species level effects. Extra curvature parameter $\beta_2$ applies only to exponential-quadratic models, which include a temperature function within the exponent (see Table 3.2 for equations and symbol definitions).
Table 3.4. Best fit mixed effects models for each type of statistical model applied to marine copepod larval growth, development and egg rate data, including species-level effects. AIC is Akaike Information Criteria, $\Delta_i$ is the AIC difference and $\omega_i$ is the Akaike weight. Values for group level parameters are shown, along with standard errors (subscript values in brackets). All parameter values required the inclusion of species-specific terms (Appendices 3.1-3.3), except those in square brackets which are fixed across species. The overall best fit model is shown in farthest right column (Best Model), and is defined as that with the highest Akaike weight (see Table 3.2 for definitions of parameters). For growth rates, the power model was the best fit, with the exponential-quadratic model also providing a good fit. For larval development rates, the exponential-quadratic model was the best fit, and also for egg development rates.

<table>
<thead>
<tr>
<th>Rate</th>
<th>Model</th>
<th>Group parameter</th>
<th>AIC</th>
<th>$\Delta_i$</th>
<th>$\omega_i$</th>
<th>Best Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\beta_0$</td>
<td>$\beta_1$</td>
<td>$\beta_2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth Rates</td>
<td>Power</td>
<td>-1.64_{(0.14)}</td>
<td>1.37_{(0.15)}</td>
<td>54.36</td>
<td>0.0</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Exponential-quadratic</td>
<td>-1.65_{(0.14)}</td>
<td>1.47_{(0.20)}</td>
<td>-0.14_{(0.18)}</td>
<td>55.82</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Exponential</td>
<td>-1.65_{(0.15)}</td>
<td>0.09_{(0.01)}</td>
<td>117.18</td>
<td>62.8</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Arrhenius</td>
<td>-1.65_{(0.14)}</td>
<td>-0.68_{(0.05)}</td>
<td>105.00</td>
<td>49.6</td>
<td>0.00</td>
</tr>
<tr>
<td>Larval Development Rates</td>
<td>Power</td>
<td>-0.68_{(0.06)}</td>
<td>1.43_{(0.11)}</td>
<td>979.0</td>
<td>31.3</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Exponential-quadratic</td>
<td>-0.66_{(0.06)}</td>
<td>1.57_{(0.06)}</td>
<td>[0.34_{(0.05)}]</td>
<td>947.67</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Exponential</td>
<td>-0.69_{(0.07)}</td>
<td>0.11_{(0.00)}</td>
<td>981.12</td>
<td>33.5</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Arrhenius</td>
<td>-0.69_{(0.07)}</td>
<td>-0.77_{(0.03)}</td>
<td>967.42</td>
<td>19.8</td>
<td>0.00</td>
</tr>
<tr>
<td>Egg Development Rates</td>
<td>Power</td>
<td>-0.82_{(0.10)}</td>
<td>1.40_{(0.11)}</td>
<td>-0.160</td>
<td>40.1</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Exponential-quadratic</td>
<td>-0.77_{(0.10)}</td>
<td>1.61_{(0.07)}</td>
<td>0.37_{(0.07)}</td>
<td>-40.21</td>
<td>0.0</td>
</tr>
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<td></td>
<td>Exponential</td>
<td>-0.81_{(0.11)}</td>
<td>0.11_{(0.01)}</td>
<td>15.84</td>
<td>56.1</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Arrhenius</td>
<td>-0.80_{(0.11)}</td>
<td>-0.80_{(0.04)}</td>
<td>0.870</td>
<td>41.1</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Figure 3.2
Figure 3.2. Compiled data, together with overall best fit line and group-specific best fit lines (i.e. incorporating the random effect parameter values for species, stage, sex and study for each data set) for (A) growth rates vs. temperature, including naupliar (both across-stage NI-NII, NII-NIII etc. and across-naupliar rates (NI-NVI)), copepodite (across-stage and across-copepodite rates (CI-CVI)) and total (across NI-CVI) growth rates, (B) development rates vs. temperature, including naupliar (stage-specific and across-naupliar rates), copepodite (stage-specific and across-copepodite rates) and total (average from NI-CVI) rates, and (C) egg development rates vs. temperature.

Figure 3.3. (A) Comparison of best fit lines for growth rates of nauplii ($\ln g = -1.43_{(0.18)}+1.70_{(0.17)}*T_c$) and copepodite ($\ln g = -1.54_{(0.15)}+1.23_{(0.15)}*T_c$) stages vs. temperature using the power model (plotted on a log$_{10}$-log$_{10}$ scale). (B) Comparison of best fit lines for development rates of nauplii ($\ln D = -0.47_{(0.08)}+1.68_{(0.08)}*T_c+0.47_{(0.07)}*T_c^2$), copepodite ($\ln D = -0.83_{(0.08)}+1.51_{(0.07)}*T_c+0.32_{(0.05)}*T_c^2$) and egg ($\ln D = -0.77_{(0.10)}+1.61_{(0.07)}*T_c+0.37_{(0.07)}*T_c^2$) stages vs. temperature using the exponential-quadratic model (plotted on a log$_{10}$-log$_{10}$ scale). $g$ = growth rate (day$^{-1}$), $D$ = development rate (day$^{-1}$), $T_c$ = centred temperature ($\ln T$-$\ln15$), subscript values in brackets are standard errors.
Figure 3.3
Figure 3.4.
Figure 3.4. (A) Comparison of the exponential-quadratic model derived for the data set of growth (ln$g = \frac{1.65(0.14)+1.47(0.20)\times T_c-0.14(0.16)\times T_c^2}{T_c}$) and development rates (ln$D = \frac{-0.73(0.06)+1.57(0.06)\times T_c+0.32(0.04)\times T_c^2}{T_c}$). Combined data for both nauplii and copepodites were used for growth, and combined egg, nauplii and copepodite data for development. Although the power (shown in Figure 3.3) is the best fit model for growth, the AIC value for the exponential-quadratic shown here is similar, hence the demonstration of the alternative here. (B) Values of curvature ($\beta_2$) of the exponential-quadratic model applied to both growth and development rates. The values for $\beta_2$ are significantly different, resulting in a concave curvature for growth ($\beta_2 = -0.14$) and a convex curvature for development ($\beta_2 = 0.32$). Error bars give 95% confidence intervals. $g$ = growth rate (day$^{-1}$), $D$ = development rate (day$^{-1}$), $T_c$ = centred temperature (ln$T$-ln15), subscript values in brackets are standard errors.
temperature, with growth producing a concave curve and development a convex curve (Figure 3.3 A). These shapes, driven by parameter $\beta_2$, were subsequently compared and found to be significantly different (2 sample t-test, $t = 4.12$, $p<0.0001$, Figure 3.3 B).

Unlike development, the exponential-quadratic model for growth required species-specific values of parameter $\beta_2$, suggesting that this curvature is more variable than for development. This can be seen from the wider confidence intervals in Figure 3.3 B. It is important to note that despite this extra variability, there is still a significant difference between the parameter $\beta_2$ for growth and development rates (Figure 3.4 B).

_Predicting temperature-size change_

Using the best fit models for stage-specific rates of growth (power) and development (exponential-quadratic), and using $\exp^{(\text{g}D)}$ (see Equation 3.6), we predicted the ratio of $M_i$ to $M_0$ (i.e. relative increase in mass over a stage, Figure 3.4). As such this indicates the TSR for a particular stage. We found that this ratio did not decline monotonically, but rather followed a concave response, with a peak size towards the lower end of the temperature scale (at $\sim 11^\circ C$ in this case), and a steep decrease below this temperature, with a shallower, approximately linear decrease above these temperatures (Figure 3.5).

**Discussion**

Our analysis shows that for a wide range of marine copepod species, intraspecific growth and development rates have different temperature dependence, and therefore these rates must be at least partially decoupled. Although decoupling of these has been suggested previously (Atkinson 1994, Sibly and Atkinson 1994, van der Have and de Jong 1996, Forster et al. 2011a), our study is the first to systematically test for these
Figure 3.5. Relative change in the ratio of the mass at the end of a stage ($M_i$) to the initial mass ($M_0$), i.e. ($M_i/M_0$), vs. temperature in marine copepods. The graph is indicative of the response of body mass to temperature for any particular stage. The ratio was predicted from $\exp(g/D)$, using best fit lines for stage-specific development rates and growth rates vs. temperature. Hatched area shows portion of the reaction norm which follows an approximately linear temperature-size response. All y-axis values have been converted to percentage change from the maximum value.
differences, across multiple species. Further, these differences are maintained throughout ontogeny (Figure 3.3). On a log-log scale, development rate shows a convex response to temperature, whereas growth shows a linear or concave response: at lower temperatures the rate of decrease in development rate is reduced, but the rate of decrease in growth rate continues to decline linearly or becomes steeper. Conversely, at higher temperatures the convex shape of development results in the rate of increase in development rates being more rapid than that for growth rates: the latter continues to increase linearly or flatten on a log-log scale. This suggests that these two rates are controlled by mechanisms with different temperature dependence.

Temperature dependence of growth rate

Why should development rate be more temperature-sensitive than growth rate? Van der Have and de Jong (1996) suggested a mechanism based on cellular processes: growth depends primarily on the rate of protein synthesis, whereas development depends on DNA replication, and that these two processes differ with respect to the size of molecules involved. Protein synthesis is limited by the diffusion of massive ribosomal subunits into the cytoplasm, whereas the much smaller DNA polymerase enzymes are limited by the time taken to find the DNA template (i.e. an enzymatic process). Diffusion is less temperature sensitive than enzymatic processes: therefore, as diffusion is the limiting rate for growth (protein synthesis) but enzymatic process is the limiting rate for development (DNA replication), the former is less sensitive to temperature than the latter (van der Have and de Jong 1996). Our results show growth to be best modelled by a power model. Both the power and exponential-quadratic model produce similar AICs, and receive stronger support from the data than exponential-based models (see Table 3.4). The mechanistic basis for using power functions is that many biological
processes are controlled by physical processes, such as diffusion and viscosity, that follow a power-form response to temperature, rather than chemical processes, that follow Arrhenius type functions (Ahlgren 1987). Our results may be indicative of growth being limited by a process such as diffusion, which supports the hypothesis of van der Have and de Jong (1996). The Akaike weights show the exponential-quadratic model with a species-specific curvature term ($\beta_2$) also provides a good fit to growth data, suggesting that the thermal response of growth may vary considerably between species; thus the relationship between temperature and growth may be more variable than between temperature and development.

Temperature dependence of development rate

The exponential-quadratic model applied to development rates has a convex form, unlike growth. The curvature term ($\beta_2$) does not require a species-specific term and is relatively invariable (as shown by the narrow confidence intervals in Figure 3.4 B). We find that development rate is more temperature sensitive at higher temperatures than growth, but that it is not best modelled by an exponential function, as would be expected for a thermal response limited by enzymatic rates (as suggested by van der Have and de Jong (1996) and proponents of the Metabolic Theory of Ecology (Gillooly et al. 2001, Gillooly et al. 2002)). Unlike for growth, all other models types, including power, exponential and Arrhenius functions perform poorly at modelling development rate data; thus the exponential-quadratic form is the only acceptable model (Table 3.4).

Modelling growth and development rates

Many researchers have modelled the effect of temperature on growth within-species using an exponential function (Escribano and McLaren 1992, Escribano et al. 1997, Campbell et al. 2001) and development using a power function (Belehradek 1926,
McLaren 1969, Corkett and McLaren 1970, Hart 1990, Peterson 2001). Our work, however, suggests that a power model is a better fit for growth rates and that development is more curved and complex than a power function. As an exponential-quadratic model also describes growth well (although with significantly different parameter estimates than development, Table 3.4), it may be most suitable to apply this same model type to both rates. In the case of development, this model can be applied with a fixed estimate for parameter $\beta_2$ of 0.32 (Figure 3.4 B). Unlike development, growth would require a species-specific variable for $\beta_2$, with an initial variable estimate of -0.14 (Figure 3.4 B). If growth does not require the extra concave curvature provided by $\beta_2$, and can be modelled instead by a power model, this will be obvious from the statistical output ($\beta_2$ not significantly different from 0). These power model types are simpler than other equation forms, such as the Sharpe-Schoolfield equation that would require data for the viability of the different copepod species across their thermal range, which was not available, nor practical to collect. When making broad predictions regarding life-history rates across a range of species, it is important to attain a balance between complexity and simplicity of a model. Using the equation forms suggested here, along with a linear mixed effects model, captures the changes in these rates across different copepod species whilst requiring few parameters and thus strikes this balance.

Ontogenetic rate changes
To date, few studies have compared the temperature dependence of growth and development rates within a species (Smith-Gill and Berven 1979, van der Have and de Jong 1996, Davidowitz and Nijhout 2004, Walters and Hassall 2006, de Jong 2010). These studies examine these rates at a coarse resolution, e.g. using total time from the initial larval stage to adult emergence (van der Have and de Jong 1996, de Jong 2010),
but there is a general lack of knowledge about how these rates might change through ontogeny. We have tested this across a range of marine copepod species and have shown that the equation forms that best describe the thermal response of growth and development are maintained throughout ontogeny. However, the thermal response of development rates are more consistent through the life cycle than is growth. The convexity and slopes of development are similar throughout ontogeny (Figure 3.3 B), suggesting an equal thermal sensitivity of different life stages. This is further supported by evidence of the “equiproportional rule” in copepods: at different temperatures, specific life stages always occupy the same fixed proportion of the total larval time (Hart 1990). In contrast, the thermal response of naupliar growth is somewhat steeper than copepodite growth (Figure 3.3 A). This suggests there may be an ontogenetic effect on the thermal sensitivity of growth. As an organism gets larger, the thermal sensitivity of its accrual of mass per time (growth rate) becomes reduced, but the rate at which is passes through life stages maintains the same thermal response. This is a tentative conclusion, however, as the differences between the slopes for naupliar and copepodite growth are not significant at a 5% level; more naupliar growth rate data are needed to confirm this.

**Potential inadequacies in current ecological theory**

The fact that growth and development rates do not have the same thermal response has important implications. For instance, a central proposition in the Metabolic Theory of Ecology (MTE) is that physiological rates (e.g. respiration rate, growth rate, rate of molecular evolution) follow an Arrhenius function, and have the same temperature dependence (Brown et al. 2004). Although metabolic rates (thus respiration rates) may scale with temperature following an Arrhenius-type response (Gillooly et al. 2001),
proponents of the MTE have extrapolated beyond this by assuming that many other physiological and population rates have this same temperature dependence (Brown et al. 2004, Anderson et al. 2006). The Arrhenius function is now widely used to correct many different life-history rates to temperature (Clarke and Johnston 1999, Gillooly et al. 2001, Brown et al. 2004), yet our data suggest that this should be conducted with caution.

A previous meta-analysis of planktonic larval development times also found a power model was better supported than the Arrhenius (O'Connor et al. 2007). They suggested the lack of intraspecific mass correction could provide an explanation as to why the Arrhenius was not the best fit model. The Arrhenius equation assumes that rates are mass-normalised (Gillooly et al. 2002), and although this was not possible in our analysis either, the use of random intercept term for species allows for differences in absolute rates between different species caused by parameters such as mass, therefore this mass correction across species should not be necessary. We did not have the available data to mass correct for the temperature-size responses within species. However, we can make some predictions of the impact of our TSR thermal reaction norm on growth and development rates. According to the MTE, rates scale with the product of temperature (an Arrhenius function) and mass^{-1/4}, i.e. within a species, smaller individuals have relatively higher rates. Our data incorporates changes in rate associated with changes in mass within the temperature dependence, thus we would expect temperature dependence to be exaggerated in the relationships we find. Effectively, empirical data for both growth and development rates should show more curvature than the Arrhenius model would predict, because mass correction was not possible. In fact, we find the empirical data for growth and development are best
modelled by power models, which are inherently less curved than exponential functions (such as the Arrhenius). Further, we find the development rate model for eggs to be best described by an exponential-quadratic model which is not significantly different to that of copepodite stages, despite eggs showing less change in mass with temperature than later stages (Forster et al. 2011a). Intraspecific temperature-dependent size changes are therefore unlikely to explain the poorer fit of the Arrhenius function. The MTE in its current form does not allow for differences in the form of the temperature dependence of growth and development rates which we observe, and consequently it does not predict the widely observed TSR outcome, which is the result of such decoupling (Forster et al. 2011a).

*Predicting temperature-size changes*

There is currently no single agreed shape for the response of ectothermic body mass to temperature. A linear decrease in volume with increasing temperature has been suggested in protists (Atkinson et al. 2003), whereas more complex thermal reaction norms have been suggested for some metazoans (David et al. 1994, Karan et al. 1998, Davidowitz and Nijhout 2004, de Jong 2010). Many studies of metazoans use negative linear models to express change in body mass with temperature, often with mass having only been recorded over a small thermal range (2-3 temperatures, e.g. Partridge et al. 1994, Fischer et al. 2004, Stillwell and Fox 2005). Using the thermal response of stage-specific development and growth rates, our study predicts that the relative mass increase (the ratio $M_i/M_0$) follows a concave response to temperature (Figure 3.5). Among the marine copepods, we found a highly positively skewed thermal reaction norm, with the greatest relative body size increase at a low temperature and a long tail of reducing size ratios with increasing temperatures. It might be that this tail, with a relatively linear
decrease, is responsible for the TSR being typically described as a simple linear function of body size (Partridge et al. 1994, Blanckenhorn and Llaurens 2005).

Interestingly, our predicted thermal reaction norm of body mass ratios shown in marine copepods is similar in form to that predicted using data for the tobacco hornworm moth Manduca sexta (Davidowitz and Nijhout 2004). Davidowitz and Nijhout (2004) demonstrated a peak in size at a low temperature, with a steep drop from this peak as temperature declines, but a less steep decline in size with increasing temperature. This shape is also commonly found in Drosophila species (data for both wing length and mass, Ray 1960, David et al. 1997, Karan et al. 1998, Petavy et al. 2001), aphids (Lamb et al. 1987), aquatic insects (Vannote and Sweeney 1980), leeches (Young and Ironmonger 1982) and frogs (Smith-Gill and Berven 1979). It may be that this mass thermal reaction norm is in fact common in ectotherms, but not widely reported due to a lack of data at the colder temperatures where size decreases. The prolonged development time associated with cold temperatures often makes the rearing of organisms more challenging, and probably explains the scarcity of data. To compare our predicted response with empirical data for marine copepods, we searched the literature for adult mass data, where mass had been measured at >3 temperatures. We found that for the 6 species where data were available, all followed the temperature-size rule, decreasing in size over the majority of their thermal range. Furthermore, a concave thermal reaction norm was present in 3 species: Acartia tonsa (Hansen et al. 2011), Pseudodiaptomus dubia (Li et al. 2009) and Sinocalanus tenellus (Kimoto et al. 1986). In those species not displaying the predicted concave shape: Calanus sinicus (Uye 1988), Paracalanus sp. (Uye 1991) and Pseudocalanus newmani (Lee et al. 2003), data were not available for the very lower end of their thermal range, which may explain
why the decline in size at the lowest temperatures was not observed. The response of adult size to temperature clearly now requires more extensive examination across more ectothermic taxa and thermal ranges.

Body size is a primary determinant of many ecological properties, including fecundity, mortality and growth rates (Fenchel 1974, Blueweiss et al. 1978, Hirst and Kiørboe 2002, Brown et al. 2004, Kiørboe and Hirst 2008), species interactions (Arendt 2007) and food web structure and dynamics (Warren and Lawton 1987, Yodzis and Innes 1992, Woodward et al. 2010a, Woodward et al. 2010b). Changes in the size of organisms with temperature will therefore impact on many different ecosystem processes. The world is warming at an unprecedented rate in human history (IPCC 2007), and our study highlights the potentially important impact of temperature change on copepod size. Although they represent only a single taxon, copepods are the most abundant zooplankton in the marine biota, forming the principal trophic link to marine fishes (Huntley and Lopez 1992, Turner 2004, Richardson 2008). Our results point towards a marked decrease in copepod size with increasing temperature across the majority of the thermal range, which may alter their role as both predators and prey, given the size dependency of such processes (Hansen et al. 1994). However, our analysis also suggests that some copepod species living towards their lower thermal limit may actually increase in size with an increase in temperature. Further, as the magnitude of the size change is larger in older life stages than younger stages (see Forster et al. 2011a), predicting ecosystem consequences of temperature change is challenging. To give an example, copepod adults (temperature-sensitive older life stage) are important prey for early larval fish, yet the size of these early stages are likely to be less temperature-sensitive than the adult copepods (Forster et al. 2011a); thus the size
changes in the predators and prey may be dissimilar. This could impact on the fishes’ prey choice, altering the structure of food webs and impacting on populations across the whole food chain. Although the TSR is a phenotypic plastic response, short-term seasonal fluctuations in temperature can affect ecosystem dynamics (Sims et al. 2004), and could therefore drive longer term changes in community structure.

Using a systematic approach, we have shown that the fundamental physiological rates, growth and development, have different temperature dependence when examined across a range of species within marine pelagic Copepoda, with clear implications for wider ecological theory and for assessing the potential impacts of global warming on species size.
CHAPTER 4

The Temperature-size Rule emerges from ontogenetic differences between growth and development rates

Introduction

The temperature-size rule (TSR) describes the smaller adult size achieved in an ectothermic species reared at warmer temperatures. Adult size is in effect a product of growth rate (increase in weight per time) and development rate (increase in life stage per time), and the TSR signals that these rates must be decoupled (Sibly and Atkinson 1994, van der Have and de Jong 1996, Kingsolver and Huey 2008). Much of the focus of the TSR has been on explaining the ultimate reason for size change (Walters and Hassall 2006, Kingsolver and Huey 2008, Arendt 2011). However, to understand why size changes, we first need a clearer understanding of how size changes are generated. In particular, there remains a lack of data available on growth and development rate decoupling through ontogeny. Decoupling of these rates is most often inferred from differences between size at some final life stage and the time taken to reach this mature stage (Partridge et al. 1994, Blanckenhorn 2000, Stillwell and Fox 2005), without considering the ontogenetic timing of these size changes at a higher resolution. Previous work on ectotherms has found temperature acclimated adults show a greater temperature-size response than do acclimated progeny, such as eggs (Chapter 2, Forster et al. 2011a). A recent analysis of marine pelagic copepod data has shown development rates to have greater temperature dependence across all life history stages than growth rates (Chapter 3, Forster et al. 2011b). Further, there was weak support for growth rate being more temperature dependent at smaller, early life stages than later stages (i.e.
slopes of natural-logged growth rates against temperature were steeper at early life stages), suggesting a size-dependent or ontogenetic component in these crustaceans. Similarly, analyses of the interaction between growth and development during ontogeny for the tobacco hornworm moth *Manduca sexta* have shown the TSR to emerge only during later larval stages (Davidowitz et al. 2004, Davidowitz and Nijhout 2004, Nijhout et al. 2006, Diamond and Kingsolver 2010). However, we still lack experimental data that focuses on the timing of ontogenetic temperature-size changes at a high resolution. Mortality rates are significantly higher during early larval stages of many species (McConaugha 1992, Cornell and Hawkins 1995, Hirst and Kiørboe 2002): this is an intense period of selection with important fitness consequences for species. It is therefore essential that we gain a better understanding of how growth and development rates, along with size, change during ontogeny and the degree to which they are temperature dependent. In this study we test these ideas in detail using *Artemia franciscana* (Kellogg) as a model crustacean. We examine size, growth and development rates at a high temporal resolution through ontogeny.

*The impact of acclimation on the TSR*

The majority of studies investigating the TSR through growth and development rates expose eggs or early larval stages to novel temperatures and observe the effect on adult size (Smith-Gill and Berven 1979, Partridge et al. 1994, de Jong 2010). However, organisms are capable of acclimating to different temperature regimes; for example, the metabolic rate of fishes transferred from a low to high temperature initially increase substantially but then reduces toward an acclimatory rate (Johnston and Dunn 1987). A previous meta-analysis examining the TSR in organisms acclimated to their thermal environments found body size of egg and early larval stages to be less temperature
dependent than adult stages (Forster et al. 2011a). Further, data on size changes through ontogeny in two copepod species, acclimated to different temperatures for multiple generations, showed size to effectively “reset” at the beginning of each generation, i.e. eggs showed no temperature-size response yet adult stages followed the TSR (see Forster et al. 2011a). This suggests that the drivers of the TSR, growth and development rates, remain decoupled, although the rates themselves have not been directly measured across multiple generations. This study tests this directly by measuring these rates over two generations in *A. franciscana*, to see the degree to which these two rates are decoupled.

Whether patterns in temperature-size responses are similar across closely-related species needs to be addressed. *Artemia franciscana* are within the Anostracans; Anostracans are the closest example to primitive crustacean morphology, and provide the nearest case of the presumed ancestral state (Browne et al. 1991). It may be that other crustaceans exhibit similar size changes through ontogeny as *Artemia*. We therefore need to compare the ontogenetic temperature-size data across crustaceans more broadly.

We address the following questions: 1. Do the temperature dependence of growth and development rates in *Artemia franciscana* vary through ontogeny, and how does this impact the temperature-size response? 2. Does the impact of temperature on these rates differ between the first and second generation? 3. Is the ontogenetic basis of the temperature-size response similar across crustaceans?

**Methods**
Experimental design

Batch cultures of *Artemia franciscana* were established using decapsulated cysts. These cysts had been collected from the Great Salt Lake (Utah, USA), disinfected, and decapsulated (provided in this state by the company Waterlife). A minimum of ~300 cysts were placed in 1L beakers containing 900mL GF/F filtered seawater with a salinity of 30 at a range of constant temperatures (20, 22.5, 25, 27.5, 30, 32.5 and 35°C). Air stones were used in each beaker to ensure the water was sufficiently aerated. Cultures were maintained at fixed temperatures using Grant SUB Aqua 26 water baths (held within +/- 0.2°C of control temperature). Upon hatching, stage 1 nauplii were transferred from the batch cultures into a minimum of two separate replicates at each temperature (with n = 50 per replicate), initiating the first generation of the experiment. As cultures did not survive for long at 35°C, these were not continued. After hatching, nauplii were provided *ad libitum* with the cyanobacteria *Arthrospira plantensis*. Replicates were fed a minimum of 10mL of saturated *A. plantensis* solution (where no more cyanobacteria could be suspended in solution) every day such that a green colouration was visible and was maintained in the cultures at all times. All individual *A. franciscana* were staged and their total body length measured on a daily basis using a light microscope.

Larvae were largely staged according to the methods of Weisz (1946), using the number of segments. The first three segments appear at the same time at stage 3 and therefore stage 1 and 2 were defined by observation of colour and ocellus pigmentation. At stage 1 the nauplius has an orange colour given by yolk content; at stage 2 this colouration has gone and the ocellus becomes pigmented, allowing these two stages to be separated.
From stage 3 to 11 developmental stage was determined following the methods employed by Weisz (1946). Within each segment formation, there are four stages:

Stage a: Transverse ring of thick mesoderm under smooth epidermal layer.

Stage b: Partial transverse constrictions appear externally in epidermis.

Stage c: Constrictions become complete and deepen with cylindrical shape, in thoracic segments this is accompanied with the development of appendage buds.

Maturity: Appendages are independently motile.

From stage 3 to 11, stage was determined by counting the number of thoracic segments which had reached stage c. For stages 12 to 17, because segments are added to the post-abdominal section, and are generally flatter than thoracic segments, stages are more difficult to separate. Unlike Weisz (1946), we therefore determined stages 12 to 17 by appendage motility (i.e. when segments had reached maturity, rather than stage c). At stage 12, the first five sets of appendages are independently motile; at stage 13 the first six pairs etc, until all but 1 pair of appendages are motile, at stage 17. Once larvae reached stage 17, we ceased staging as *A. franciscana* continue to grow without the addition of further segments or appendages, making accurate assessment difficult.

Dry weights were calculated from lengths using the length-weight regression line in Figure 4.1 of Reeve (Reeve 1963). As the original regression equations were not given in this paper, we reconstructed these by extracting data from along them using the graphics program “Grab It!”, and determining equations that overlaid their original responses. The length-weight equation for *Artemia* consisted of two parts, the length range 0.4 to <1.6mm, described by the equation:
And the length range ≥1.6 to 4mm, described by:

\[ \log_{10} DW = 0.4926 \times e^{1.338 \times \log_{10} L} + 0.0048 \times e^{14.4163 \times \log_{10} L} \quad \text{Equation 4.1} \]

where \( DW \) = dry weight (μg) and \( L \) = length (mm). The point of inflexion (at 1.6mm) corresponds to approximately stage 10, suggesting this change in length-weight regression slope from Equation 4.1 to Equation 4.2 may be driven by changing growth patterns from thoracic to abdominal growth.

Once animals reached the adult stage in the cultures we inspected for the presence of their nauplii on a daily basis. A total of 50 nauplii were removed from each temperature replicate with a Pasteur pipette, and placed into new beakers; this was the initiation of the second generation. In a small number of cases (3/12 cultures) there were insufficient nauplii from the experimental culture; in these cases we supplemented with nauplii from other batch cultured adults, maintained at the same temperature as the replicate in question and at saturated food conditions. Development and growth experiments were started for the second generation replicates, the time at first appearance of nauplii defining \( t = 0 \). Body lengths and stage were determined daily on live individuals, as conducted for the first generation, and the same feeding regime was used also. Across both generations water in all replicates was changed weekly; between these changes, any water loss via evaporation was replaced using distilled water to maintain salinity.

\[ \text{Calculating Growth and Development Rate} \]
Individual dry weights were estimated from length measurements using equations calculated from Reeve (1963, Equation 4.1, 4.2), and average weight calculated for each replicate at each observation point. Weight-specific growth rates \((g, d^{-1})\) were determined as:

\[
g = \frac{\ln(DW_t/DW_0)}{t}
\]

Equation 4.3

where \(DW_t = \) dry weight at time \(t\), \(DW_0 = \) dry weight at previous observation point, and \(t = \) time between observations (days).

Although measurements were taken daily, as in some instances >3 stages can pass in a single day, growth rates were calculated during ontogeny by combining data for 3 stages together: specifically stages 3-5, 6-8, 9-11, 12-14 and 15-17. We term each of these “phases”. As the two initial stages have very rapid development (<24 hours for transition through both stages at most temperatures) we did not include these. Growth rates are presented in Appendix 4.1.

Development times were calculated as median stage-specific development times, i.e. from initial nauplii introduction to the point at which 50% of individuals reached stage 2, then from this point to the point at which 50% of individuals reached stage 3, etc. These median development times were calculated (for each replicate) from stage frequency data, following the methods of Campbell et al. (2001). Median development times were calculated for each stage from 1 to 17 inclusively. These development rates were then grouped into the phases (Appendix 4.2), to allow direct comparison with growth rates.
Model fitting

Growth rates and development rates were available for different phases (e.g. stages 3-5, 6-8 etc), replicates and for 1st and 2nd generations. A linear mixed effects model was used to account for the differences in phase and generation number, incorporating phase as a random effect and generation number as a fixed effect (see Chapters 2 and 3). Replicates were not significantly different (p>0.05) thus this was not included as a factor within the mixed effects model. We applied a range of statistical models to the data (power, exponential and Arrhenius) to determine which best described the data. We centred the data for both growth and development rates around the mid-temperature of the experiment (25°C), following the methods used in Chapters 2 and 3. Using a log-likelihood ratio test, we then discerned which variation of these equations best modelled data for each rate. These procedures were followed to determine whether ontogeny (i.e. phase) impacts the intercept of growth and development and/or the slopes. Similarly, these procedures were used to determine whether the two generations show differences, both in their intercepts, and/or their slopes. The Akaike weights were subsequently calculated for each model type, to determine which model (power, exponential or Arrhenius) best described the data.

Comparison of weight within stage

To estimate the temperature effect on weight at stage, and to discern the importance of generation number on organism size, we described the effect of temperature on dry weights for each stage using a linear mixed effects model and an exponential equation form. A previous analysis of multicellular species data for weight vs. temperature has shown this equation form to best describe temperature-size data (Forster et al. 2011a). Further, we confirmed here, using Akaike weights, that other model forms (power and
Arrhenius) did not provide a better fit to the data. We followed the same method used for modelling growth and development rates, except using individual stages (3, 4, 5 etc.), rather than phases. The equation applied was of the form:

$$\ln DW = \ln a + bT_c + \epsilon$$  \hspace{1cm} \text{Equation 4.4}$$

where $DW = \text{dry weight (μg)}$ and $T_c = \text{the centred temperature (the natural-logged experimental temperature (°C) minus ln25)}$. Stage-specific weights are available in Appendix 4.3. Having calculated the stage-specific slopes for all larval stages of $A. franciscana$, we converted these slopes to % change in weight per °C using the formula $(\exp^{\text{slope}} - 1)*100$. We collected the eggs produced as the second generation at each temperature (> 50 eggs for each temperature) and measured their diameter ($E$). Eggs were near-spherical and volume calculated as $4/3*\pi(E/2)^3$ (see Appendix 4.4). The slope of egg volume against temperature was calculated using generalised least squares regression and Equation 4.4.

**Comparison of ontogenetic temperature-size response for crustaceans**

To compare the temperature-size response of *Artemia franciscana* with other species, we searched the wider literature for data on weight at stage vs. temperature in other crustaceans, measured through ontogeny. We searched the ISI Web of Knowledge using the search terms “larva* AND temperature AND (mass OR size OR weight)” along with previous data collected for copepod species (Forster et al. 2011b) and other multicellular organisms (Forster et al. 2011a). We included data where size had been measured for ≥ 3 temperatures at ≥ 2 larval stages. We applied a linear mixed effects model to each species in turn, using Equation 4.4 and following the same methods as
applied to the *A. franciscana* data.

**Results**

*Artemia franciscana* were successfully reared over two generations at fixed temperatures ranging between 22.5 and 32.5°C. Although those reared at 20°C reached adulthood and reproduced at the end of the first generation, the 2nd generation did not reach maturity. Growth rate changed during ontogeny, with two distinct trajectories: the first being early larval growth during the formation of thoracic segments (stages 1-11), and the second trajectory during the formation of abdominal segments (stages 12-17); growth was distinctly faster during this second period (as demonstrated by the steeper slope of ln weight vs. time, Figure 4.1A). Our phases were defined so as not to combine stages across this division. Such marked shifts though ontogeny were not present in development rates (Figure 4.1B).

**Model fitting**

The Akaike weights revealed the power model to be the best fit model for both growth (with phase-specific slopes and intercepts, Akaike weight of 0.52) and development rates (with phase-specific intercepts only, Akaike weight of 0.53). These models have been shown to be the most appropriate for other crustaceans, i.e. marine copepods (Forster et al. 2011b), and planktonic larval species (O’Connor et al. 2007).

Furthermore, the exponential and Arrhenius models were a poorer fit in all cases (Akaike weights <0.33). Growth rates (*g*, day⁻¹) and development rates (*D*, day⁻¹) were therefore modelled as functions of temperature (*T*, °C) using the power equation. The inclusion of generation number within the power equation did not improve the fit for either growth or development, and therefore this identifier was removed from the
Figure 4.1. (A) *Artemia franciscana* progression of ln(dry weight) over time (days) at different temperatures. Early larval stages (where thoracic segments are added) show a shallower slope than later larval stages (abdominal growth), i.e. growth rate is lower in the earlier stages. Data are from individual replicates during the 1<sup>st</sup> generation. (B) *Artemia franciscana* increase in development stage (1-17) with time. Data represent the median development times for the same replicate from the 1<sup>st</sup> generation (as in A). Development rates do not show two distinct trajectories during ontogeny as does growth.
Figure 4.2.
Figure 4.2. *Artemia franciscana* weight-specific growth rates vs. temperature (°C) across 5 ontogenetic phases (stages 3 to 5, 6 to 8, 9 to 11, 12 to 14, 15 to 17). Triangles are mean values for the 1st generation, squares are means for the 2nd generation. Panel 6 shows the best fit models (i.e. power) for each of the phases. All regressions are fitted through data from both generations combined. Regression equations are provided in each panel, where $g = \text{growth rate (day}^{-1})$ and $T_C$ is temperature $T$ (°C) centred around 25°C (i.e. $T_C = (\ln T - \ln(25))$. Note the log_{10}-log_{10} scale, error bars represent 95% confidence intervals.

Figure 4.3. *Artemia franciscana* stage-specific development rates vs. temperature (°C). Rates are presented as averages across the 5 phases (stages 3 to 5, 6 to 8, 9 to 11, 12 to 14, 15 to 17). Triangles are mean values for the 1st generation, squares are means for the 2nd generation. Panel 6 shows the best fit models (power model) for each of the phases. All regressions are fitted through data from both generations combined. Regression equations are provided in each panel, where $D = \text{development rate (day}^{-1})$ and $T_C$ is temperature $T$ (°C) centred around 25°C (i.e. $T_C = (\ln T - \ln(25))$. Note the log_{10}-log_{10} scale, error bars represent 95% confidence intervals.
\[ \ln D = 0.78 + 1.97 \times T_C \]

\[ \ln D = 0.84 + 1.97 \times T_C \]

\[ \ln D = 0.95 + 1.97 \times T_C \]

\[ \ln D = 0.80 + 1.97 \times T_C \]

\[ \ln D = 0.66 + 1.97 \times T_C \]

Figure 4.3.
linear mixed effects models. The lack of improved fit suggests no significant difference between 1\textsuperscript{st} and 2\textsuperscript{nd} generation for growth or development rates. For growth rates, the best fit model required the inclusion of phase as a random parameter within both the slope and intercept term. This suggests that growth rates vary between phases (intercept), i.e. some have faster weight-specific growth than others, but also that different phases have a different temperature dependence (i.e. slopes, see Figure 4.2).

By contrast, the fit of the development model was not improved with the incorporation of phase as a random effect in the slope term, the best fit model required phase to be incorporated within the intercept term only (Figure 4.3). This suggests that some phases develop more quickly than others, but they have the same temperature dependence regardless of phase (i.e. slopes are similar).

\textit{Comparison of weight within stage}

We compared the temperature-size response across different stages of \textit{Artemia} using the linear mixed effects model applied to Equation 4.4. This revealed a temperature dependence of stage-specific weight, but no significant improvement of the model with the addition of generation number. This was supported in the results of the models applied to growth and development: generation number did not appreciably change the thermal response of these rates. To demonstrate this, we present the stage-specific best fit models in Figure 4.4, but including the generation number term in the model, to show the slopes of these models are near-identical for the 1\textsuperscript{st} and 2\textsuperscript{nd} generation. The lowest temperature (20°C) was excluded from this analysis as the reared individuals failed to reach maturity in the second generation. Furthermore, size decreased at this temperature in later stages (Figure 4.5); thus it was excluded to maintain the simplicity of the exponential model (Equation 4.4). The stage-specific temperature-dependent
Figure 4.4. *Artemia franciscana* larval stage dry weights (µg, log10 y-axis) vs. temperature (°C). Regression lines represent the best fit to the data using a linear mixed effects model and Equation 4.4. Early larval stages (1 to 8) show an inverse TSR, with TSR being established from stage 12 onwards (asterisks denote negative slopes which are significantly different from zero). These slope values and confidence intervals are shown in the first panel of Figure 4.6. Best fit lines are given separately for the 1<sup>st</sup> and 2<sup>nd</sup> generation. There was no significant effect of generation on dry weight values, as including this within the mixed effects model did not improve the fit.
Figure 4.5. (A) *Artemia franciscana* dry weight (µg, log₁₀ y axis) vs. temperature (°C) for stage 17, the final larval stage. Triangles represent the 1st generation, squares the 2nd generation. The best fit model (linear mixed effects model, Equation 4.4) was calculated and shows the TSR equivalent to -2.96% dry weight °C⁻¹. (B) *Artemia franciscana* egg volume (mm³, log₁₀ y axis) vs. temperature (°C). The best fit line (generalised least squares regression model, Equation 4.4) shows no TSR, with a change in volume equivalent to 0.08% °C⁻¹.
slopes revealed an inverse temperature-size response at early larval stages (Stages 1-7, Figure 4.4). The temperature-size response became flat during intermediate larval stages (Stages 8-10), before establishing the more typical TSR at stages 11 onwards (Figure 4.4), with a significant decrease in weight with increasing temperature for stage 12 onwards. We compared final larval size data to egg size in Figure 4.5. Final larval size (excluding 20°C) showed a significant negative slope of dry weight vs. temperature (-2.96% °C⁻¹, 95% CIs ± 0.62%), whereas the slope of egg volume vs. temperature was not significantly different from zero (0.08% °C⁻¹, 95% CIs ± 0.40%).

Comparison of ontogenetic temperature-size response for crustaceans

Along with data for A. franciscana, we collected temperature-size data for 10 other crustacean species including 7 copepod, 2 crab and 1 daphnid species. The % change in weight.°C⁻¹ is plotted for each species through ontogeny in Figure 4.6. This revealed a general pattern of declining slopes with increasing stage. These patterns were very similar in all 11 crustacean species. Early larval stages show an inverse or no TSR, whereas later stages show a strong TSR, with weight changes varying between -1 to -4.5% per °C (Figure 4.6). Data for 7 out of the 8 species where very early larval or egg sizes were available (Stages 0-2, Figure 4.6) show no significant change in progeny size with temperature. In many cases the adult stage showed the strongest temperature-size response, although some showed a reduction in the response into the final stage(s).

Discussion

Temperature dependence of growth and development

There were clear differences between the temperature dependence of growth and development rates in Artemia franciscana. While the temperature dependence of growth
Figure 4.6. The temperature-size response through ontogeny in a variety of crustacean species; slopes are expressed as % change in weight per °C for a given stage. Species-specific life stages were assigned arbitrary values from egg (0) to first instar (1) onwards. Therefore there is no relation, for example, between stage x of a crab species and stage x of a copepod species, but these are comparable within a group (copepod-copepod, crab-crab etc.). Arrows indicate the adult stage. Daphnia pulex data are embryonic stages (development within mother) therefore adult stage is not indicated. Error bars represent 95% confidence intervals.
rates decrease with size/life stage (see slopes in Figure 4.2), development rates had similar temperature dependence throughout ontogeny (see slopes in Figure 4.3). Other development rate data from the crustacean literature supports this outcome more widely. Copepods maintain the temperature dependence of development during ontogeny; this is in effect the widely observed equiproportional development rule, where specific life stages occupy a fixed proportion of the total life cycle across different temperature regimes (Hart 1990). As earlier larval stages of *Artemia franciscana* show a greater temperature dependence of growth rate than later larval stages, this suggests these rates are stage/size dependent (see Figure 4.2). Evidence for this in other crustaceans is scarce. Data for the crab *Carcinus maenus* follow a similar pattern, with growth rates of early zoeal stages being more temperature dependent than later larval stages (Dawirs et al. 1986). There is also weak support from data for growth rates in marine copepod species (Forster et al. 2011b); specifically, the slopes of early nauplius logged growth rates vs. temperature were found to be steeper than those of later copepodite stages, although this result was not significant at a 5% level (2 sample t-test, t = 1.94, p = 0.057; Forster et al. 2011b). A decrease in the temperature dependence of growth through ontogeny (with increasing stage/size) in *A. franciscana* has important implications for the TSR. Growth rates are more temperature dependent than development rates in the early larval stages (Figures 4.2 and 4.3), resulting in a reverse TSR: body size in early larval stages increases with increasing temperature (Figure 4.4). Only during later stages, when the temperature dependence of growth is less than that of development, is the TSR established. Therefore the appearance of the TSR is not determined solely by the temperature dependence of growth rates changing through ontogeny, but on growth having a lower temperature dependence than development rate in later larval stages. This highlights the importance of following changes in both
growth and development rates throughout ontogeny.

Potential mechanisms of rate change

Does a mechanism exist to explain why the temperature dependence of growth (the slopes in Figure 4.2) decreases with increasing size in *Artemia*? Previous mechanistic models to explain changes in growth rate with size and temperature have been based on the von Bertalanffy (1957) growth equation:

\[
\frac{dW}{dt} = kW^m - lW^n \quad \text{Equation 4.5}
\]

where \( W \) = body weight, \( k \) is the coefficient of anabolism, \( l \) is the coefficient of catabolism and \( m \) and \( n \) are exponent parameters. Increasing temperature can alter maximal body size by changing either the coefficients or the exponents. Previous work by Perrin (1995) and Strong and Daborn (1980) has produced two mutually exclusive mechanisms based on the von Bertalanffy (1957) growth rate model to explain changes in growth rates associated with temperature. Perrin (1995) showed optimal life history to follow the TSR when the temperature dependence of the catabolism coefficient \( l \) is greater than that of anabolism \( k \), assuming exponents \( m \) and \( n \) are constants (0.75 and 1 respectively). Conversely, Strong and Daborn (1980) used data for the isopod *Idotea baltica* to argue that smaller size is driven by a decrease in \( m \) (from approximately 1.0 to 0.7) and increase in \( n \) (0.7 to 1.0) with increasing temperature, resulting in different allometries of anabolism and catabolism. Our results suggest that neither of these proximate mechanisms are sufficient to explain the change in growth rates in *A. franciscana*. Perrin’s (1995) model assumes a decelerating rate of weight-specific growth through ontogeny for small size to be optimal at higher temperatures. This is not
the case in *A. franciscana*, where growth rates are faster during the later stages in which abdominal segments are added (Figure 4.1), nor is it the case in a large number of species including amphibians, cnidarians, crustaceans, fish, insects, molluscs and reptiles (see review by Angilletta et al. (2004)). Strong and Daborn’s (1980) model implies that the temperature for maximal growth rate decreases with increasing size. We do not find support for this, as growth rate was always at its maximum at the highest temperature (32.5°C) in *A. franciscana*. Both interpretations of the von Bertalanffy (1957) growth equation are therefore inadequate at describing changes in growth rates in *A. franciscana*. Both coefficient terms and exponents would have to change to accommodate differences in growth rates across different phases and at different temperatures (Kozłowski et al. 2004). The lack of mechanistic explanation provided by the von Bertalanffy (1957) highlights the problems associated with this model type; indeed, a mechanistic explanation for why the temperature dependence of growth decreases with increasing size remains elusive.

**The impact of acclimation on the TSR**

The temperature dependence of growth and development rates for any particular phase of *Artemia franciscana* did not change between the first and second generation, with similar ontogenetic patterns in the decoupling of growth and development rates in both (see Figure 4.4). Further, generation number did not have a significant effect on body size through ontogeny (i.e. there were no size differences between first and second generation of organisms). We therefore suggest that acclimatory compensation of growth and development rates to novel thermal environments may be extremely rapid. Is this supported by data for other species in the wider literature? Although there is a lack of growth and development rates measurements over multiple generations, we can
infer the acclimatory responses of these rates by examining available data for body size. Data for *Drosophila melanogaster* size vs. temperature showed the effect of generation (1st vs. 2nd generation) to have significant effects on organism size; however, these size changes were extremely small and explained only 0.23% of the variation in body weight found (compared to 82% of variation explained by temperature; Karan et al. 1998). Similarly, small but significant changes have been shown to occur in egg and adult size in *D. melanogaster*, driven by differences in the parental thermal environment (Crill et al. 1996). Fischer et al. (2003a) showed that the butterfly *Bicyclus anynana* lays larger eggs at cooler temperatures, but that the effect of oviposition temperature does not significantly alter size at later larval stages when reared at a common temperature. Data for the yellow dung fly *Scatophaga stercoraria* showed maternal acclimation temperature did not have a significant effect on offspring growth rates (Blanckenhorn 2000). Similarly, data for the hawkmoth *Manduca sexta*, where eggs were hatched at different temperatures then reared at a common temperature, showed the hatch temperature to affect initial larval size, but that this disappeared by the fourth instar (Potter et al. 2011). These studies, and our own, suggest rapid acclimation of growth and development rates in ectothermic species.

*The concave thermal reaction norm*

We found size to decline in later stages at the lowest temperature (Figure 4.5A). This concave thermal response of adult has previously been found in other ectotherm species, including *Drosophila* (data for both wing length and mass, Ray 1960, David et al. 1997, Karan et al. 1998, Petavy et al. 2001), aphids (Lamb et al. 1987), aquatic insects (Vannote and Sweeney 1980), leeches (Young and Ironmonger 1982), frogs (Smith-Gill and Berven 1979), copepods (Kimoto et al. 1986, Hansen et al. 2011) and a
moth (Davidowitz and Nijhout 2004). This suggests there may be common temperature-size patterns in adult ectotherms, but that these are not simply linear or exponential terms. Indeed, applying empirical relationships between growth and development rates data have previously also resulted in the prediction of this concave shape (Davidowitz and Nijhout 2004, Forster et al. 2011b). We found A. franciscana did not attain adult stage at the lowest temperature in the 2nd generation; thus this lower temperature may be harmful over multiple generations. Low survivability, coupled with long generation times, make rearing ectotherms and obtaining data at lower thermal limits more difficult, which may explain why the majority of studies do not show a concave shape (Kingsolver and Huey 2008). Further, the low survivability associated with cold stress suggests that this aspect of the TSR may not be relevant in the field, as maintaining populations at these lower temperatures over multiple generations was not possible.

Comparison of ontogenetic temperature-size response for crustaceans

Examining the temperature-size response through ontogeny in A. franciscana, we found no relationship in eggs, an inverse TSR in early larval stages, and a significant TSR established at stage 12 (Figures 4.4 and 4.6). Although the establishment of a significant temperature-size response occurs at the same point as the shifts from slower to more rapid growth (and from thoracic segments being added to abdominal segments, Figure 4.1), this appears largely circumstantial: the change from a negative temperature-size response is cumulative, with stage 1 showing the most negative temperature-size response, and this getting less negative with increasing stage, until a significant temperature-size response is established at stage 12. Other crustacean species follow similar patterns. Early larval stages show little or no temperature dependence of their size (and sometimes a reverse TSR), whereas later stages show the more typical TSR,
with size declining with increasing temperature. This suggests that the temperature-size relationship is commonly “reset” at the beginning of each generation (Forster et al. 2011a). Indeed, the data of Leandro et al. (2006) for *Acartia tonsa* (see our Figure 4.6) show that individuals acclimated for at least two generations to their thermal environment show this same pattern. This lends further support to the theory that crustaceans follow a common pattern of size responses to temperature, with initial size being relatively temperature independent even when organisms are maintained at temperatures for multiple generations.

Although we have restricted our analysis to crustaceans, data from other ectothermic groups has shown egg size to be less temperature dependent than adult size (e.g. see the synthesis of Forster et al. 2011a). Further support for the TSR emerging only in later larval stages comes from the insect *Manduca sexta* (Davidowitz et al. 2004, Davidowitz and Nijhout 2004, Diamond and Kingsolver 2010). It should be noted, however, that some species have significant changes in egg and/or early larval size with temperature (Crill et al. 1996, van Voorhies 1996, Ernstring and Isaaks 1997, Blanckenhorn 2000, Fischer et al. 2003b, Hassall et al. 2006a, Steigenga and Fischer 2007b), with size changes following the TSR. The fact that egg and early larval stages are temperature dependent in some ectotherms, but typically not in crustaceans, shows that different groups with different life history patterns respond to temperature in different ways. This suggests different proximate mechanisms bring about temperature-size changes in different taxa, which in turn gives weight to the idea that the TSR is an adaptive response (Atkinson 1994, Atkinson et al. 2003), i.e. there is a fitness benefit to smaller size at warmer temperature and organisms achieve this through a variety of mechanisms.
CHAPTER 5

Achieving temperature-size changes in a unicellular organism through temporary decoupling of growth and development rates

Introduction

The temperature-size rule (TSR) is common to both metazoans (Atkinson 1994, Forster et al. 2011a) and single celled organisms, having been found in bacteria and many protists (Montagnes and Franklin 2001, Atkinson et al. 2003). Despite the demonstration of this rule in a wide range of organisms, there is a lack of experimental study on how the TSR is established in some ectothermic species. There has recently been a significant amount of work on metazoans, especially arthropods. Studies have investigated how size changes occur during ontogeny in the brine shrimp *Artemia franciscana* (Forster and Hirst 2011) and the tobacco hornworm *Manduca sexta* (Petersen et al. 2000) and how temperature acclimation status impacts on body size in *Drosophila melanogaster, A. franciscana* and *M. sexta* (Karan et al. 1998, Forster and Hirst 2011, Potter et al. 2011). Also, the effect of parental rearing temperature on size has been studied in the dungfly *Scatophaga stercoraria*, the beetle *Notiophilus biguttatus* and the butterfly *Bicyclus anynana* (Ernsting and Isaaks 1997, Blanckenhorn 2000, Steigenga and Fischer 2007a). Further, meta-analyses of adult and progeny size (Forster et al. 2011a) and of growth and development rates (Forster et al. 2011b) have suggested the mechanism by which the TSR is established and maintained in metazoa. However, research focusing on unicellular organisms is much more limited. A meta-analysis of protist data has shown these organisms to follow the TSR, on average decreasing in size by 2.5% for every 1°C of warming (Atkinson et al 2003).
Nevertheless, we are still yet to understand how these size changes are brought about; studies of unicellular organisms typically allow species to acclimate to new temperatures before carrying out size measurements (e.g. 5 generations, Montagnes and Franklin 2001). If we wish to understand how the TSR in single celled organisms is produced, and eventually its ultimate cause, we first need an understanding of the process of size adjustment during acclimation.

*How do unicellular organisms change size with temperature?*

Addressing “how” organisms change size with temperature, simple models have demonstrated that the mechanism underpinning the TSR must be different in unicellular and multicellular organisms (Forster et al. 2011a). This difference is highlighted by the equation that links size and rates:

\[
\frac{g}{D} = \ln \frac{M_A}{M_P}
\]

(Equation 5.1)

Where \(g\) is the growth rate of the individual (day\(^{-1}\)), \(D\) is the development rate (day\(^{-1}\), i.e. 1/time between divisions) \(M_A\) the mass of the adult, and \(M_P\) the mass of a single progeny. The term “progeny” refers to the daughter cells just after binary fission of the mother cell in protists. Dividing in half requires the TSR to equally impact adult and progeny size in unicells at acclimation. This is turn means the rates driving the TSR, growth and development, can only become temporarily decoupled during acclimation in unicells (see Figure 5.1). This temporary decoupling suggests a fundamentally different mechanism of the TSR in unicellular organisms compared to metazoans, where rates remain decoupled (Forster et al. 2011a). Currently, this disparity between uni- and multicellular organisms remains theoretical: we still require testing of changes in adult and progeny size, along with growth and development rates, during the acclimation
Figure 5.1. A hypothetical example of the effect of temperature change on a unicellular organism which adheres to the TSR, where $M_a$ = adult mass, $M_p$ = progeny mass and subscript numbers represent generation number. The organism starts at 10°C, $M_a/M_p$ is a fixed ratio and thus $g/D$ is fixed too. The organism is then displaced into an environment at 20°C (indicated by the arrow), as cell size must change, the $g/D$ ratio must become temporarily decoupled. Finally, $g/D$ returns to a fixed state of 2 (in this example at the 4th generation, between $M_{p4}$ and $M_{a4}$) and adult and progeny size attain an acclimated size ($M_{pn}$ to $M_{an}$).
phase in unicellular organisms. We need to understand when, and for how long, adult and progeny sizes become decoupled, and whether growth and development rates do indeed become decoupled. We carry out such research here by measuring cell size changes in the unicellular species *Cyclidium glaucoma*, along with estimates of its growth and development rates, at different temperatures during thermal acclimation.

**Temperature, size and population abundance**

Aside from being a general phenomenon requiring explanation, it is also important to understand the potential impact of the TSR on other life history rates. Of particular importance in a field setting, we need to ascertain the impact of increased temperatures on population abundance and biomass production. There have been few studies examining the impact of temperature on these traits in unicellular organisms; mesocosm experiments investigating the impact of temperature on freshwater phytoplankton found higher temperatures to be associated with a reduced total biomass (Yvon-Durocher et al. 2011). Further, previous ecological theory predicts carrying capacity to decrease with increasing temperature, following an Arrhenius function (Savage et al. 2004). Here, we compare peak population abundances and biomass in this unicellular protist species across a range of temperatures to see whether these traits do indeed scale negatively with temperature.

Using the ciliate species *Cyclidium glaucoma*, we shall address the following questions: How do adult and progeny cell sizes change when acclimating to a novel thermal environment? How do growth and development rates change during this acclimatory period? Finally, do maximum population abundance and biomass scale negatively with temperature in this protist?
Methods

Experimental design

10 sterile culture flasks (Corning® 30mL flasks) were prepared for Cyclidium glaucoma, using 30mL of sterilised 30psu filtered sea water, passed through a 0.20μm Minisart® filter. To each flask, 1 wheat grain, cut into two, was added. Wheat grains were boiled vigorously for 3 minutes prior to their addition to sterilize them. 500μL inoculum was then added to each of the 10 sterile culture flasks. Batch cultures of C. glaucoma used to inoculate these flasks were originally isolated, cloned and established in culture for a previous investigation (Finlay et al., 2006). These batch cultures were maintained at 17°C for 2 weeks prior to experimentation. To allow the initiation of growth of the cultures, inoculated C. glaucoma flasks were maintained at 17°C for a further 10 days, in accordance with pilot experiments, which indicated this was the minimum lag phase associated with this species. After this time, cultures were placed at a range of temperatures (8, 13, 17, 21 and 25°C), with 2 flasks as replicates at each temperature. 500μL sub-samples were taken from each culture over the course of 2 weeks (including the point of introduction, \( t_0 \)). During the first 3 days, samples were taken every 4 hours to obtain high temporal resolution results during the period we assumed acclimation would be most likely. Frequency of sub-sampling was then gradually reduced over the remainder of the two week period, such that the final sub-samples were taken 3 days apart. C. glaucoma samples were preserved in 50μL formalin for later counting.

Sub-samples were analysed to calculate population abundances and volumes. Each was placed on a Sedgewick Rafter cell, which divides each sample into squares with 1μL volumes. For each sub-sample, the number of individuals were counted in 50 randomly selected squares, and the mean number of cells calculated per 1μL (Appendix 5.1).
individual cells were randomly selected, photographed under a 100x magnification optical microscope and then measurements of length and width (µm) made from these photographs using QCapture Pro (QImaging Software). Cell volume was calculated for each individual, assuming a standard geometric shape (prolate spheroid):

\[ V = \frac{4}{3} \pi a^2 b \]  

Equation 5.2

where \( V \) = cell volume, \( a \) = equatorial radius (cell width/2), and \( b \) = polar radius (cell length/2). Having estimated cell volumes for each individual, mean cell volumes (MCVs) were calculated for the 30 cells per sample (total of ~ 7225 cells, Appendix 5.2). MCVs and population abundances were subsequently plotted against time for each temperature and replicate (Appendix 5.3). Further, to determine the relationship between MCV and population abundance, temperature and time, we constructed a general linear model (GLM):

\[ \ln MCV = \ln t + \ln P + T + \ln P \times T \]  

Equation 5.3

where \( MCV \) = mean cell volume (µm³), \( t \) = time (hours), \( P \) = population abundance (cells µL⁻¹) and \( T \) = temperature (°C). MCVs, time and population abundance were natural-logged to maintain the homoscedasticity of residuals. Further, these equation forms for each parameter were shown to fit the data well (Figure 5.2) and thus deemed appropriate models. Using these parameters, we determined whether each parameter had a significant effect on of MCVs. When the interaction term (\( \ln P \times T \)) was not significant (p<0.05), this was removed from the GLM.
Adult cell sizes were calculated from the mean size of the largest 20% of the cell size measurements. Similarly, progeny cell sizes were calculated from the mean size of the smallest 20% of cells. Using these measurements for adult and progeny volumes, we similarly applied the GLM (Equation 5.3) to the data. We found a clear impact of population abundance upon the cell sizes of the organisms in the cultures. During the temperature acclimation period, population size was changing (log phase of population growth). We wished to remove the impact of this from the cell size change, such that thermal acclimation could be calculated singularly. We corrected data to the population abundance, ensuring we were correcting only for population size and not temperature by using the slope of population abundance for the GLM applied to 17°C only, as this was the culture in which temperature was in effect unchanged. Cell volumes were all corrected to the temperature-independent peak population abundance (13.4 cells μL⁻³).

Corrected cell volumes were subsequently plotted against temperature (across all treatments) at each observation time point, and the linear regression for each used to calculate percentage volume changes from that at 15°C (following Atkinson et al. 2003). These percentage changes in volume were then plotted against time. Acclimated data were determined from working from the last observation point to the point at which confidence intervals for a prior observation point no longer overlapped. Using the determined point of acclimation, we calculated acclimated population abundance-corrected MCVs for each replicate. We then estimated when cell size became acclimated at each temperature and for each replicate by determining the first observation point where MCV was not significantly different from the acclimated MCV.

_Determining Development and Growth Rates_
As individually grown protists often have low survivability (Kimmance et al. 2006), we could not design our experiment so as to follow a single cell (and its progeny) over time. Our experiment therefore relied upon populations with development and growth rates derived during the log period of growth, assuming mortality to be negligible during this phase. The natural logged population abundances were plotted against time during the non-acclimated period (i.e. from sampling commencing until the point when size was defined as having become acclimated). To calculate the development rate (which we take as being equivalent to the doubling rate, and the reciprocal of development time), we calculated the slopes for each replicate population and transformed them to development rates by dividing by ln(2). These rates were subsequently plotted against temperature to show the temperature dependence of development rate. In the literature, population growth rates are often seen as synonymous with development rates, as it is assumes cell size does not change. However, to ensure we captured size changes, the mean population abundances at each time interval (hours) was multiplied by the mean cell volume at the same time interval, giving a value for the total volume of protists per microlitre. The natural log of total protist volumes (μL⁻³) were subsequently plotted against time and linear regression applied to each replicate during the non-acclimated phase. The slope of these linear regressions represented the mean growth rate for each replicate. The natural log of mean growth rates were plotted against temperature and slopes compared with those for development. To ensure population growth and development rates provided a reasonable estimate of individual growth and development rates, we calculated g/D at the pre-experimental temperature (17°C). If population rates were the same as individual rates we would expect a value of approximately 0.69 (i.e. ln (Mᵣ/Mᵢ) = ln (2/1), Equation 5.1). Our calculated value was in good agreement with this (0.72 CIs ±
Figure 5.2 *Cyclidium glaucoma* 3D scatter plots of ln mean cell volume (MCV) vs. (A) ln time (t, hours) and temperature (T, °C); (B) temperature (T, °C) and ln population abundance (P, individuals μL⁻¹). Meshes indicate best fit models using equation forms applied to the data in the general linear model (GLM), i.e. ln MCVs vs. ln t, ln P and T. Meshes provide good fits to the data (p<0.0001 for all parameters).
0.14), showing population growth and development rates to be reasonable estimates of individual rates.

The TSR and Maximum Population Abundance

To compare our temperature-size results with those for the protists described in the meta-analysis conducted by Atkinson et al. (2003), we calculated acclimated MCVs at peak population abundance, and calculated the linear regression line between these values and temperature. We calculated the maximum population abundance, defined as the point at which the slope of ln population abundance vs. time was not significantly different from zero. We compared these carrying capacities across the different experimental temperatures. We calculated the product of peak population abundance (cells μL⁻³) and MCV at this peak to determine the maximum biomass, and examined whether a decrease in cell size was associated with a concomitant decrease in maximum biomass.

Results

Size, temperature and population abundance

Mean individual cell volume increased with time when subjected to temperatures less than the 17°C to which they had been previously acclimated and decreased with time at temperatures greater than this, thereby following the TSR. Changes in MCV were also associated with changes in population; during the exponential growth phase at 17°C and higher temperatures, these changes in population abundance were accompanied by decreased MCV, suggesting a negative relationship between the two (see example in Figure 5.3). Application of the general linear models to ln MCVs revealed all three factors (Equation 5.3) had a significant effect on MCV (p < 0.001 in all cases).
Figure 5.3. *Cyclidium glaucoma* population abundance (LHS) and mean cell volume (RHS) vs. time (hours). Population abundance (*P*) and mean cell volumes (MCVs) are plotted on log_{10} scales. Solid lines represent moving averages for population abundances and dashed lines represent moving averages for MCVs. Moving averages were calculated from the average of the previous 5 data points. Data presented are for a single replicate at 17°C.
Figure 5.4. *Cyclidium glaucoma* slopes of mother and daughter cell volumes vs. temperature plotted against time. Mother cell volumes were calculated from the mean of the largest 20% of cells in a sample (n = 2x3), daughter volumes were calculated from the smallest 20% of cells in a sample (n = 2x3). Slopes were calculated as % changes in size per °C from that at 15°C, using linear regression of cell volumes vs. temperature at each time interval. Thick horizontal lines are time periods where confidence intervals overlap for daughter (solid line) and mother (dashed line) data. Thinner lines either side = 95% confidence intervals.
Population abundance had a negative effect on size, though the interaction term between population abundance and temperature was not significant (p = 0.73). This suggests that the effects of population abundance and temperature on MCVs are additive and therefore independent of one another.

**Adult and Progeny Size**

Having applied the GLM at 17°C to adult and progeny size data (to remove the effect of population abundance on cell sizes), the % change in volume from that at 15°C showed temperature acclimation in both adult and progeny size. The temperature dependence of adult and progeny size were determined as being acclimated at ~70.5 hours and ~120 hours respectively (Figure 5.4).

Once the latter became acclimated, there was no discernible difference between the confidence intervals of the adult or progeny response to temperature. Using 120 hours as the defined acclimation point, we calculated the acclimated MCVs for each experimental temperature and replicate. We then determined when size became acclimated for each temperature treatment and for each replicate. Only 8°C showed a significant difference in non acclimated size from acclimated MCV, with acclimation being completed after 102.5 hours.

**Calculating Growth and Development Rates**

We compared the rates of development and growth during the acclimatory phase (i.e. before steady state of size is achieved) in Figure 5.5. Both development and growth rates peaked at 21°C, and therefore linear regression was only applied up to this temperature. Development rate had a stronger temperature dependence than growth, i.e.
the former had a steeper slope (Figure 5.5C, $t = 3.31, p = 0.011$). Population abundances ($P$) and MCVs reached steady state at all temperatures except 13°C. At this point, $P$ and MCVs did not change, such that slopes were zero at steady state (e.g. from ~200 hours in the example in Figure 5.3). At steady state, development rates (determined by $P$) and growth rates (determined by the product of $P$ and MCV) were therefore in equilibrium, such that these rates became coupled at acclimation.

The TSR and Maximum Population Abundance

_Cyclidium glaucoma_ followed the temperature-size rule, with size change equating to 3.6% (CIs ± 0.45%) reduction of volume per °C from the size at 15°C (Figure 5.6A). The acclimated MCV data corresponded to a point at or near the peak population abundance, except at 13°C, where individuals exhibited poor population growth. These data were confounded by low population abundances and were therefore excluded from the TSR calculation. Fixed maximum population abundances were achieved at 8, 17, 21, and 25°C. Similarly to the TSR measurements, 13°C was excluded from maximum population abundance calculations as ln $P$ was still increasing at the end of the experimental time. There was no significant regression slope between population abundance and temperature (Figure 5.6B). The peak biomass was similarly plotted against temperature (Figure 5.6C). This showed the highest biomass to be associated with the lowest temperature, and biomass to decrease linearly with increasing temperature (Figure 5.6C).

Discussion

Size acclimation in _Cyclidium glaucoma_

The unicellular organism _Cyclidium glaucoma_ followed the temperature-size rule,
Figure 5.5
Figure 5.5 Development and growth rates in the protist species *Cyclidium glaucoma* during the log growth phase. (A) Development rates (equivalent to doubling rates) vs. temperature. (B) Growth rates (the product of doubling rates and mean cell volume) vs. temperature. Both development and growth rates are plotted on log_{10} scales. (C) Slope parameters, calculated for the linear portion of plots A and B, $D$ is development rate, $g$ is growth rate. Error bars are 95% confidence intervals.

Figure 5.6 (A) Temperature-size changes in the protist *Cyclidium glaucoma*. Mean cell volumes (MCVs) were calculated for acclimated samples (“acclimated” being defined as the point at which ln mean cell volume across each time interval no longer had a slope significantly different from zero). Error bars represent 95% confidence intervals for each mean. The mean cell volume for sample 13A in *C. glaucoma* (given in grey) was not included within the linear regression, as the culture had not attained maximum population abundance. (B) *Cyclidium glaucoma* maximum population abundances ($P$). Mean values at each temperature were calculated over the range at which the regression of ln population abundance vs. time had a slope which was not significantly different from zero. (C) Maximum biomass, calculated from maximum population abundance*mean cell volume. Error bars are 95% confidence intervals.
Figure 5.6
showing a negative linear relationship between mean cell volume and temperature, with a 3.6% decline in the volume of cells at 15°C per 1°C increase in temperature (± 0.45% CIs, Figure 5.6A). This change in cell volume is not significantly different to the meta-analysis of protists size responses made by Atkinson et al. (2003), which showed cell size to change linearly by approximately -2.5% °C⁻¹ (± 0.78 % CIs) from the volume at 15°C. *C. glaucoma* therefore appears to be a reasonable model organism in which to study the establishment of the TSR in unicellular organisms, on the basis that the degree to which it changes size with temperature is fairly typical. Data for adult and progeny size revealed thermal acclimation to occur rapidly after approximately 70.5 hours and 120 hours respectively. The slowest doubling rate, at 8°C, was 165 hours (± 81hrs, 95% CIs), yet MCVs were no longer significantly different from acclimated MCVs after 120 hours, suggesting rapid intra-generational temperature acclimation. Referring to Figure 5.1, we can therefore estimate that the “size acclimation” phase requires only a single generation before temperature-size rule changes are established, and that these size changes are subsequently maintained beyond this point (as seen from the horizontal slopes in Figure 5.4 beyond 120 hours). This is the first example, of which we are aware, where the thermal acclimation period in a single celled organism has been determined, and one of few studies which has focused on acclimatory changes associated with novel environments (Finlay et al. 2006).

Although size changes are seen in both adult and progeny, we found progeny took longer to reach an acclimated state than did adults (Figure 5.4). This is likely to be caused by the lag between a steady acclimated size being reached in adults, and the production of enough progeny to dominate the measurement of the smallest 20% of cells. Further, we found growth and development rates to be significantly decoupled
during the acclimatory period (Figure 5.5). This significant decoupling of growth and development during thermal acclimation has not been recorded previously in unicells. Typically, growth and development rates are treated synonymously in the unicellular literature as these two rates are assumed to be completely coupled. For example, Jensen and Moestrup (1997) show a linear relationship between cell size change and division rate associated with temperature, suggesting growth and development rates are coupled, but these measurements were taken at the end of the exponential growth phase, thus they were likely size acclimated, such that \( M_g/M_P \) and thus \( g/D \) was constant (Equation 5.1). This same coupling in the relationship was shown in the dinoflagellate species *Gonyaulax tamarensis* (Watras et al. 1982) but again at the end of the exponential growth phase.

Our results regarding size and rate acclimation to temperature reveal both similarities and differences between uni- and multicellular organisms. Both groups display rapid thermal acclimation: *Cyclidium glaucoma* appears thermally acclimated in size within one generation. Similarly, multicellular organisms appear to acclimate their size to their thermal environment within a single generation (Karan et al. 1998, Forster and Hirst 2011). Further, these size changes are subsequently maintained in *C. glaucoma* across further multiple generations, which has also been found in multicellular organisms (Karan et al. 1998, Forster and Hirst 2011, Potter et al. 2011). However, relative size changes differ between these two groups. *C. glaucoma* shows acclimated size changes in progeny and adult which are not significantly different from one another (-4.07%°C\(^{-1}\) (CIs ± 0.34%) and -3.76%°C\(^{-1}\) (CIs ± 0.28%) respectively, Figure 5.4), whereas acclimated progeny size in metazoans are commonly less temperature dependent than adult sizes (Forster et al. 2011a), resulting in acclimated development and growth rates
having different temperature dependence (Forster et al. 2011a, 2011b).

**Temperature-size changes in unicellular organisms**

What does this mean for our understanding of the temperature-size rule? Proximate mechanisms previously suggested to explain the TSR have been driven by either decoupling of growth and development rates (Sibly and Atkinson 1994, van der Have and de Jong 1996, Walters and Hassall 2006), or the different thermal sensitivities of anabolism and catabolism (Strong and Daborn 1980, Perrin 1995, Woods 1999, Karl and Fischer 2008). As the ratio $M_d/M_P$ changes with temperature in multicellular organisms, growth and development (and possibly anabolism vs. catabolism) have to be decoupled and have different temperature dependence across multiple (acclimated) generations (Forster et al. 2011a). However, this cannot be the case in protists, as sizes return to an acclimated state, and $M_d/M_P$ becomes a constant (Figure 5.4). This requires $g/D$ to also be a constant across temperatures when acclimated (Equation 5.1). Our results provide empirical evidence to support the conceptual scheme of Forster et al. (2011b), showing that uni- and multicellular organisms achieve size changes associated with temperature through different mechanisms. Across multicellular organisms, size changes have been shown to be brought about through changes in cell size (van Voorhies 1996, Stelzer 2002), changes in cell number (Noach et al. 1997, Arendt 2007) or both (French et al. 1998, Blanckenhorn and Llaurens 2005). Further, even within an organism, specific size changes have been shown to occur in some cell types but not others (Atkinson et al. 2006), showing that multicellular organisms cannot be seen as analogous to a population of single cells. These differences within individuals and between single and multicellular organisms demonstrate both similarities and differences in the proximate mechanism for the TSR, but clearly there is not a universal
mechanism across these groups. From this we are led to suggest that the TSR is an adaptive response, i.e. that smaller size at warmer temperatures confers a fitness advantage to the majority of species. The next important step is to work out why being smaller at warmer temperatures and larger at colder temperatures is advantageous.

The impact of population abundance on cell size

Along with temperature-driven size changes in *Cyclidium glaucoma*, we found population abundance to significantly affect cell sizes. The results from the GLM show this to be independent of temperature, as there was no interaction between these two parameters. The negative correlation between cell size and population abundances were present across the entire exponential growth phase at higher temperatures (e.g. Figure 5.3). We excluded the impact of population abundance in progeny and adult temperature-size calculations by corrected size changes for population abundance using the results of the GLM applied to 17°C, the pre-experimental temperature. However, uncorrected data are also presented in Figure 5.6. The data for the MCV at 13°C replicate was a distinct outlier from the rest of the data. At 13°C, samples used to calculate MCVs for *C. glaucoma* were not at peak abundance, unlike all other temperatures. Decreased MCVs were associated with higher population densities in *C. glaucoma*, thus this explains this apparent outlier in these results.

If increased population abundances are associated with decreased MCVs in *Cyclidium glaucoma*, how do we know there is a true temperature-size effect and that size changes are not simply driven by increasing population abundances? Firstly, we find from the general linear model that temperature has a significant effect on size, independent of population abundance. Secondly, along with *C. glaucoma*, decreasing MCV with
increasing population abundance has previously been observed in *Alexandrium ostenfeldii* (Jensen and Moestrup 1997), *A. tamarense*, and four diatom species (Prakash et al. 1973), suggesting this may be common in protist species.

Why do MCVs change with population abundance in *C. glaucoma*? One factor potentially driving size changes is food concentration: as population abundances increase, food concentrations decrease and MCVs are reduced. Many studies have been conducted observing predator-prey interactions using protists as model organisms. In these predator-prey interactions, we find a cyclical population response, where protist populations increase to a peak and then crash as food becomes scarce (Sharon and Morin 1993). If decreased food concentrations were driving the changes in size associated with the exponential growth phase seen in *C. glaucoma*, we would expect to see a crash in population abundance following a peak. However, when we observe replicates 17B and 25A, where population abundances peak within the first 100 hours of the experiment, we see no such crash in population abundance over the subsequent 500 hours, suggesting food concentrations can support these population abundances and thus size changes are not driven by food shortages. Also, once population abundances peak, MCVs are maintained (e.g. from 200 hours onwards, in Figure 5.3), whereas one would predict continued cell size reduction as cells become more food limited. Further, a previous study investigating temperature-food interactions on cell volumes of *Oxyrrhis marina* (Kimmance et al. 2006) found an interactive effect between food and temperature, yet our general linear model found no significant interaction between population abundance and temperature, suggesting changes in size related to population abundance were not food driven.
The fact that cell sizes do change with population abundance in *Cyclidium glaucum*, and have been shown to change during the exponential growth phase in a number of other protist species (Prakash et al. 1973, Jensen and Moestrup 1997), has important implications for our understanding of the TSR. Firstly, it shows a highly plastic response in cell volume in protist species; cell size can respond rapidly to changes in environmental conditions and population abundances. Secondly, it shows that measuring protist species size during the exponential growth phase is likely to be associated with high variability in MCVs, driven by the additive effects of changes in population abundance and temperature. As Jensen and Moestrup (1997) point out, this exponential growth phase is not indicative of “balanced growth in a constant environment”. One problem with measuring size changes in protists compared with multicellular organisms, is that unicellular organisms have to be grown in cultured populations. Individually grown protists have low survivability (Kimmance et al. 2006), making measurement of size and development of an individual extremely challenging. A previous meta-analysis of protist data attempted to resolve this problem by only recording cell size data taken during the log phase of population growth (Atkinson et al. 2003), yet we suggest this phase to be associated with high variability of MCV, driven by population abundance. The confounding factor of population density might be overcome using chemostat in future experiments. A chemostat allows population abundances to be maintained and maintains organisms in a physiological steady-state. In this way, temperature effects on cell size could more easily be separated from population abundance.

*Temperature and biomass*
The maximum population abundance data for *Cyclidium glaucoma* revealed no clear pattern relating this to temperature (Figure 5.6B). The regression of population abundance vs. temperature was not significantly different from zero. As maximum population abundance does not change with temperature, this suggests peak population abundance to be temperature-insensitive at an intraspecific level. Although the temperature dependence of intraspecific peak population abundance has received little attention, previous theoretical work focused on carrying capacity predicts this to scale negatively with temperature (Savage et al. 2004). Further, these same models predict carrying capacity to scale negatively with body size. We may expect similar predictions for the impact of temperature and body size on peak population abundance, yet this is not the case in *C. glaucoma*, which shows temperature and size-independent peak population abundances. Although peak population abundance does not change with temperature, this is not the case for biomass. As biomass is a product of size (Figure 5.6A) and population abundance (Figure 5.6B) the lowest temperature was associated with the highest biomass values, with a linear decrease in maximum supported biomass as temperatures increased. This may suggest a less efficient transfer of energy associated with higher temperatures, or differences in mortality rates. Data focusing on the impact of temperature on these important life history traits is lacking. Further research is required at the intra-specific and food web level to determine the potential impacts of warming on size, population abundance and biomass.
CHAPTER 6

Quantitatively Testing the Adaptive Mechanisms of the Temperature-size Rule

Introduction

This thesis has focused on developing our understanding of how the temperature-size rule (TSR) is brought about. The mechanism of the TSR has been shown to be different in uni- and multicellular organisms: using a conceptual model the rates of growth and development have been shown to return to their coupled state in uni- but not multicellular organisms. This has been confirmed with experimental data for two case studies (Chapter 4 and 5). Further, a meta-analysis of data for growth and development rates in marine copepods has also shown support for this difference in multicellular organisms. Even within multicellular organisms, we find the timing of size change to be different; for example, egg sizes do not change in crustaceans (Chapter 5) but do in other arthropods (Ernsting and Isaaks 1997, Fischer et al. 2003b). The evidence thus far does not point towards a universal proximate mechanism: the same outcome of adult temperature-size change is achieved through different means, and does not come about from some physiological limitation imposed upon all ectotherms. This suggests the TSR to be an evolutionarily beneficial response to temperature, which increases species fitness, i.e. it is adaptive. In this final chapter we investigate potential adaptive mechanisms that have been purported as the ultimate reason as to why the TSR exists. We review these proposed adaptive mechanisms, identify testable hypotheses and use the most complete set of temperature-size data available to quantitatively test these hypotheses.
1. Compound Interest Hypothesis

One potential adaptive mechanism driving the TSR relates to the impact of season length on intraspecific body size. In species that have a seasonal reproductive phase, organisms should attempt to increase their body size to maximise their reproductive output during this favourable seasonal phase (Partridge and French 1996, Fischer and Fiedler 2002). However, this is dependent on the generation time of the species. In ectotherms with short generation times, fitness (number of offspring produced) is maximised by increasing the number of generations completed during the growing season, i.e. following the law of “compound interest”. Thus it is more beneficial to reproduce earlier, even at the cost of being smaller at maturation (Fischer and Fiedler 2002). In those species with longer generation times, where only a single generation can be completed in a year, fitness is maximised by maximising body size at maturity, thus maximising reproductive output. Thus those species which complete many generations in a single year (multivoltine) should maximise their reproductive output at warmer temperatures by reproducing early at a smaller size, whereas those species completing only a single generation in a year should maximise their reproductive output by reproducing at a larger size. This presents an easily testable hypothesis:

a) Monovoltine species exhibit a weaker temperature-size response than multivoltine species.

2. Temperature and Latitude

If the TSR were an adaptive plastic response to maximise performance at different temperatures, one may expect the most plastic species to be those that inhabit environments where temperature changes are greater during the growing season. These
thermal generalists are favoured where temperature changes systematically with time (Huey and Kingsolver 1993), such as in a seasonal environment. We may therefore expect species living at latitudes where temperatures are more seasonally variable to have a more plastic thermal response than those living in aseasonal (i.e. subtropical, tropical) environments. Thermal specialists, adapted to living a relatively constant temperatures, have narrower thermal tolerances (Kingsolver 2009). Assuming the TSR to be adaptive, they should therefore not have developed the mechanisms of temperature-size change present in their generalist counterparts. Consequently, species living at higher latitudes (i.e. > 23.5 degrees from the equator) should exhibit a stronger temperature-size response than those living at lower latitudes, where the thermal environment is more constant.

b) The TSR is strongest in species found at higher latitudes.

3. MASROS

Another potential adaptive explanation for the TSR is built on the impact of temperature on oxygen supply and demand. MASROS (maintaining aerobic scope and regulating oxygen supply) was originally defined by Atkinson et al. (2006), suggesting the TSR to be part of an “integrated adaptive suite of acclimatory responses at all levels of organization that maintains scope for aerobic activity.” This builds on previous work by Woods (1999) on the “oxygen hypothesis”, but unlike Woods is not restricted to changes in cell size and can apply to the whole or part of an organism. There are gross differences in the environmental oxygen availability between aquatic and terrestrial environments. Following the oxygen supply index (Verberk et al. 2011), oxygen supply is over 5 orders of magnitude greater in terrestrial than aquatic systems. We may
therefore expect, *a priori*, that if oxygen availability is an important control of the temperature-size response, then there will be differences between organisms from aquatic and terrestrial environments. Further, due to the increased total demand for oxygen (for metabolism, Glazier 2007), with increasing body mass, we may expect this to be influential also. Increasing the rate of supply of oxygen in aquatic systems (e.g. through ventilation), where oxygen is much less available and the medium is more dense, may impart heavy costs with increasing temperature and size. Indeed, the largest aquatic amphipods are found in polar regions, where low metabolic demand can be more readily met by available oxygen (Chapelle and Peck 1999, Peck and Chapelle 2003), whereas the largest terrestrial insects are found in the warm tropics (Makarieva et al. 2005). Outliers to the TSR also exist, Orthoptera (crickets and grasshoppers) typically follow a reverse temperature-size response, increasing in size with increasing temperature (Willott and Hassall 1998, Walters and Hassall 2006). By analysing temperature-size responses across environments, and by size and taxa, we should gain a better understanding of the mechanisms, and the degree to which some species are more susceptible in their response. We therefore test these hypotheses by examining the TSR in different habitats:

c) Aquatic (marine and freshwater) ectotherms have a stronger temperature-size response than terrestrial ectotherms.

d) Large aquatic ectotherms have a stronger temperature-size response than smaller species.

We quantitatively tested these four hypotheses (a-d) using large sets of data collected for uni- and multicellular ectotherms.
Methods

Data collection

Data for multicellular organisms were compiled using an expanded dataset from Forster et al. (2011a). Newly-published data were added using the ISI Web of Knowledge using the search terms: “(adult OR pupa* OR larva*) AND temperature AND (weight OR *mass OR size)”. We compiled data for progeny size using the search terms: “(egg OR progeny OR hatchling) AND temperature AND (weight OR *mass OR size)”. Entomological journals were individually searched for extra data sets. Data were included for both sexes where available, and for multiple studies of a single species. Only laboratory studies were included in which sizes were measured at a range of constant temperatures, but food concentrations had been maintained at or above saturation (therefore removing the confounding impact of food limitation). We were careful to only included studies where food supply was considered to be non-limiting.

The minimum period of acclimation for the inclusion of adult mass data was set so that only individuals who had been raised from egg or first larval stages were included. We assume progeny are acclimated if produced at the experimental temperature, i.e. the parental generation were introduced to temperatures prior to copulation and maintained at these temperatures until egg laying. We only included non-harmful temperatures within the analysis, limiting the data to temperatures where animals survived to adulthood and where there was no evidence of growth rate declining with increasing temperature. Adult data were collected as lengths, volumes, dry, wet or carbon mass. Measurements were subsequently converted to dry mass (mg) using appropriate conversions (see Appendix 6.1). Similarly to the adult data, progeny sizes were converted to dry masses (see Appendix 6.2).
Data for unicellular organisms was compiled using the dataset from Atkinson et al. (2003), combined with published data for bacteria (see Appendix 6.3). Data were searched for using the ISI Web of Knowledge using the search terms: “(protist OR protozoa* OR unicell*) AND temperature AND (*volume OR *mass OR size)”. Further, individual relevant journals were searched (e.g. Journal of Aquatic Microbial Ecology, Journal of Plankton Research). Data for Blepharisma americanum were from our own previously unpublished results (Appendix 6.4). Similarly to the multicellular data, size data were converted to dry mass: all data are presented in Appendix 6.3.

**Modelling the temperature-size response**

We required an appropriate model to describe the intra-specific temperature-size response that could be applied to all species. Here we describe fitting the response of adult dry mass to temperature. Research has suggested a range of models as the best descriptor of the body mass thermal reaction norm (Karan et al. 1998, Atkinson et al. 2003, de Jong 2010). We therefore required a method to apply this range of equation forms (linear, exponential, Arrhenius and power) to the full data set intraspecifically to determine which best described the empirical data. Further, we need to account for differences within each species, driven by the sex of individuals and by the fact that different studies may have been carried out on the same species (see Chapter 3). We therefore used a linear mixed effects model (O’Connor et al. 2007):

\[
\begin{align*}
\ln M &= \beta_{0ij} + \beta_{1ij} (\ln T - \ln 20) + \epsilon_{ij} \\
\beta_{0ij} &= \beta_0 + u_{0i(b/a)j} \\
\beta_{1ij} &= \beta_1 + u_{1ij}
\end{align*}
\]  

**Equation 6.1**
where $M$ = dry mass (mg), $T$ is temperature ($^\circ$C), $i$ indexes the species (within which study and sex are nested) and $j$ the temperature. $\beta_0$ and $\beta_1$ are intercept and slope fixed effects respectively, $u_{0i}$ and $u_{1i}$ are species-specific random effects terms which allow for intra-specific differences in the intercepts and slopes respectively, assumed to be normally distributed, $\epsilon$ is the error, assumed to be normally distributed. Within the species-specific intercept term, $u_{0i}$, the effects of sex ($a$) and study ($b$) were nested, to account for differences within species driven by different studies and by different sexes (see Chapter 3). These factors had highly correlated slope and parameter terms in all statistical models for growth and development rates (i.e. correlation between $a_0$ and $a_1 > 0.9$, $b_0$ and $b_1 > 0.9$). Therefore, to avoid over-parameterisation of these models, we allowed random variation in intercepts only for sex and study for all mixed effects models, following the methods of Chapter 3. Temperatures were centred to improve the interpretation of parameter terms and to reduce the correlation between slope and intercept terms. A centring temperature of $20^\circ$C was applied, as this temperature is within the boundaries used in most studies in the database and therefore required minimal extrapolation. Subtracting this centring temperature from each of the model types allowed each rate to be examined in terms of changes from that at $20^\circ$C.

We initially tested for linearity by applying a power model with a fixed slope parameter, following the methods outlined in Chapter 2. As the best-fit parameter $\beta_1$ was significantly different from 1 (-0.41 ±0.03 (95% CIs)), a simple linear model (as an alternative model type) could be rejected.

To determine the best fit model for mass data, power, exponential and Arrhenius
models were subsequently fitted to the data. Average species masses varied greatly; therefore in each model type intercepts were allowed to vary randomly to account for species-specific masses. These models were initially fitted assuming a fixed slope, assuming similar relative changes in mass with temperature. However, these models were also fitted allowing slopes to vary randomly, thus allowing species-specific changes in mass with temperature.

Initially, the best equation was chosen for each model type (power, exponential, Arrhenius) using modified likelihood ratio tests to determine whether each model type required slopes with species-specific random effects to improve fit. Having selected the best equation, the model types were compared by using Akaike’s Information Criterion (AIC). Akaike weights ($\omega_i$) were used to indicate the best fitting model. Similar methods were applied to multicellular progeny data and unicellular data, except without the nested term “sex”.

**Associated parameters**

Having identified the best model for multi- and unicellular data, the species-specific slope terms (equivalent to the species-specific temperature dependence of mass) were used to test hypotheses a-d. The effect of habitat type (freshwater, marine and terrestrial), dry mass, voltinism and latitude were determined for multicellular data. For unicellular species the effect of dry mass was examined only, as all species were aquatic, multivoltine, and occur over wide geographical ranges. Data for dry masses were calculated as geometric means for each species. Data for voltinism was extracted from the wider literature on each individual species. Latitudes were determined by the collection site used within each study. The data for these parameters were incorporated
within a series of general linear models with the basic structure:

$$\Delta m = H + \log DM + V + L + T_m$$  \hspace{1cm} \text{Equation 6.2}$$

where $\Delta m$ is the species-specific change in mass, determined by the linear mixed effects model, $H$ is the discrete variable “habitat” (freshwater, marine, terrestrial), $DM$ is the species dry mass (mg), $V$ is voltinism (1, 2, 3 or >3 generations per year), $L$ is latitude and $T_m$ is mid experimental temperature. Latitude was incorporated within the model in two forms, firstly as a continuous variable, using absolute values of latitude and secondly as a discrete value, dividing the data into tropical (0-23.5°), temperate (23.5-66.5°) and polar (>65°) regions. Beyond this simple structure, we allowed the interaction of these parameters and determined the GLM which best described the data. Using the species-specific slopes of mass change, calculated from the linear mixed effects model, we also were able to compare slopes by taxa (e.g. Decapoda, Orthoptera, Amphibia). We commonly used order as the phylogenetic level for comparisons, but in some cases applied levels which better accommodated life history types (such as subclass Copepoda).

**Results**

*Modelling the temperature-size rule*

A summary of the data included within the analysis is provided in Table 6.1. Adult multicellular data were best modelled using an exponential form equation, with species-specific intercept and slope terms (Table 6.1). Similarly, progeny data were best modelled using an exponential model, although an Arrhenius function also provided a good fit. For the purposes of the general linear model, we used the species-specific
slope outputs for the exponential model for progeny data, such that they were directly comparable to the adult data. The slopes of ln(dry mass) vs. temperature were subsequently transformed to percentage change in dry mass per °C for ease of interpretation, using the formula $(\exp(slope)-1)*100 = % \text{ change in mass per} \ °C$. Unicellular data were best modelled using a power model, with species-specific slopes and intercepts (Table 6.1). These species-specific slope terms were therefore used in the GLM.

*The general linear model*

Application of equation 6.2, allowing individual terms and all potential interactions revealed similarities in unicellular, progeny and adult multicellular data. In all cases, we found voltinism was not a significant variable in the GLM for multicellular data (Figure 6.1). Given that voltinism has previously been shown to be a significant factor in butterflies (Fischer and Fiedler 2002), we examined the Lepidoptera group singularly (Figure 6.1c). This supports voltinism not being significant in determining the strength of the TSR. Latitude was also not a significant explanatory variable of the strength of the TSR. This is highlighted in Figure 6.2, where there was no significant effect of latitude on mass change with temperature. This was also the case when latitude was incorporated as a discrete variable (tropical, temperate and polar data).

Application of the GLM also revealed important differences between uni- and multicellular organisms. For the unicellular and progeny data, inclusion of dry mass did not prove to be a significant factor in explaining inter-species differences in slopes. Further, habitat type was not a significant explanatory variable for progeny mass changes. However, there were significant differences between different habitats, and a
Table 6.1. Summary of the data used in the linear mixed effects model. P = progeny, A = adult.

<table>
<thead>
<tr>
<th>Organism Type</th>
<th>Environment Type</th>
<th>Number of Species</th>
<th>Number of Studies</th>
<th>Number of Data Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unicellular</td>
<td>Freshwater</td>
<td>25</td>
<td>57</td>
<td>295</td>
</tr>
<tr>
<td>Unicellular</td>
<td>Marine</td>
<td>36</td>
<td>44</td>
<td>319</td>
</tr>
<tr>
<td>Multicellular (P)</td>
<td>Terrestrial</td>
<td>16</td>
<td>25</td>
<td>90</td>
</tr>
<tr>
<td>Multicellular (P)</td>
<td>Freshwater</td>
<td>8</td>
<td>10</td>
<td>33</td>
</tr>
<tr>
<td>Multicellular (P)</td>
<td>Marine</td>
<td>9</td>
<td>11</td>
<td>49</td>
</tr>
<tr>
<td>Multicellular (A)</td>
<td>Terrestrial</td>
<td>54</td>
<td>124</td>
<td>880</td>
</tr>
<tr>
<td>Multicellular (A)</td>
<td>Freshwater</td>
<td>32</td>
<td>60</td>
<td>247</td>
</tr>
<tr>
<td>Multicellular (A)</td>
<td>Marine</td>
<td>22</td>
<td>27</td>
<td>149</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>202</strong></td>
<td><strong>358</strong></td>
<td><strong>2062</strong></td>
</tr>
</tbody>
</table>

Table 6.2. Comparison of model types to determine best model. AIC is the Akaike Information criterion, $\Delta_i$ is the AIC differences, $\omega_i$ is the Akaike weight. Values for fixed effect parameters are shown, along with standard errors (subscript values in brackets). In all cases, the best fit models required species-specific slope and intercept terms. The overall best fit model is shown in farthest right column (Best Model), and is defined as that with the highest Akaike weight.

<table>
<thead>
<tr>
<th>Mass</th>
<th>Model</th>
<th>Fixed Effects</th>
<th>AIC</th>
<th>$\Delta_i$</th>
<th>$\omega_i$</th>
<th>Best Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multicellular Progeny Mass</td>
<td>Arrhenius</td>
<td>-4.45(678)</td>
<td>70.7</td>
<td>0.25</td>
<td>0.46</td>
<td>•</td>
</tr>
<tr>
<td></td>
<td>Exponential</td>
<td>-4.45(677)</td>
<td>70.4</td>
<td>0.00</td>
<td>0.52</td>
<td>•</td>
</tr>
<tr>
<td></td>
<td>Power</td>
<td>-4.45(677)</td>
<td>77.6</td>
<td>7.17</td>
<td>0.01</td>
<td>•</td>
</tr>
<tr>
<td>Multicellular Adult Mass</td>
<td>Arrhenius</td>
<td>.085(318)</td>
<td>-124</td>
<td>6.90</td>
<td>0.03</td>
<td>•</td>
</tr>
<tr>
<td></td>
<td>Exponential</td>
<td>.086(318)</td>
<td>-131</td>
<td>0.00</td>
<td>0.97</td>
<td>•</td>
</tr>
<tr>
<td></td>
<td>Power</td>
<td>.083(318)</td>
<td>-40.7</td>
<td>90.7</td>
<td>0.00</td>
<td>•</td>
</tr>
<tr>
<td>Unicellular Mass</td>
<td>Arrhenius</td>
<td>-14.0(388)</td>
<td>53.3</td>
<td>8.20</td>
<td>0.02</td>
<td>•</td>
</tr>
<tr>
<td></td>
<td>Exponential</td>
<td>-14.0(388)</td>
<td>56.6</td>
<td>11.4</td>
<td>0.00</td>
<td>•</td>
</tr>
<tr>
<td></td>
<td>Power</td>
<td>-14.1(388)</td>
<td>45.1</td>
<td>0.00</td>
<td>0.98</td>
<td>•</td>
</tr>
</tbody>
</table>
Figure 6.1. The effect of voltinism (generations per year) on the temperature-size response in (A) progeny (B) adult (C) Lepidoptera. In all cases, there was no significant difference between the strength of the temperature-size response and the number of generations per year. Error bars = 95% confidence intervals.
Figure 6.2. The effect of latitude (degrees from the equator) on the temperature-size response in (A) multicellular progeny, (B) multicellular adults. In both cases, there was no significant effect of latitude on the temperature-size response.
significant interaction between habitat and dry mass in multicellular adult data. Using the species-specific percentage mass changes (PCM) for each species, we determined these size changes were best described using the generalised linear model:

\[ PCM = H + \log(DM) \times H \]  

Equation 6.3

where “*” indicates an interaction between dry mass and habitat. To ensure that differential effects in different taxa were not driving the mass-dependent changes, we also applied the GLM to data for arthropods only, and found the same GLM to be the best fit. The best fit model applied to the entire adult multicellular data set showed there to be a significant difference in percentage mass change between freshwater and terrestrial temperature-size change (\( p < 0.0001 \)) but not between marine and freshwater; thus we grouped these as “aquatic species” as this did not significantly alter the results of the GLM. The GLM showed not only differences between aquatic and terrestrial habitats in absolute terms but also between the mass-dependence of the temperature-size response in these groups. There was a negative interaction between mass and aquatic species but a positive interaction between mass and terrestrial species. We subsequently plotted the percentage mass changes against temperature for the different habitats to illustrate these differences (Figure 6.3). Plotting the aquatic and terrestrial temperature-size data against dry mass highlights the significant and opposite responses in these two groups (Figure 6.4). In aquatic species, as adult dry body mass increases, the temperature-size response becomes increasingly negative. In terrestrial species, as mass increases the temperature-size response becomes significantly less negative, and some of the larger species have a reverse response. The mass dependence of these
Figure 6.3. Temperature-size response of individual species in terrestrial, freshwater, and marine environments. Size changes are expressed as a percentage change from that at 20°C, n = number of species.
Figure 6.4. Species-specific temperature size responses (% change in mass per °C) expressed as a function of the organism size (dry mass) in aquatic (marine and freshwater) and terrestrial environments. Terrestrial species show a significant positive regression \( PCM = -1.72 + 0.54 \times \log_{10} DM, R^2 = 0.15, df = 53, p<0.01 \); aquatic species show a significant negative regression \( PCM = -3.90 - 0.53 \times \log_{10} DM, R^2 = 0.14, df = 53, p<0.01 \). \( DM \) = dry mass, (mg), \( PCM \) = percentage change in mass °C⁻¹.
Figure 6.5. The temperature-size response as a function of body mass for aquatic organisms, including both uni- and multicellular organisms. As there is no significant change in the temperature-size response with mass in unicellular species, the mean response is given by the horizontal line (-1.80%°C⁻¹), the significant negative regression for multicellular organisms is shown.
Figure 6.6. A) Comparison of the percentage change in mass per °C in aquatic and terrestrial species. Aquatic species (mean = -3.65%°C⁻¹) show a significantly stronger temperature-size response than terrestrial species (mean = -1.43%°C⁻¹). Within the aquatic group, there is no significant difference between marine and freshwater species. Note how species within the order Diptera are found in both terrestrial and freshwater environments, with differences between these two reflecting the broader patterns across environment types. B) Mean ± 95% CIs for the percentage change in mass per °C in aquatic and terrestrial species. Size changes are significantly different between these two habitats (p <0.0001).
responses applies only to multicellular organisms; the much smaller unicellular organisms show no significant mass-dependence to their temperature-size response when plotted as a percentage change in mass per °C (Figure 6.5). Although terrestrial and aquatic species both have a response of around -2.5% °C⁻¹ at body sizes between 0.01 to 0.1 mg dry mass, the responses of metazoans from these two habitats diverge with increasing species’ sizes. In aquatic species the response becomes increasingly negative, reaching around -5% °C⁻¹ when body mass is 100 mg dry mass. In terrestrial species the response progressively reduces, reaching around -1% °C⁻¹ when body mass is 100 mg dry mass. These differences in the adult temperature-size response are not the result of differences in the responses of their progeny, as progeny mass data revealed no significant difference by environment type. Differences in adult responses between habitats must therefore be controlled by growth and development through ontogeny during the juvenile stages (Forster et al. 2011a).

Examination of the data by taxonomic group highlights the difference between aquatic and terrestrial species (Figure 6.6). The taxa showing the strongest negative temperature-size response are the Amphibia, which are larger aquatic species; the Orthoptera, which are largest terrestrial species, have the most positive value. Differences between aquatic and terrestrial species are further exemplified by examining the Diptera, the only taxa in which we have species representing both of these habitats (Figure 6.6). In this single taxa, we found a significant difference in the temperature-size response between aquatic (-4.54 ±1.03 (95% CIs)) and terrestrial species (-1.63 ±0.44 (95% CIs), 2 sample t test, t = 5.98, df = 21, p<0.0001).

Discussion
1. Compound Interest Hypothesis

a) Monovoltine species exhibit a weaker temperature-size response than multivoltine species.

We found no support for voltinism driving the TSR. According to Fischer and Fiedler (2002), one may expect a reduced temperature-size response in monovoltine species, as fitness is maximised by increasing size over fast reproduction in species with only a single generation in a year. In obligately monovoltine species, generation time is fixed, such that there is little fitness increase by maturing early (Meats 1971). In a population where numbers are increasing, fitness in maximised by reproducing early as these individuals will go on to form a larger fraction of the total population (Lewontin 1965, Atkinson 1994). Conversely, species which are monovoltine do not gain such benefits as they will not produce more generations in a year by reproducing earlier. Species capable of multiple generations should show strong temperature-size responses as speed of reproduction should be at a premium over size at warm temperatures, as this allows their offspring to make up a larger fraction of the population. Inclusion of voltinism within the GLM for multicellular progeny and adult data did not explain differences in the percentage mass change. We find no significant difference in the strength of the temperature-size response between species with 1, 2, 3 or more than 3 generations per year. This is highlighted in Figure 6.1. The compound interest hypothesis has previously been tested by Fischer and Fiedler (2002) using the butterfly species *Lycaena hippothoe*. The authors took populations which exhibited monovoltinism and compared the temperature-size response with multivoltine populations. They found a stronger temperature-size response in the multivoltine populations, which supports the predictions of the compound interest hypothesis. However, when we examined data for
a wider range of Lepidoptera (16 species), we found no such link between the strength of the temperature-size response and voltinism. Consequently, we find no support for the compound interest hypothesis as the ultimate explanation of the TSR.

2. Temperature and Latitude

b) The TSR is strongest in species found at higher latitudes.

We found no evidence for the TSR to change with latitude in multicellular organisms. Inclusion of latitude in the GLM did not significantly improve the fit of the model, either as a continuous or discrete variable. Further, plotting latitude against the strength of the temperature-size response in multicellular organisms highlights the lack of support for latitude as an explanatory variable (Figure 6.2). This suggests thermal generalists, found in seasonal habitats do not have a more plastic temperature-size response than those living in aseasonal environments. Any adaptive mechanism to explain the TSR must therefore be present in all species, irrespective of geographical location.

3. MASROS

c) Aquatic (marine and freshwater) ectotherms have a stronger temperature-size response than terrestrial ectotherms.

d) Large aquatic ectotherms have a stronger temperature-size response than smaller species.

Our quantitative analysis of the TSR supports both of the hypotheses associated with maintaining aerobic scope in multicellular adult species. Marine and freshwater species
show a significantly stronger temperature-size response than do terrestrial species, and large aquatic species show a stronger temperature-size response than smaller species. The negative response in aquatic species only occurs in multicellular adults. Further, in terrestrial species we find the opposite trend, with a significant positive response between the strength of the temperature-size response and dry mass. Unicellular organisms exhibit no negative response in the strength of the TSR, with a constant change of approximately -1.9% °C⁻¹. Similarly, we find no significant effect of mass or environment type on progeny, with a constant change of approximately 1% °C⁻¹.

How can these patterns be explained in terms of maintaining aerobic scope? Terrestrial species are generally less limited by oxygen supply (it is far more abundant and easily taken up from air) than are aquatic species (Makarieva et al. 2005). Expressed as the Oxygen Supply Index (Verberk et al. 2011) values are 1.05 x 10⁻¹⁴ mol.m⁻¹.s⁻¹ at the respiratory surface in freshwater at 20°C, 0.84 x 10⁻¹⁴ mol.m⁻¹.s⁻¹ in marine (salinity 34), but over 5 orders of magnitude greater in terrestrial organisms, at 2.30 x 10⁻⁸ mol.m⁻¹.s⁻¹. Thus, although as temperature increases it drives up the metabolic demand for oxygen, there is a far greater supply of oxygen available for terrestrial species. As metabolic rate increases with increasing temperature, the additional oxygen demands can therefore be met with fewer costs in terrestrial species. Further, the costs of increasing ventilation rate in air are much less than those in water, due to the differences in density of the media. For example, Tench (Tinca tinca) expend approximately a third of resting metabolic energy on ventilation (Pauly 2010). Given the highly significant difference between the temperature-size response in aquatic (-3.65% °C⁻¹) and terrestrial (-1.43% °C⁻¹) habitats, the obvious driver of these differences is oxygen. This supports the idea that oxygen, at least in part, drives temperature-size changes.
Beyond the absolute differences in the TSR between aquatic and terrestrial species, we also find the strength of the TSR to increase with increasing mass in aquatic species, which is not the case in terrestrial species. Further, this is only the case in multicellular aquatic species (Figure 6.5). As aquatic species become larger, they can no longer only rely on simple diffusion to transport oxygen into the body and require the active uptake of oxygen through respiratory structures. We hypothesise that large aquatic species have a greater proportion of their energy budgets committed to ventilation than smaller species which are more reliant on diffusion. This increase in active ventilation rate to maintain aerobic scope in larger aquatic species therefore costs a greater proportion of the energy budget. Given this, larger aquatic species will minimise these costs by maturing at a smaller size, resulting in an enhanced temperature-size response.

Although some of the species we consider do either move to the terrestrial domain (e.g. many emerging aquatic insects such as Diptera, Odonata), or in the case of the Amphibia (e.g. *Rana* spp.), the TSR is established during ontogeny, and shifts to air breathing are at or beyond the point the temperature-size response has developed or quantified here. In unicells, relying on diffusion, changes in oxygen supply relative to demand with increased temperature are limited by the surface area to volume ratio, and do not have the extra cost of ventilation. Therefore size changes may simply be an adaptive response to maintain the aerobic scope of cells.

The pattern of temperature-size change with mass in terrestrial species is more difficult to explain. As species dry mass increases, the strength of the temperature size response decreases (Figure 6.4), showing an opposite pattern to aquatic species. One potential cause of this pattern could be linked with the maximum metabolic rate of these
organisms. The largest species of terrestrial species in this analysis exhibiting a positive or no temperature-size response were large flying insects. In these species, oxygen supply must be sufficient to maintain respiration during flight, where oxygen consumption is extremely high (Nicolson and Louw 1982). Further, flight metabolic rate has been found to be independent of ambient temperature (Heinrich 1992). Therefore, maximum metabolic rate during flight is likely to put an upper limit on body size in these large flying insects, rather than oxygen supply relative to temperature. Ventilatory systems in place to support flight should easily meet the required aerobic scope at different temperatures. Thus adult body size in these species, where oxygen does not limit size, should show no temperature-size response. Smaller species, on the other hand, relying on simple diffusion, show similar temperature-size responses as aquatic species.

The size-dependent patterns found in the data also help to answer some of the more puzzling aspects of the TSR. Although most species (~90% according to our analysis) of species show a negative temperature-size response, orthopteran species have long been recognised as an outlier, rather showing an increase in size at higher temperatures (Atkinson 1994, Willott and Hassall 1998, Hassall et al. 2006b). In fact, our analysis suggests they are part of a continuum of reduced (and eventually reversed) temperature-size responses at larger body sizes in terrestrial species. Similarly, the enhanced Amphibia temperature-size response can be shown to be a consequence of their greater size. Further experimental data collected for large ectothermic animals reared at different fixed temperatures would be a useful addition to this analysis.
Our quantitative analysis of the TSR has important implications with regard to the potential impact of climate change in different habitats. Most species (90%) follow a negative temperature-size response. However, aquatic species show a significantly greater response than do terrestrial species. Species size has an additional impact: larger species have a reduced size response in terrestrial species, but an increased response in aquatic species. Given the increased frequency of extreme climatic events such as heat-waves (IPCC 2007), size responses may have significant impacts on these systems through modifying the overall size structure, as well as the size dependent biogeochemical rates and food web processes (Hansen et al. 1994, Woodward and Warren 2007).

Using a large data set (Table 6.1) to analyse potential adaptive mechanisms of the temperature-size rule has pointed towards oxygen having a significant role in determining the temperature-size response of ectotherms. This supports the results in the preceding four experimental chapters, which suggest no universal proximate mechanism.


CHAPTER 7

General Conclusions

This thesis has explored the mechanism of the temperature-size rule using a conceptual model. The model has highlighted that the temperature-size response of adult body size is determined by three factors: progeny size, growth and development rate. This simple conceptual model, combined with meta-analyses and experimental data, has supported the theory of the TSR being adaptive, as multiple proximate mechanisms exist to drive temperature-size changes in different ectothermic organisms. Furthermore, oxygen has been shown to play a central role in driving temperature-size changes. Here, I summarise my main findings and discuss potential directions of future work.

To understand how the TSR is brought about, a simple conceptual model was outlined in Chapter 2. The conceptual model showed the TSR to be dependent on four interlinked factors: growth rate, development rate, adult mass and progeny mass. In this second chapter, the model showed that for temperature-size changes to occur, rates of growth and development must become at least temporarily decoupled. Given the different reproductive methods in uni- and multicellular organisms, the model shows how the TSR is established to be different in uni- and multicellular organisms. In unicellular organisms, the rates of growth and development can only be temporarily decoupled due to the constraints imposed by dividing in half. After a period of acclimation, the ratio of adult to progeny size must return to the fixed value of 2. If this were not the case, cells would continue to become smaller or larger until they were no longer viable.
This is not the case in multicellular organisms. As the ratio of adult to progeny mass needn’t return to a fixed value, rates of growth and development can remain decoupled. This is shown to be the case using empirical data: progeny mass is less temperature dependent than adult mass, such that the ratio of $M_A/M_P$ reduces with increasing temperature. This suggests that growth and development rates remain decoupled. Using data for marine pelagic copepods, chapter 3 of the thesis bolsters the findings of chapter 2. The temperature dependence of growth is shown to be less than that of development throughout ontogeny. This shows these rates to be decoupled in multicellular organisms.

In chapter 4 the establishment of the TSR during ontogeny is determined in detail using the brine shrimp *Artemia franciscana* as a case study. This crustacean has distinct ontogenetic stages, thus changes in mass and stage could be determined independently. This showed the strength of the TSR to increase throughout ontogeny. Further, acclimation was not found to be significant. In fact, progeny size was found to be effectively “reset” at the beginning of each generation. This was supported by data for a range of crustaceans. However, the analysis in chapter 2 found other groups, such as Coleoptera and Lepidoptera, to change progeny size significantly with temperature. This suggests the proximate mechanism of temperature-size changes to differ not only across broad groups (uni- and multicellular species) but also in different taxa within these groups.

Using the ciliate *Cyclidium glaucoma* as a case study for unicellular species (Chapter 5), temperature-size responses were found to be established rapidly, with size changes being established within a single generation. The size changes were only decoupled
within the first generation, with the new acclimated size being established beyond this. We could therefore refine our conceptual model in chapter 2: in unicells, size changes are established rapidly and subsequently maintained. Therefore growth and development rates are only briefly decoupled. Further, we found population abundance to independently significantly influence size, suggesting plasticity in body size in unicellular organisms is a common approach in adapting to environmental changes. The temperature-size rule is ubiquitous and common to all ectothermic groups. However, this thesis finds little support for a common proximate mechanism underpinning the TSR. The life-history rates which drive these size changes are decoupled in multicellular organisms over multiple generations, but are only briefly decoupled in unicellular organisms. Further, changes in size in multicellular organisms differ across different groups. Crustaceans show no significant temperature-size change in progeny mass, yet across all metazoans we find a significant decline in progeny mass with temperature of approximately 1%°C⁻¹. The lack of a common proximate mechanism points towards the TSR being adaptive.

In Chapter 6 of the thesis, the potential adaptive mechanisms driving the TSR are examined. By performing a quantitative analysis on a large set of TSR data, we find significant effects of environment and body mass on the strength of the TSR. Importantly, aquatic organisms have a significantly stronger temperature-size response than do terrestrial species. Given the large differences in oxygen availability between terrestrial and aquatic environments, this difference suggests oxygen supply and demand to be an important driver of the TSR: as temperature increases and drives up metabolic rates, aquatic species must expend a greater proportion of their energy budget maintaining aerobic scope, thus fitness is maximised by maturing at a smaller size.
Directions for future study

Given the lack of support for a unifying proximate mechanism for the TSR highlighted in this thesis, along with the important role of oxygen highlighted in Chapter 6, I suggest future study should be target on developing our understanding of the interaction between oxygen and temperature. Until recently, there has been debate over the influence of temperature on oxygen supplied to organisms, focused on aquatic environments (Chapelle and Peck 1999, Peck and Chapelle 1999). In particular, there was disagreement over the importance of oxygen solubility vs. partial pressure. This has now been resolved using the Oxygen Supply Index (Verberk et al. 2011), which determines oxygen supply from a product of oxygen partial pressure, solubility and diffusivity. Such an index could help determine the importance of oxygen in determining the TSR. Potential avenues of exploration for determining the role of oxygen in the TSR include the following:

1. Within the quantitative analysis of the available temperature-size data in Chapter 6, we found a basic lack of available data for large aquatic species (with adult dry weights >10mg). In particular, there are no data for marine species. Given the mass-dependent temperature-size response in aquatic systems, further studies of the TSR in large aquatic species would be extremely useful evidence for confirming this response.

2. The importance of oxygen in determining temperature-size responses during ontogeny could be neatly explored using ectothermic taxa which span both aquatic and terrestrial environment. The order Isopoda would provide a good case study. These crustaceans are found in both aquatic and terrestrial environments, have short generation times and development can be determined through number of moults. The
establishment of the TSR could thus be determined across environments where oxygen supply varies. It may be, for example, that the TSR is established earlier in ontogeny in aquatic species, where oxygen supply is more limited. Other potential taxa to study include nematodes and gastropods.

3. Given the potential importance of oxygen supply vs. demand in determining the TSR, more focus needs to be put on examining the interaction of these two factors in determining size changes. Such work has been conducted on the fruit fly *Drosophila melanogaster* (Frazier et al. 2001), rearing individuals from egg to adult in a range of oxygen (10%, 20%, 40%) and temperature (15-31.5°C) treatments. Extending this work to include aquatic species would help to assess the relative importance of oxygen in determining the TSR, and highlight the oxygen-limitation in aquatic organisms vs. terrestrial organisms. Following on from point 2, a simple case study could be designed using an Order which inhabits both aquatic and terrestrial environments.

4. In unicells and small metazoans, in both aquatic and terrestrial environments, diffusion meets oxygen demands without the need for special adaptations such as ventilation of specialised respiratory organs. It has been long demonstrated that, without the complication of a boundary layer of stagnant water enveloping an aquatic organism, a body radius of up to ~1mm is sufficient to meet metabolic oxygen requirements by diffusion through its body surface (Schmidt-Nielsen 1979, Woods 1999), though this maximum size will be reduced by a boundary layer. The thickness of the boundary layer relative to organism volume increases for smaller species (Vogel 2003), and thus we expect variability in microplankton size response to temperature to be more influenced by water movement than by species size. By designing replicate experiments at
different temperatures and varying flow rates in microplankton species, one could determine the importance of water movement (i.e. the boundary layer thickness) in determining the TSR in species which rely only on diffusion to transport oxygen.

5. Beyond examining the impact of temperature-oxygen interactions at the whole organism level, an understanding is required at the cellular level to provide a complete understanding of the impact of oxygen in determining the TSR. Research into the oxygen partial pressure inside eggs of the lepidopteran species *Manduca sexta* has indicated oxygen to become limited at higher temperatures (Woods and Hill 2004). Such research, using microelectrodes, during ontogeny in both aquatic and terrestrial species would provide important evidence into the impact of high temperatures on oxygen availability.
REFERENCES


How do organisms change size with changing temperature? The importance of reproductive method and ontogenetic timing

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Summary

1. The ‘temperature-size rule’ (TSR) is a widely observed phenomenon within ectothermic species: individuals reared at lower temperatures grow more slowly, but are larger as adults than individuals reared at warmer temperatures. Although the TSR is common and of widespread ecological importance, it is not known whether there is a general physiological mechanism causing the TSR or even if species share a similar pattern of thermal response across ontogeny.

2. We constructed a conceptual model to show that binary division forces growth (g) and development (D) rates to return to a fixed ratio in unicellular organisms exposed to a change in temperature. After a period of decoupling during thermal acclimation, these rates must be restored to maintain a fixed ratio of adult:progeny size. However, the relationship between adult and progeny size need not be fixed in multicellular organisms at different temperatures, and hence growth and development rates need not have a fixed ratio either.

3. We conducted a meta-analysis on data of metazoan ontogenetic responses to temperature which demonstrates that adult size shows a much stronger temperature–size response than progeny size, and reveals variation in size response among other life cycle phases.

4. This study shows fundamental differences in the operation of the TSR in unicellular and multicellular organisms, suggesting that a general physiological mechanism causing the TSR is unlikely. Our findings also reveal the value of analysing shifts in size through the life cycle and across generations: these will yield a more complete quantitative description of how, and potentially provide clues to why, body size responds to temperature.

Key-words: development, growth, multicellular, plasticity, reaction norms, temperature–size rule, unicellular

Introduction

Body size is fundamental to the functioning of all organisms, impacting on all aspects of life including growth, reproduction and mortality (Kingsolver & Huey 2008). Therefore, understanding what drives species body size is a critical aspect of ecology. One widespread pattern of body size in ectothermic organisms is the ‘temperature–size rule’ (TSR). The TSR refers to how, within a species, lower rearing temperatures leads to increased size at a given developmental stage (Atkinson 1994). This phenotypically plastic response has been found to occur in the majority of ectotherms (83% of those examined) including a bacterium, protists, plants and animal groups including molluscs, arthropods, amphibians and fish (Atkinson 1994). Furthermore, a meta-analysis of protist data (Atkinson, Ciotti & Montagnes 2003) found that for each 1 °C increase in temperature, cell volume decreased by 2.5% of their volume at 15 °C. Changes in size have been described as the ‘third universal ecological response to global warming’ (Daufresne, Lengfellner & Sommer 2009), alongside shifts in species range and changes in phenology. A feature of current climate change is the predicted increase in frequency and intensity of heat-waves (IPCC, 2007); therefore, understanding how organisms will respond to increasing temperature in the short-term and longer term and the mechanisms underpinning these responses is critical.

Growing to a smaller final size at warmer temperatures seems counterintuitive and has been termed a ‘life-history puzzle’ (Sevenster 1995). One might expect that as organisms
have faster growth rates at higher temperatures, they should delay maturation to exploit the increase in fecundity, survival and mating success associated with larger size (Sibly & Atkinson 1994; Kingsolver & Huey 2008). Indeed, increased growth rate associated with improved food conditions results in larger adults, whereas the increased growth rate associated with higher temperature results in reduced adult size (Kindlmann, Dixon & Dostalkova 2001). Attempts to explain why temperature differences result in body size changes often consider the problem with respect to the maximization of fitness (population growth rate, \( r \), and offspring production, \( R_0 \)), and include the interplay of multiple traits such as growth, fecundity, development and mortality (Sibly & Atkinson 1994; Kozlowski, Czarnoleski & Danko 2004; Kiærboe & Hirst 2008). Rather than focusing on why the TSR occurs, others have instead focused on the question of how body size changes. For example, Davidowitz & Nijhout (2004) formulated a physiological (endocrine-based) model for holometabolous insects. However, we still lack a general model to explain the TSR (Angilletta, Sears & Steury 2004): such a general model would account for differences across taxa, changes in size during ontogeny and changes in size across genera. Here, we will explore how critical differences between methods of reproduction, and in growth and developmental responses to temperature between different ontogenetic stages, affect attempts to derive a universal mechanistic TSR model.

### How does body size change?

The TSR indicates that when juveniles grow in cooler environments, they develop into larger adults (Atkinson 1994); consequently, although the rate of development (passing through life stages) and growth (accumulation of mass) from embryo to adult decreases with decreasing temperature, there must be a relatively larger decrease in the development rate. Although this seems obvious, previous general models of how size changes with temperature have often not explicitly indicated that these two rates are decoupled [e.g. explanations based on the von Bertalanffy growth equation (Von Bertalanffy 1957; Perrin 1995)].

Van der Have & de Jong (1996) argued that the TSR must be a result of mismatch in the temperature dependence of growth rate and development rate (which they use synonymously with differentiation rate). They then built a biophysical model, assuming development and growth rates to have independent thermal reaction norms under non-limiting food conditions. van der Have & de Jong (1996) state, ‘As a proximate model, the biophysical model applies to all eukaryotes, including protists in which ‘differentiation’ consists only of cell divisions.’ Van der Have & de Jong (1996) also suggest that progeny size may be impacted by different rates of differentiation and growth of oocytes (e.g. vitellogenin synthesis in insects; (Ernsting & Isaias 1997)). From this assumption, they argued that the same effect of temperature on oocyte production as on the size at metamorphosis could be predicted; that is, larger eggs will be produced at lower environmental temperature.

Van der Have & de Jong (1996) made an important advance in analysing the TSR by explicitly treating growth and development as separate rates. However, any general mechanistic model needs to explain fundamental differences in the establishment of the TSR in different organisms. In this study, we show how reproduction by binary division (i.e. cell dividing into two equally sized progeny) results in fundamental differences in the operation of the TSR between unicellular and multicellular organisms. Secondly, we perform a meta-analysis to measure the effects of temperature on adult vs. progeny size in metazoans, and show that progeny size in multicellular organisms does not follow the same response as adult size. Thirdly, we explore size responses to temperature across ontogeny in multicellular organisms, to examine whether these organisms exhibit systematic changes in size throughout the whole life cycle or whether size responds mostly during specific stages after which these changes are maintained. Analysis of shifts in size throughout the life cycle and across generations will yield a more complete quantitative description of how, and potentially provide clues to why, body size responds to temperature.

We specifically addressed the following questions:

1. How do constraints of a unicellular vs. multicellular life cycle affect the adjustments of size and therefore growth and development associated with the TSR?
2. Does the TSR affect adult and progeny mass of multicellular organisms equally?
3. Does the TSR have consistent effects throughout ontogeny?

### The conceptual model

We constructed a model building on the linear equation used by van der Have & de Jong (1996) to link growth and development with adult and progeny size:

\[
g = \left( \frac{M_A}{M_P} \right) / t \tag{eqn 1}
\]

Where \( g = \) mean juvenile growth rate (day\(^{-1}\)), \( t = \) development time (days, e.g. egg hatch to maturation, or time between subsequent divisions in unicellular organisms), \( M_A = \) mass of adult, and \( M_P = \) mass of a single progeny. We use the term ‘progeny’ to refer to young at the point of inception. This is the daughter cell just after binary division of the mother in a unicellular organism, or the newly produced egg, or the propagule at the point of budding in a multicellular organism. Using the inverse of development time \( t \) in eqn 1 converts this parameter to a mean rate of development, \( D \) (\( D = 1/t \)). Individual growth from progeny to adult can then be expressed as:
Thus, the ratio of adult to progeny mass provides a straightforward way to determine the effect of temperature on two fundamental biological rates (growth and development rate) and to test van der Have & de Jong (1996) hypothesis: that development and growth rates have independent thermal reaction norms under non-limiting food conditions. We consider the implications of this growth equation to the TSR in unicellular and multicellular organisms.

UNICELLULAR ORGANISMS

Most unicellular organisms reproduce by binary division (Adolph 1931), a term we use to encapsulate binary fission in prokaryotes, and mitosis in unicellular eukaryotes. In binary division, an ‘adult’ cell (of mass \( M_A \)) divides into two ‘daughter’ cells (of mass \( M_P \)), each with a mass half that of the adult, i.e. \( M_A = 2M_P \). Thus, at a fixed temperature across generations, and with other conditions constant, \( M_A/M_P = 2 \), eqn 2 then simplifies to:

\[
g \over D = 2
\]  

(eqn 3)

At a fixed temperature, unicellular organisms must have a fixed ratio of growth to development rate, thus, referring to Fig. 1, \( g/D_{(cold)} = g/D_{(warm)} = 2 \). This is in clear disagreement with the assumption of the van der Have and de Jong model, that development and growth rates have independent thermal reaction norms under non-limiting food conditions. In fact, binary division imposes strict limits on adult and progeny size ratio and forces \( g/D \) to return to a fixed ratio of 2. However, most unicellular organisms obey the TSR, becoming larger at cooler temperatures and smaller at warmer temperatures (Atkinson 1994; Montagnes & Franklin 2001; Atkinson, Ciotti & Montagnes 2003). Therefore, within generations, the size of unicells must change when exposed to a new temperature and \( g/D \) must become temporarily decoupled (see Fig. 1). After \( g/D \) adjustment is complete, the rates must become coupled once more; these rates cannot be considered independent as binary division requires that total temperature compensation occurs (i.e. Eqn 3 is restored) to prevent cells continuing to get smaller or larger \textit{ad infinitum}. These conclusions are not qualitatively affected by altering the growth function from linear increase in mass per unit time, to exponential or von Bertalanffy (see Appendix S1 in Supporting Information).

MULTICELLULAR ORGANISMS

Application of eqn 2 is more complex for multicellular organisms. As they do not replicate by simple binary division of the adult, the progeny mass is not restricted to be a fixed proportion of adult mass and in many species, individual organisms are able to produce progeny that can vary in size (Blanckenhorn 2000; Atkinson et al. 2001; Fischer et al. 2003; Fischer, Zwaan & Brakefield 2004). Therefore, unlike unicells, individual progeny are not so strictly constrained by maternal size, thus the ratio \( M_A/M_P \) need not be fixed across different temperatures and consequently growth and development rates would not need to return to a fixed ratio (eqn 2). If this were the case, growth and development rates could change independently with temperature, which supports the assumption of the biophysical model applied by van der Have & de Jong (1996). There is much evidence confirming a temperature–size response in adults (see review in Atkinson 1994), but less evidence for eggs (see review in Atkinson et al. 2001). We show this potential temperature independence of \( g \) and \( D \) in Fig. 2.
in which adult mass is assumed to change with temperature more than progeny mass, thus $M_{a}/M_{P}(\text{cold}) > M_{a}/M_{P}(\text{warm})$.

If adult and progeny mass show different temperature–size responses in metazoans, this would suggest a fundamental difference between the TSR in unicellular and multicellular organisms: unicellular organisms living at a fixed temperature must have a constant ratio of $g/D$, whereas multicellular organisms have a variable ratio of $g/D$.

Is this supported by experimental data in the literature? We conducted a meta-analysis on metazoan adult and progeny size data for a wide range of species, and tested whether the thermal responses of these data sets differ. Where data were available, changes in mass were also examined separately throughout ontogeny, described in the Methods below.

**Materials and methods**

Detailed accounts of methods are provided in Supporting Information (Appendix S2). We collected published data to assess the extent to which mass of both adults and progeny of ectotherms varies with temperature (after allowing a period for size acclimation). We only include data where individuals were grown at constant temperatures and at food conditions believed to be saturated. We designated a minimum acclimation period for both progeny and adult data (Appendix S2) to ensure that sizes were acclimated to the temperature at which they were recorded. Initially, we included data when presented as masses for either progeny, adults or both from single studies (Tables S1 and S2 respectively in Supporting Information). To test for differences in the response of adult and progeny size to temperature more rigorously, and act as direct comparison with size-acclimated unicellular organisms where $M_{a}/M_{P}$ is fixed, we analysed a sub-set of this entire data set, specifically those data in which adult and progeny sizes are described on single species by the same study group: we term this ‘paired data’ (Appendix S2 and Table S3). We also consider the period of acclimation more closely in this set (Appendix S2).

An appropriate model for the response of adult and progeny size to temperature was required which could be applied across all species. There is conflicting opinion as to the form that the body mass thermal reaction norm should take within a species, and many different equation forms have been proposed (Karan et al. 1998; Atkinson, Ciotti & Montagnes 2003; de Jong 2010). We therefore applied a range of equation forms (linear, exponential, Arrhenius and allometric) to the full data set to determine which best described the empirical data, using a linear mixed effects model (see Appendix S3 for details and in-depth results). An information theoretic approach was used to determine which model best fit the adult and progeny data; Akaike weights were used, which determine the best fit while accounting for the complexity of the model. We next analysed both the full data set and the paired data to examine if the slopes of the temperature–size response in adults and progeny were significantly different, comparing the mean slopes for the full data set and conducting a paired t-test on individual species in the paired data set.

Beyond examining progeny and adult masses, an appreciation of where in the development schedule changes in the mass to temperature relationship occur in metazoans will provide insight into the causes of these changes. Within the data, two studies had individual masses and times for multiple larval stages between egg and adult (including prior acclimation), which allowed us to examine how the response of mass to temperature varies throughout ontogeny. These were both for copepods, *Acartia tonsa* (Leandro, Tiselius & Queiroga 2006), and *Calanus finmarchicus* (Campbell et al. 2001), and included egg, six nauplii stages and six copepodite stages, the final stage being the adult. To determine the mass vs. temperature relationship for each stage, the best-fit model type, as shown from our analysis of all progeny and adult data, was applied to each species individually (see Appendix S3).

**Results**

**ADULT TO PROGENY SIZE RATIOS OF MULTICELLULAR ORGANISMS**

For the larger unpaired dataset, we collected progeny data for 33 different species (Table S1) and adult data for 85 different species (Table S2). Within this larger set, there were adult and progeny paired data for 15 (sub)species which fulfilled the more rigorous requirements (Table S3). We found that an exponential model with species-specific intercepts and slopes provided the best fit to both the adult and progeny mass vs. temperature responses (see Appendix S2), with the basic form:

$$\ln M = a + bT$$  \hspace{1cm} (eqn 4)

where $M$ = mass, $T$ = temperature, $a$ is the mean intercept and $b$ is the mean slope term.

According to the fitted slopes for this best fit model, adult mass had a significantly more negative slope ($b = -2.60 \times 10^{-2}$, 95% CIs = ±0.57 $\times 10^{-2}$) than progeny mass ($b = -0.90 \times 10^{-2}$, 95% CIs = ±0.61 $\times 10^{-2}$) across the entire data set ($t$-test, $t = 6.19$, $P < 0.001$, Fig. 3, Appendix S3). This is equivalent to a 0.9% decrease in mass °C$^{-1}$ in progeny, but a 2.5% decrease in mass °C$^{-1}$ in adults, with the mag-

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A consequence of this is that multicellular organisms must have a $g/D$ ratio, which varies substantially across temperatures, i.e. growth and development rates have a different temperature dependence. In contrast, in unicells reproducing by binary division, we know that $g/D$ must be fixed (eqn 3).

TIMING OF SIZE ADJUSTMENT DURING ONTOGENY

The effect of temperature on the size of specific larval stages in the copepod species *Acartia tonsa* and *Calanus finmarchicus* shows some variation between these two species (Fig. 5); however, there are general patterns in these responses. There is no discernible effect of temperature on size of progeny (represented by early larval stages) in either species. The mass vs. temperature relationships exhibit a generally increasing negative trend throughout ontogeny when examined in relation to time (Fig. 5a, b). When examined with respect to mass, the majority of the temperature-dependence of size has been completed by approximately 20% of the adult mass (Fig. 5c, d). The majority of the temperature–size effect has been completed before the last 3–4 larval stages, despite these stages accounting for the majority of mass accrual (approximately 80% of total mass) due to the exponential nature of mass accrual with time exhibited in copepod species (Escrivano & Mclaren 1992).

Discussion

Using a conceptual model, we have shown that unicellular organisms acclimated to different temperatures must have a ratio of growth to development rate which is a constant; this

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**Fig. 4.** Change in mass of ectotherms as a function of temperature for: (a) progeny, (b) adult, and (c) adult to progeny mass ratio. Symbols give individual data points in a and b, while in c, the symbols do not give individual values, but rather indicate which species the line is for. Progeny and adult data fitted with exponential best-fit models, adult to progeny mass ratio determined for each species by dividing results from the best fit equation for adults at a specific temperature by the best fit equation for progeny at the same temperature. To improve visualization, data for (c) were converted to percentage change in mass with temperature.
Reproductive method and ontogenetic timing

Our synthesis of adult and progeny size in multicellular organisms shows that the ratio of these is not constant at different temperatures. Combining this evidence with our conceptual model that unlike in unicells, multicellular organisms can maintain different temperature dependence for growth rate relative to development rate. Our meta-analysis demonstrates that progeny mass shows a reduced response to temperature compared with adult mass. Although both show a negative response, adult mass has a significantly more negative slope than that of progeny. Furthermore, when the paired data were compared, the ratio of $M_f/M_p$ was consistently negative across the 15 (sub)species, with the average progeny temperature-size response not being significantly different from zero. This novel finding suggests that it is incorrect to assume a temperature-size effect on progeny size in multicellular organisms that is of similar magnitude to adults. van der Have & de Jong (1996) proposed that ‘the same effect of temperature on oocyte production as on size at metamorphosis could be predicted, that is, smaller eggs will be produced at higher environmental temperature’. This statement requires clarification: we find that the magnitude of this change is consistently larger in adults than in progeny. This is the case even after allowing for acclimation of both adult and progeny size. Referring to eqn 2, this means that growth and development rates have a different temperature dependence in multicellular organisms, with development being more temperature-sensitive than growth.

There is evidence from two copepod species that early larval stages (i.e. beyond egg stage) show no size response to temperature, whereas later stages show strong negative relationships. The data suggest that thermal selective pressures act increasingly during the maturation of the two copepods (Fig. 5) and the unequal effect of temperature on growth and development rates only begins acting on size during post-embryonic growth. Although temperature-size effects are cumulative during ontogeny, the majority of the temperature-size response is established by the point at which approximately 0.2 of the adult weight has been achieved. This is in contrast to larval development in the butterfly Lycaena tityrus, where the TSR is only established during the final larval stage associated with the largest (approximately 80%) increase in mass (Karl & Fischer 2008). Furthermore, ontogenetic size changes in Fig. 5 indicate that these copepod species may be seen as adjusting size in every generation: changes in size are effectively being reset or considerably muted at

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**Fig. 5.** Slopes of the relative changes in mass with temperature (ln Mass vs. Temperature) for consecutive larval developmental stages of the copepods: (a) *Acartia tonsa* (Leandro, Tiselius & Queiroga 2006), and (b) *Calanus finmarchicus* (Campbell *et al.* 2001) size changes as a proportion of time to adult. (c) *Acartia tonsa* (Leandro, Tiselius & Queiroga 2006), and (d) *Calanus finmarchicus* (Campbell *et al.* 2001) size changes as a proportion of adult mass. Larval stages comprise six nauplii stages (NI–NVI) and six copepodite stages (CI–CVI), the CVI stage is the adult. Error bars indicate 95% confidence intervals.
egg/progeny stage. This, again, is not the case in butterfly species, which show marked changes in egg size at different temperatures (Fischer et al. 2003; Fischer, Bauerfeind & Fiedler 2006). This suggests that although the TSR applies to the majority of metazoans (Atkinson 1994), there may be taxon-specific changes in size with temperature, which impact on different life stages to different extents.

How do these differences between unicellular and multicellular organisms impact on the potential causes of the TSR? The results of our conceptual model, combined with the meta-analysis reveal that unicellular organisms are restricted in the adjustment of their rates of growth and development. The ratio of $g/D$ must return to a constant of two in a two species living at a fixed temperature, thus any temperature-induced changes in this ratio is limited to a temporary acclimation phase. In multicellular organisms, the ratio of $g/D$ need never be a fixed constant when comparing across different temperature regimes because these organisms alter their adult-progeny size ratio (see eqn 2 and Fig. 4c). Although there must be limits imposed on size changes set by physical constraints, such as maternal ovipositor/birth canal diameter (Atkinson et al. 2001), this does not impose strict limits on the ratio of $M_g/M_p$, and therefore $g/D$, as it does in unicells. Indeed, the results from the paired meta-analysis show the ratio of $M_g/M_p$ can change substantially over a species’ thermal range in multicellular organisms. Consequently, there can be large alterations in the ratio of $g/D$ (eqn 2). For example, $M_g/M_p$ data for *Pseudocalanus newmani* show that development rate must increase by more than twice the rate of growth over this copepod’s total thermal range.

It is important to note that despite the different restrictions imposed by reproductive method in unicellular and multicellular organisms, both follow the TSR. Therefore, despite the restrictions imposed by binary division, rates of $g/D$ must temporarily decouple to facilitate size change, even if they must eventually return to a fixed ratio. This suggests that there must be significant fitness benefits to this thermal plasticity, as it occurs in different groups through different means. Thus, although the proximate mechanism for the TSR differs between these two groups, the ultimate explanation for the TSR may still be the same. Despite many hypotheses having been proposed (Angilletta, Sears & Steury 2004; Atkinson, Morley & Hughes 2006; Walters & Hassall 2006; Kingsolver & Huey 2008), we are yet to find a general, ultimate cause for the phenomenon of the TSR. To understand the variation in size responses to temperature, we propose that more attention be directed to fuller quantitative descriptions of responses throughout the period of population growth in unicells, and ontogeny in multicellular organisms. In unicells, for example, by identifying the number of cell generations until $g/D$ adjustment is complete and the amount of $g/D$ adjustment per cell cycle per °C, we can partition variation in size responses among species to the different mechanisms (average thermal sensitivity of size per cell cycle per °C, number of cell divisions to complete acclimation), and seek patterns in these among taxa and ecological niches. Likewise, in multicellular organisms, differences between species in the period of $g/D$ adjustment, as shown in Fig. 5 for two species of copepod, can help identify variation, or indeed similarities, between species and taxa. Another potential benefit from quantifying trends in TSR across ontogeny is to identify particular stages or size ranges when selection for size response to temperature may be particularly intense. Berven & Gill (1983) suggest that temperature-dependent variation in adult size in *Rana sylvatica* may be a consequence/correlate of temperature-dependent selection on offspring size plasticity. By quantifying which developmental phases actually show a size response to temperature (Fig. 5), particular parts of the life cycle may be examined to see whether or not there are particular temperature-dependent selection pressures that affect those developmental phases or size classes.

Any universal mechanism explaining the TSR must be applicable to all ectothermic groups. We have shown that fundamental differences exist between unicellular and multicellular organisms in the way size changes are brought about. This suggests that there is no universal physiological mechanism to explain the TSR.

References


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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1. Progeny mass data.

Table S2. Adult mass data.

Table S3. Paired mass data.

Appendix S1. Applying different growth equations.

Appendix S2. Methods used in data collection.

Appendix S3. Modelling the data.

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Growth and Development Rates Have Different Thermal Responses

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Abstract: Growth and development rates are fundamental to all living organisms. In a warming world, it is important to determine how these rates will respond to increasing temperatures. It is often assumed that the thermal responses of physiological rates are coupled to metabolic rate and thus have the same temperature dependence. However, the existence of the temperature-size rule suggests that intraspecific growth and development are decoupled. Decoupling of these rates would have important consequences for individual species and ecosystems, yet this has not been tested systematically across a range of species. We conducted an analysis on growth and development rate data compiled from the literature for a well-studied group, marine pelagic copepods, and use an information-theoretic approach to test which equations best describe these rates. Growth and development rates were best characterized by models with significantly different parameters: development has stronger temperature dependence than does growth across all life stages. As such, it is incorrect to assume that these rates have the same temperature dependence. We used the best-fit models for these rates to predict changes in organism mass in response to temperature. These predictions follow a concave relationship, which complicates attempts to model the impacts of increasing global temperatures on species body size.

Keywords: growth, development, rates, temperature-size rule, copepod.

Introduction

Development (passing through life stages) and growth (increase in mass) are fundamental to all living organisms. The rate at which individuals mature, along with their size, determines higher-level properties, such as population abundance (Di Cola et al. 1999), dispersal distance (O’Connor et al. 2007), and energy flow (Silvert and Platt 1978). It is therefore vital that we understand how these rates respond to variation in temperature, especially in the context of global warming. Average global air temperatures are expected to increase by between 1.1° and 6.4°C this century, whereas shorter-term fluctuations in temperature as a result of climate change are becoming increasingly common (IPCC 2007). Furthermore, climate models predict that the average sea surface temperature will have increased by 2°–3.5°C by the end of this century, with the Arctic showing even greater increases (up to 8°C warmer; Richardson 2008). More than 99% of species are ectotherms (Pincheira-Donoso et al. 2008), in which metabolism is driven primarily by body size and environmental temperature (Brown et al. 2004). Changes in the latter will impact on organisms’ body temperature and in turn drive changes in development and growth rates. We therefore need to be able to make broad predictions as to how these rates change with temperature and the impacts of these changes on individual organisms.

There is currently no consensus on how growth and development rates respond to temperature nor on the most efficient way of modeling these relationships. Within species, models based on linear (Montagnes et al. 2003), allometric (Belehradek 1926; McLaren 1969; Corkett and McLaren 1970; Hart 1990; Peterson 2001), and exponential (Escribano and McLaren 1992; Escribano et al. 1997; Campbell et al. 2001) functions have often been applied to describe how these rates change with temperature. More complex relationships with a mechanistic basis—for example, Arrhenius (Gillooly et al. 2002; Brown et al. 2004) and Sharpe-Schoolfield (van der Have and de Jong 1996; de Jong 2010) equations—are also commonly used. When attempting to make broad predictions about the response of fundamental rates to temperature in individual species, it is important to balance accuracy and simplicity. We aimed to find a model that accurately describes the data without over-parameterization and without requiring large quantities of additional information that is difficult and time-consuming to collect. We used an information-theoretic approach to find out which of these models was best supported by the data and hence best described the
relationship between temperature and both growth and development rates.

Accurately describing the thermal response of growth and development rates across the life cycle of an organism (e.g., from egg to adult) will provide valuable information on how these fundamental rates are likely to respond to climate change. Simply examining them as averages across the entire life cycle, however, fails to acknowledge important changes in these rates through ontogeny (Forster et al. 2011). We addressed this by comparing growth and development rates using data from egg and early and late larval stages. We focused our efforts on quantifying these processes using marine pelagic Copepoda; these are the dominant mesozooplankton in the world’s oceans and are a key component of the ocean food web (Mauchline 1998; Richardson 2008).

Studying this group offers many advantages, because they have a fixed number of molts and exhibit determinate growth. Growth and development rates can therefore be determined across easily identifiable and distinct stages throughout ontogeny. Furthermore, detailed laboratory studies of these rates in marine copepods have been conducted for many years (see Hart 1990; Peterson 2001), and a large amount of data are available for many species.

Examining the thermal response of growth and development rates separately has important implications for the understanding of the temperature-size rule (TSR; Atkinson 1994). The TSR describes the phenotypically plastic response within ectothermic species, in which individuals attain larger adult body sizes when reared at cooler temperatures than when reared at warmer temperatures. The TSR is demonstrated in over 83% of the ectothermic species investigated, including bacteria, protists, insects, vertebrates, and crustaceans (Atkinson 1994), including our target group, marine copepods (Kimoto et al. 1986; Uye 1988, 1991; Campbell et al. 2001; Hansen et al. 2011). It has been suggested that the TSR is driven by growth and development rates having differing temperature dependence within a species (Atkinson 1994; van der Have and de Jong 1996; Davidowitz and Nijhout 2004; Forster et al. 2011): growth rate (accumulation of mass) increases with temperature but is outpaced by the relative increase in development rate (passing through life stages), resulting in smaller adult size at warmer temperatures. A recent analysis that considered marine, freshwater, and terrestrial metazoans revealed that intraspecific changes in size with temperature are significantly greater in adults than in progeny (Forster et al. 2011), further implying that growth and development rates must be decoupled through the egg to adult period across a wide range of taxa.

Although the decoupling of growth and development rates has been confirmed experimentally in a handful of species (e.g., *Drosophila melanogaster* [van der Have and de Jong 1996], *Rana pipiens* [Smith-Gill and Berven 1979], *Chorthippus brunneus* [Walters and Hassall 2006], and *Manduca sexta* [Davidowitz and Nijhout 2004]), we need to test whether this result holds more generally for a range of species, because the thermal responses of these rates have wider importance with respect to the general ecological theories of metabolism. The metabolic theory of ecology (MTE; sensu Brown et al. 2004) assumes that biological rates are intimately linked with metabolism, such that rates as varied as heart rate, growth, development, and mortality (when mass corrected) follow a thermal response modeled by the Arrhenius function (Brown et al. 2004). Decoupling of growth and development for a range of species would suggest fundamental differences in the mechanistic processes underlying these rates (van der Have and de Jong 1996). This would suggest inadequacies in theories built upon the assumption that life-history rates share a common temperature dependence (Gillooly et al. 2001, 2002; Brown et al. 2004). Furthermore, if we find that these rates do not follow an Arrhenius-type function, it would suggest fundamental flaws in the assumptions associated with this model type. Systematically and comprehensively determining the thermal response of growth and development rates allows us to test both of these points.

Changes in size with temperature have been described as the “third universal ecological response to global warming” (Daufresne et al. 2009, p. 1). Because body size is a key determinant of food web structure and dynamics (Woodward et al. 2005; Barnes 2008; Woodward et al. 2010a, 2010b; Yvon-Durocher et al. 2010), any temperature-mediated changes in organism size could have important impacts at the ecosystem level. Accurately determining the general thermal response of growth and development rates to temperature across a range of copepod species will allow us to estimate the thermal reaction norm (the pattern of phenotypic expression of a single genotype) of individual body mass to temperature. These insights could ultimately be used to scale up to the higher levels of biological organization, such as communities and food webs (Woodward et al. 2010a).

The aims of this study are therefore to address the following questions: (1) Which equation(s) best describe the response of growth and development rates to temperature, and is there evidence to suggest different responses to temperature for these rates? (2) Are the shapes of these responses maintained across different developmental stages? (3) Based on the relationships determined, how do we predict organism size will vary with temperature?

### Methods

Growth and development rate data for marine planktonic copepods were collected from the literature. We limited the search to include only laboratory studies conducted at a
range of different temperatures with high (saturated) food conditions; the protocols are described in detail in appendix A, available online. In brief, growth rates were compiled from data sets as mass-specific growth rates (g day⁻¹), calculated as the slope of ln mass against time, or calculated using the molt rate method (see Hirst et al. 2005), typically by applying the formula ln (M₁/₀/M) /SD_i, where M is the stage mass (carbon content or dry mass), i is life stage, and SD_i is stage duration of stage i. Growth rates calculated using the molt rate method are subject to errors and have all been corrected following the revised methods of Hirst et al. (2005). Finite growth rates (G day⁻¹), based on production-to-biomass ratios, were converted to instantaneous mass-specific growth rates (using the equation g = ln (G + 1)). Growth rates were included as across-stage rates (e.g., NI-NII and CI-CII), across-naupliar rates (NI-NVI), across copepodite rates (CI-CVI), and growth rate of the entire larval development (NI-CVI, which we term total growth).

We included data for 15 species of marine copepod that fulfilled our selection criteria (see app. A), with 312 individual data points. Development data are typically presented in the literature as development times, either as stage durations or median development times (MDTs). MDTs represent the total time to a stage, rather than a stage-specific duration; thus, we converted these data to stage durations. Development data were collected for eggs, each of the six nauplius stages (NI-NVI), and the five juvenile copepodite stages (CI-CV). Stage durations were then converted to development rates as 1/stage duration (in days). Development rates were included as stage-specific rates, across-naupliar rates (NI-NVI), across-copepodite rates (CI-CVI), or rate across the entire larval development (NI-CVI, which we term total development). Egg development rates were examined separately. We compiled larval development rate data for 24 species (1,059 data points) and egg data for 34 species (345 data points; app. A).

We focused on examining the effect of temperature on rates under nonextreme situations. We excluded growth or development data for low temperatures at which individuals did not attain maturity, and we removed those data for high temperatures from the point that rates began to decrease with increasing temperature (as judged by a decrease in rates from one temperature to the next highest). These criteria enabled us to account for the different thermal tolerances between species while avoiding temperatures at which severe resource limitation might occur (Atkinson et al. 2003). This approach also maximized parsimony, because fewer parameters were required to model the species-specific rate changes. This screening process excluded relatively few data from the analysis (~5% of data; app. A).

We used a linear mixed-effects model in R, version 2.10.0, to account for variation caused by different covariates (temperature, species, study, sex, and stage), with temperature fitted as a fixed effect and species, study, sex, and stage treated as random effects (see app. B, available online, for details). The two reproductive methods employed by marine pelagic copepods (releasing eggs into the water column and carrying eggs in sacs) were initially included in the model but did not improve fit and were thus subsequently excluded (see app. B). One of the primary assumptions of a mixed-effects model is that the residual error for each model type is normally distributed (ε ~ N[0, σ^2]; O’Connor et al. 2007). Because this was not the case with our data (variance increased with temperature), we natural log–transformed the data before fitting the different models (see app. C, available online); thus, exponents became slope terms, and slopes became intercept terms (see “statistical model” in table 1).

Centering data about a fixed temperature when using mixed models improves the interpretability of each model and reduces the correlation between parameters (O’Connor et al. 2007). We adopted the centering method as applied by O’Connor et al. (2007); see table 1. A centering temperature of 15°C was applied, because this temperature is within the boundaries used in most studies in
Table 2: Best-fit mixed-effects models for each type of statistical model applied to marine copepod larval growth, development, and egg rate data, including species-level effects

<table>
<thead>
<tr>
<th>Rate, model</th>
<th>Group parameter</th>
<th>β₀</th>
<th>β₁</th>
<th>β₂</th>
<th>AIC</th>
<th>Δ₁</th>
<th>ω₁</th>
<th>Best model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth rate:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allometric</td>
<td></td>
<td>1.64(0.14)</td>
<td>1.37(0.13)</td>
<td>54.36</td>
<td>0.0</td>
<td>.67</td>
<td></td>
<td>•</td>
</tr>
<tr>
<td>Complex allometric</td>
<td></td>
<td>1.65(0.14)</td>
<td>1.47(0.20)</td>
<td>-.14(0.14)</td>
<td>55.82</td>
<td>1.5</td>
<td>.33</td>
<td></td>
</tr>
<tr>
<td>Exponential</td>
<td></td>
<td>1.65(0.13)</td>
<td>.09(0.01)</td>
<td>117.18</td>
<td>62.8</td>
<td>.00</td>
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<td></td>
</tr>
<tr>
<td>Arrhenius</td>
<td></td>
<td>25.6(0.69)</td>
<td>[-.72(0.02)]</td>
<td>143.81</td>
<td>89.5</td>
<td>.00</td>
<td></td>
<td></td>
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<tr>
<td>Larval development rate:</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Allometric</td>
<td></td>
<td>.68(0.08)</td>
<td>1.43(0.11)</td>
<td>979.0</td>
<td>31.3</td>
<td>.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex allometric</td>
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<td>.66(0.06)</td>
<td>1.57(0.06)</td>
<td>[.34(0.05)]</td>
<td>947.67</td>
<td>0.1</td>
<td>1.00</td>
<td>•</td>
</tr>
<tr>
<td>Exponential</td>
<td></td>
<td>.69(0.07)</td>
<td>.11(0.00)</td>
<td>981.12</td>
<td>33.5</td>
<td>.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arrhenius</td>
<td></td>
<td>28.8(0.37)</td>
<td>[-.78(0.02)]</td>
<td>982.9</td>
<td>35.2</td>
<td>.00</td>
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</tr>
<tr>
<td>Egg development rates:</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allometric</td>
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<td>.82(0.10)</td>
<td>1.40(0.11)</td>
<td>-.160</td>
<td>40.1</td>
<td>.00</td>
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<tr>
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<td>[.37(0.07)]</td>
<td>-40.21</td>
<td>0.1</td>
<td>1.00</td>
<td>•</td>
</tr>
<tr>
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<td>.11(0.01)</td>
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<td>56.1</td>
<td>.00</td>
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<td></td>
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<td>53.30</td>
<td>93.5</td>
<td>.00</td>
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</tbody>
</table>

Note: AIC is Akaike Information Criterion, Δ is the AIC difference, and ω is the Akaike weight. Values for group level parameters are shown, along with standard errors (subscript values in parentheses). All parameter values required the inclusion of species-specific terms (see app. B, available online), except those in square brackets, which are fixed across species. The overall best-fit model is indicated in the farthest-right column (best model) and is defined as that with the highest Akaike weight (see table 1 for definitions of parameters). For growth rates, the allometric model was the best fit, with the complex allometric model also providing a good fit. For larval development rates and egg development rates, the complex allometric model was the best fit.

the database and therefore required minimal extrapolation. Subtracting this centering temperature from each of the model types allowed each rate to be examined in terms of changes from the rate at 15°C.

We searched the literature to identify appropriate equation forms to apply to development and growth rates (table 1). Linear models could not be applied, because residuals were heteroscedastic on an arithmetic scale (app. C). Sharpe-Schoolfield equation forms required unavailable species-specific data (viability) and thus were not included. Having identified appropriate statistical models (table 1), the choice of random effects was considered. Because on occasion different studies were conducted that involved the same species, study was nested within species. Similarly, sex (male or female) was nested within study, and stage was nested within sex (see app. B for additional details). Initially, we allowed both slopes and intercepts to vary for each of the random effects (species, study, sex, and stage), but models required only random intercept terms for study, sex, and stage, because intercepts and slopes were highly correlated (app. B). Unlike study, sex, and stage, the correlation of slope and intercept terms for the covariate “species” was low; therefore, each statistical model had to be divided into different mixed-level model types, which accounted for differences between species by allowing random intercepts only, slopes only, and both intercepts and slopes. This resulted in 11 different models being fitted to each data set (see app. B). Because the complex allometric model is a modification of the allometric model, there were only two mixed models for this set, with a fixed and random quadratic term, β₂. Having applied all 11 models in table B1 to each data set, the best mixed model for each statistical model (i.e., allometric, complex allometric, exponential, and Arrhenius) was chosen by conducting a likelihood ratio test (app. B). Using the best mixed-effects model for each statistical model type, comparisons were then made across different types of model using information theory, specifically the Akaike information criteria (AIC; Akaike 1974; Burnham and Anderson 2002).

Using the best-fit models for growth and development rates as a function of temperature, it is possible to predict the effect of temperature on organism size. We assume exponential increase in mass with time, because this describes growth well for marine copepods (Huntley and Lopez 1992; Hirst and Bunker 2003):

$$g = \frac{(\ln M_i - \ln M_0)}{t},$$

where g is growth rate (day⁻¹), M is the mass at stage i, M₀ is mass in the previous stage, and t is development time (days). From the best-fit models of stage-specific development and growth rates, the relative increase in mass
The best-fit mixed-effects model for growth rates was an allometric model (see table 2), which was subsequently fitted to the data, both as a general best-fit model and as group-specific models (i.e., including specific parameter values for species, stage, sex, and study; fig. 1A). Larval development rates were best modeled using a complex allometric model with species-specific random effects for intercepts and slopes; the complex allometric model was a much better fit than all other model types (table 2). Figure 1B shows this best-fit model, along with the group-specific models, applied to the data. Similarly, egg development rates were best modeled using a complex allometric model but with the additional species-specific random effects term for parameter $\beta_2$ (table 2; fig. 1C). Examining these thermal responses throughout ontogeny (i.e., for naupliar and copepodite growth separately), we found that the allometric model was best supported by growth rate data for both naupliar and copepodite stages. Although the slope for the naupliar growth rates was steeper than that for copepodite stages, the difference between these slopes was not statistically significant (two-sample $t$-test: $t = 1.94, P = .057$; fig. 2A). Development rates for egg, naupliar, and copepodite stages were best described by a complex allometric response. The estimated parameters for the slopes and curvature were not significantly different for eggs, nauplii, or copepodites (figure 2B).

Although the allometric model was the best-fit model for growth, the complex allometric model also provided a good fit to the data (table 2). The evidence ratio $(\Omega_{\text{allometric}} : \Omega_{\text{complex allometric}})$ for these two models was 2.0; in this case, a value <2.7 suggests relatively weak support for one model over another (Burnham and Anderson 2002). We have therefore also compared the best complex allometric model for growth rate with that for development rate in figure 3. This indicates a difference in the direction of the curvature for growth rate against temperature, compared with development against temperature, with growth producing a concave curve and development producing a convex curve (fig. 3A). These shapes, driven by parameter $\beta_2$, were subsequently compared and found to be significantly different (two-sample $t$-test: $t = 4.12, P < .0001$; (between $M_i$ and $M_f$) can be calculated from rearranging equation (1), as:

$$M_f = \frac{M_i}{\exp^{\beta_D}}$$

where $D$ is development rate (day$^{-1}$; i.e., the reciprocal of time to develop $[t]$ from $M_0$ to $M_i$). We compare this size change with temperature as a percentage change from the maximum size ratio.

**Results**

Figure 1: Compiled data, together with overall best-fit line and group-specific best-fit lines (i.e., incorporating the random effect parameter values for species, stage, sex, and study for each data set) for (A) growth rates versus temperature, including naupliar (both across-stage [e.g., NI-NII and NII-NIII] and across-naupliar rates [e.g., NI-NVI]), copepodite (across-stage and across-copepodite rates [e.g., CI-CVI]), and total (across NI-CVI) growth rates; (B) development rates versus temperature, including naupliar (stage-specific and across-naupliar rates), copepodite (stage-specific and across-copepodite rates), and total (average from NI-CVI) rates; and (C) egg development rates versus temperature.
peak size toward the lower end of the temperature scale. We found that this ratio did not decrease monotonically but, rather, followed a concave response, with a peak size toward the lower end of the temperature scale. Unlike development, the complex allometric model for growth required species-specific values of parameter \( \beta_2 \), which suggests that this curvature is more variable than is the curvature for development. This can be seen from the wider confidence intervals in figure 3B. It is important to note that, despite this extra variability, there is still a significant difference between the parameter \( \beta_2 \) for growth rate and that for development rate.

Using the best-fit models for stage-specific rates of growth (allometric) and development (complex allometric) and using exp\(^{0.07} \) (see eq. [2]), we predicted the ratio of \( M_i \) to \( M_0 \) (i.e., relative increase in mass over a stage; fig. 4). As such, this indicates the TSR for a particular stage. We found that this ratio did not decrease monotonically but, rather, followed a concave response, with a peak size toward the lower end of the temperature scale (at \( \sim 11^\circ C \) in this case) and a steep decrease below this temperature, with a shallower, approximately linear decrease above these temperatures.

### Discussion

Our analysis shows that, for a wide range of marine copepod species, intraspecific growth and development rates have different temperature dependence, and therefore these rates must be at least partially decoupled. Although decoupling of these has been suggested previously (Atkinson 1994; Sibly and Atkinson 1994; van der Have and de Jong 1996; Forster et al. 2011), our study is the first to test for these differences in a systematic way across multiple species. Furthermore, these differences are maintained throughout ontogeny (fig. 2). On a log-log scale, development rate shows a convex response to temperature, whereas growth shows a linear or concave response: at lower temperatures, the rate of decrease in development rate is reduced, but the rate of decrease in growth rate continues to decrease linearly or becomes steeper. Conversely, at higher temperatures, the convex shape of development results in the rate of increase in development rates being more rapid than that for growth rates. The latter continues to increase linearly or flatten on a log-log scale. This suggests that these two rates are controlled by mechanisms with different temperature dependence.

Why should development rate be more temperature-sensitive than growth rate at warmer temperatures? Van der Have and de Jong (1996) suggested a mechanism based on cellular processes: growth depends primarily on the rate of protein synthesis, whereas development depends on DNA replication, and these two processes differ with respect to the size of molecules involved. Protein synthesis is limited by the diffusion of massive ribosomal subunits into the cytoplasm, whereas the much smaller DNA polymerase enzymes are limited by the time taken to find the DNA template (i.e., an enzymatic process). Diffusion is less temperature sensitive than are enzymatic processes; therefore, because diffusion is the limiting rate for growth (protein synthesis) but enzymatic process is the limiting rate for development (DNA replication), the former is less sensitive to temperature than is the latter (van der Have and de Jong 1996). Our results show growth to be best modeled by an allometric model (i.e., a power function). Both the allometric and complex allometric model produce similar AICs and receive stronger support from the data than do exponential-based models (see table 2). The mechanistic basis for using power functions is that many biological processes are controlled by physical processes, such as diffusion and viscosity, which follow an allometric response to temperature, rather than chemical processes, which follow Arrhenius-type functions (Ahlgren 1987).
Our results may be indicative of growth being limited by a process such as diffusion, which would lend support to the hypothesis of van der Have and de Jong (1996). The Akaike weights show that the complex allometric model with a species-specific curvature term (\(\beta_i\)) also provides a good fit to growth data, which suggests that the thermal response of growth may vary considerably from species to species; thus, the relationship between temperature and growth may be more variable than the relationship between temperature and development.

The complex allometric model has a convex form when applied to development rates, unlike when applied to growth rates. The curvature term (\(\beta_i\)) does not require a species-specific term and is relatively invariable (as shown by the narrow confidence intervals in fig. 3B). We find that development rate is more temperature sensitive at higher temperatures than is growth rate but that this is not best modeled by an exponential function, as would be expected for a thermal response limited by enzymatic rates, as suggested by van der Have and de Jong (1996) and proponents of the MTE (Gillooly et al. 2001, 2002). Unlike for growth, all other model types, including allometric, exponential, and Arrhenius functions, perform poorly at modeling development rate data, compared with the complex allometric model (table 2). Many researchers have modeled the effect of temperature on growth within species using an exponential function (Escribano and McLaren 1992; Escribano et al. 1997; Campbell et al. 2001) and on development using a power function (Belehradek 1926; McLaren 1969; Corkett and McLaren 1970; Hart 1990; Peterson 2001). Our work, however, suggests that an allometric model is a better fit for growth rates and that development is more curved and complex than a power function. Because a complex allometric model also describes growth well (although with significantly different parameter estimates than development; table 2), it may be most suitable to apply this same model type to both rates. In the case of development, this model can be applied with a fixed estimate for parameter \(\beta_i\) of 0.32 (fig. 3B). Unlike development, growth would require a species-specific variable for \(\beta_i\), with an initial variable estimate of \(-0.14\) (fig. 3B). If growth does not
require the extra concave curvature provided by $\beta_2$ and can be modeled instead by an allometric model, this will be obvious from the statistical output ($\beta_2$ not significantly different from 0). These allometric model types are simpler than other equation forms, such as the Sharpe-Schoolfield equation, that would require data for the viability of the different copepod species across their thermal range, which was neither available nor practical to collect. When making broad predictions regarding life-history rates across a range of species, it is important to attain a balance between the complexity and simplicity of a model. Using the equation forms suggested here, along with a linear mixed-effects model, captures the changes in these rates across different copepod species while requiring few parameters and thus strikes this balance.

To date, few studies have compared the temperature dependence of growth and development rates within a species (Smith-Gill and Berven 1979; van der Have and de Jong 1996; Davidowitz and Nijhout 2004; Walters and Hassall 2006; de Jong 2010). These studies examine these rates at a coarse resolution (e.g., using total time from the initial larval stage to adult emergence; van der Have and de Jong 1996; de Jong 2010), but there is a general lack of knowledge about how these rates might change through ontogeny. We have tested this across a range of marine copepod species and have shown that the equation forms that best describe the thermal response of growth and development are maintained throughout ontogeny. However, the thermal response of development rates is more consistent through the life cycle than is the thermal response of growth rates. The convexity and slopes of development are similar throughout ontogeny (fig. 2B), suggesting an equal thermal sensitivity at different life stages. This is further supported by evidence of the “equiproportional rule” in copepods: at different temperatures, specific life stages always occupy the same fixed proportion of the total larval time (Hart 1990). In contrast, the thermal response of naupliar growth is somewhat steeper than that of copepodite growth (fig. 2A). This suggests that there may be an ontogenetic effect on the thermal sensitivity of growth. As an organism gets larger, the thermal sensitivity of its accrual of mass per time (growth rate) becomes reduced, but the rate at which it passes through life stages maintains the same thermal response. This is a tentative conclusion, however, because the differences between the slopes for naupliar and copepodite growth are not significant; more naupliar growth rate data are needed to confirm this.

The fact that growth and development rates do not have the same thermal response has important implications. For instance, a central proposition in the MTE is that physiological rates (e.g., respiration rate, growth rate, and rate of molecular evolution) follow an Arrhenius function and have the same temperature dependence (Brown et al. 2004). Although metabolic rates (and thus respiration rates) may scale with temperature following an Arrhenius-type response (Gillooly et al. 2001), proponents of the MTE have extrapolated beyond this by assuming that many other physiological and population rates have this same temperature dependence (Brown et al. 2004; Anderson et al. 2006). The Arrhenius function is now widely used to correct many different life-history rates to temperature (Clarke and Johnston 1999; Gillooly et al. 2001; Brown et al. 2004), yet our data suggest that this should be conducted with caution.

A previous meta-analysis of planktonic larval development times also found that an allometric model was better supported than the Arrhenius (O’Connor et al. 2007). They suggested that the lack of intraspecific mass correction could provide an explanation as to why the Arrhenius was not the best-fit model. The Arrhenius equation assumes that rates are mass normalized (Gillooly et al. 2002), and although this was not possible in our analysis either, the use of a random intercept term for species allows for differences in absolute rates between different species caused by parameters such as mass. Therefore, this mass correction across species should not be necessary. We did not have the available data to mass correct for the temperature-size responses within species. However, we can make some predictions of the impact of our TSR thermal reaction norm on growth and development rates. According to the MTE, rates scale with the product of temperature (an Arrhenius function) and mass $^{1/4}$ (i.e., within a species, smaller individuals have relatively higher rates). Our data incorporate changes in rate associated with changes in mass within the temperature dependence; thus, we would expect temperature dependence to be exaggerated in the relationships that we find. Effectively, empirical data for both growth and development rates should show more curvature than the Arrhenius model would predict, because mass correction was not possible. In fact, we find that the empirical data for growth and development are best modeled by allometric models, which are inherently less curved than are exponential functions (such as the Arrhenius). Furthermore, we find the development rate model for eggs to be best described by a complex allometric model that is not significantly different from that of copepodite stages, despite eggs showing less change in mass with temperature, compared with later stages (Forster et al. 2011). Intraspecific temperature-dependent size changes are therefore unlikely to explain the poorer fit of the Arrhenius function. The MTE in its current form does not allow for differences in the form of the temperature dependence of growth and development rates that we observe, and consequently it does not predict the widely observed TSR outcome, which is the result of such decoupling (Forster et al. 2011).

There is currently no single agreed shape for the re-
sponse of ectothermic body mass to temperature. A linear decrease in volume with increasing temperature has been suggested in protists (Atkinson et al. 2003), whereas more complex thermal reaction norms have been suggested for some metazoans (David et al. 1994; Karan et al. 1998; Davidowitz and Nijhout 2004; de Jong 2010). Many studies of metazoans use negative linear models to express change in body mass with temperature, often with mass having only been recorded over a small thermal range (2–3 temperatures; e.g., Partridge et al. 1994; Fischer et al. 2004; Stillwell and Fox 2005). Using the thermal response of stage-specific development and growth rates, our study predicts that the relative mass increase (the ratio \( M_i : M_0 \)) follows a concave response to temperature (fig. 4). Among the marine copepods, we found a highly positively skewed thermal reaction norm, with the greatest relative body size increase at a low temperature and a long tail of reducing size ratios with increasing temperatures. It might be that this tail, with a relatively linear decrease, is responsible for the TSR being typically described as a simple linear function of body size (Partridge et al. 1994; Blankenhorn and Llaurens 2005). Interestingly, our predicted thermal reaction norm of body mass ratios shown in marine copepods is similar in form to that predicted using data for the tobacco hornworm moth *Manduca sexta* (Davidowitz and Nijhout 2004). Davidowitz and Nijhout (2004) demonstrated a peak in size at a low temperature, with a steep drop from this peak as temperature decreases, but a less steep decline in size with increasing temperature. This shape is also commonly found in *Drosophila* species (data for both wing length and mass, Ray 1960; David et al. 1997; Karan et al. 1998; Petavy et al. 2001), aphids (Lamb et al. 1987), aquatic insects (Vannote and Sweeney 1980), leeches (Young and Ironmonger 1982), and frogs (Smith-Gill and Berven 1979). It may be that this mass thermal reaction norm is in fact common in ectotherms but not widely reported because of a lack of data for the colder temperatures at which size decreases. The prolonged development time associated with cold temperatures often makes the rearing of organisms more challenging and probably explains the scarcity of data. To compare our predicted response with empirical data for marine copepods, we searched the literature for adult mass data in which mass had been measured at more than three temperatures. We found that, for the six species for which data were available, all followed the temperature-size rule, decreasing in size over the majority of their thermal range. Furthermore, a concave thermal reaction norm was present in three species: *Acartia tonsa* (Hansen et al. 2011), *Pseudodiaptomus dubia* (Li et al. 2009), and *Sinocalanus tenellus* (Kimoto et al. 1986). In those species not displaying the predicted concave shape—*Calanus sinicus* (Uye 1988), *Paracalanus* species (Uye 1991), and *Pseudocalanus newmani* (Lee et al. 2003)—data were not available for the very low end of their thermal range, which may explain why the decrease in size at the lowest temperatures was not observed. The response of adult size to temperature clearly now requires more extensive examination across more ectothermic taxa and thermal ranges.

Body size is a primary determinant of many ecological properties, including fecundity, mortality, and growth rates (Fenchel 1974; Blueweiss et al. 1978; Hirst and Kiørboe 2002; Brown et al. 2004; Kiørboe and Hirst 2008), species interactions (Arendt 2007), and food web structure and dynamics (Warren and Lawton 1987; Yodzis and Innes 1992; Woodward et al. 2010a, 2010b). Changes in the size of organisms with temperature will therefore impact on many different ecosystem processes. The world is warming at a rate that is unprecedented in human history (IPCC 2007), and our study highlights the potentially important impact of temperature change on copepod size. Although they represent only a single taxon, copepods are the most abundant zooplankton in the marine biota, forming the principal trophic link to marine fishes (Huntley and Lopez 1992; Turner 2004; Richardson 2008). Our results point toward a marked decrease in copepod size with increasing temperature across the majority of the thermal range, which may alter their role as both predators and prey, given the size dependency of such processes (Hansen et al. 1994). However, our analysis also suggests that some copepod species living toward their lower thermal limit may actually increase in size with an increase in temperature. Furthermore, as the magnitude of the size change is larger in older life stages than in younger life stages (see Forster et al. 2011), predicting ecosystem consequences of temperature change is challenging. To give an example, copepod adults (temperature-sensitive older life stage) are important prey for early larval fish, yet the size of these early stages is likely to be less temperature sensitive than the size of the adult copepods (Forster et al. 2011); thus, the size changes in the predators and prey may be dissimilar. This could impact on the fishes’ prey choice, altering the structure of food webs and impacting on populations across the whole food chain. Although the TSR is a phenotypic plastic response, short-term seasonal fluctuations in temperature can affect ecosystem dynamics (Sims et al. 2004) and could therefore drive longer-term changes in community structure.

Using a systematic approach, we have shown that the fundamental physiological rates, growth and development, have different temperature dependence when examined across a range of species within marine pelagic Copepoda, with clear implications for wider ecological theory and for assessing the potential impacts of global warming on species size.
Acknowledgments

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The temperature-size rule emerges from ontogenetic differences between growth and development rates

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Summary

1. The temperature-size rule (TSR) is a widespread phenomenon, which describes the phenotypic plastic response of species’ size to temperature: individuals reared at colder temperatures mature as larger adults than at warmer temperatures.

2. The TSR is driven by an unequal thermal response of growth and development rates. However, we currently lack an understanding of how these rates change through ontogeny and their decoupling. Further, we do not know how this decoupling varies across generations.

3. Using the brine shrimp *Artemia franciscana* as a model, we examine growth and development rates through ontogeny at different temperatures across two generations.

4. The slopes of natural-logged weight-specific growth rates against temperature are steeper in earlier than later larval stages, indicating their greater temperature dependence, whereas development rates maintain the same temperature dependence across life stages. An inverse TSR is generated in early larval stages; the typical TSR (smaller size at warmer temperatures) is only established later in ontogeny.

5. Phase-specific temperature dependence of growth and development rates is not significantly different across the 1st and 2nd generation, suggesting the TSR is primarily a within-generation outcome.

6. Ontogenetic size responses in *Artemia* are compared to other crustacean species to identify patterns within this subphylum. Data for a range of crustaceans follow the same ontogenetic pattern: early larval stages show an inverse or no TSR, with TSR being only established in later stages. Adults often, but not always, show the greatest response.

Key-words: *Artemia franciscana*, crustacean, development, growth, ontogeny, temperature-size rule

Introduction

The temperature-size rule (TSR) describes the impact of temperature on intraspecific size of ectotherms: individuals reared at colder temperatures reach maturity at a larger size than when reared at warmer temperatures (Atkinson 1994). This phenotypically plastic response has been observed in >83% of ectothermic species studied, including plants, bacteria, protists, invertebrates and vertebrates (Atkinson 1994). Adult size is in effect a product of growth rate (increase in weight per time) and development rate (increase in life stage per time), and the TSR signals that these rates must be decoupled (Sibly & Atkinson 1994; Van Der Have & De Jong 1996; Kingsolver & Huey 2008). Much of the focus of the TSR has been on explaining the ultimate reason for size change (Walters & Hassall 2006; Kingsolver & Huey 2008; Arendt 2011). However, to understand why size changes, we first need a clearer understanding of how size changes are generated. In particular, there remains a lack of data available on growth and development rate decoupling through ontogeny. Decoupling of these rates is most often inferred from differences between size at some final life stage and the time taken to reach this mature stage (Partridge et al. 1994; Blanckenhorn 2000; Stillwell & Fox 2005), without considering the ontogenetic timing of these size changes at a higher resolution. Previous work on ectotherms has found temperature-acclimated adults show a greater temperature-size response than do acclimated progeny, such as eggs (Forster, Hirst & Atkinson 2011a). A recent analysis of marine pelagic copepod data has shown development rates to have greater temperature dependence across all life history stages than growth rates (Forster, Hirst & Woodward 2011b). Further, there was weak support for growth rate being more temperature dependent at smaller, early life stages than later stages (i.e. slopes of natural-logged growth rates against temperature were steeper at early life stages), suggesting a size-dependent or ontogenetic component in these crustaceans. Similarly, analyses of the interaction between growth and development during ontogeny for the tobacco hornworm moth *Manduca sexta* have shown the TSR to emerge only during later larval stages.
We address the following questions: (i) Do the temperature dependence of growth and development rates in *A. franciscana* vary through ontogeny, and how does this impact the temperature-size response? (ii) Does the impact of temperature on these rates differ between the first and second generation? and (iii) Is the ontogenetic basis of the temperature-size response similar across crustaceans?

### Materials and methods

Batch cultures of *A. franciscana* were established using decapsulated cysts. These cysts had been collected from the Great Salt Lake (Utah, USA), disinfected and decapsulated (provided in this state by the company Waterlife). A minimum of ~300 cysts were placed in 1-L beakers containing 900 mL GF/F filtered sea water with a salinity of 30 at a range of constant temperatures (20, 22.5, 25, 27.5, 30, 32.5 and 35 °C). Air stones were used in each beaker to ensure the water was sufficiently aerated. Cultures were maintained at fixed temperatures using Grant SUB Aqua 26 water baths (held within ± 0.2 °C of control temperature). Upon hatching, stage 1 nauplii were transferred from the batch cultures into a minimum of two separate replicates at each temperature (with *n* = 50 per replicate), initiating the first generation of the experiment. As cultures did not survive for long at 35 °C, these were not continued. After hatching, nauplii were provided *ad libitum* with the algae *Arthrospira plantensis*. Replicates were fed a minimum of 10 mL of saturated *A. plantensis* solution (where no more algae could be suspended in solution) every day such that a green coloration was visible and was maintained in the cultures at all times. All individual *A. franciscana* were staged, and their total body length was measured on a daily basis using a light microscope. Post-embryonic larvae were staged in a similar manner to Weisz (1946), using number of segments and limb mobility (see Appendix S1 in Supporting Information), with development being divided into 17 stages. Beyond stage 17, measurements of development ceased, as without the addition of further segments or appendages, accurate assessment of stage could not be achieved. Once animals reached the adult stage in the cultures, we inspected for the presence of their nauplii on a daily basis. A total of 50 nauplii were removed from each temperature replicate with a Pasteur pipette and placed into new beakers; this was the initiation of the second generation. In a small number of cases (3/12 cultures), there were insufficient nauplii from the experimental culture; in these cases, we supplemented with nauplii from other batch cultured adults, maintained at the same temperature as the replicate in question and at saturated food conditions. Development and growth experiments were started for the second-generation replicates, the time at first appearance of nauplii defining *t* = 0. Body lengths and stage were determined daily on live individuals, as conducted for the first generation, and the same feeding regime was used also. Across both generations, water in all replicates was changed weekly; between these changes, any water loss via evaporation was replaced using distilled water to maintain salinity.

#### Calculating growth and development rate

Individual dry weights were estimated from length measurements using equations calculated from Reeve (1963, see Appendix S1), and average weight calculated for each replicate at each observation point. Weight-specific growth rates (*g*, day⁻¹) were determined as:

\[
g = \frac{\ln(DW_t/DW_0)}{t}
\]

where *DW*<sub>*t*</sub> = dry weight at time *t*, *DW*<sub>0</sub> = dry weight at previous observation point and *t* = time between observations (days).

Although measurements were taken daily, as in some instances > 3 stages can pass in a single day, growth rates were calculated during ontogeny by combining data for 3 stages together: specifically stages 3–5, 6–8, 9–11, 12–14 and 15–17. We term each of these ‘phases’. As the two initial stages have very rapid development (*<* 24 h for transition through both stages at most temperatures), we did not include these.
Development times were calculated as median stage-specific development times, i.e. from initial nauplii introduction to the point at which 50% of individuals reached stage 2, then from this point to the point at which 50% of individuals reached stage 3, etc. These median development times were calculated (for each replicate) from stage frequency data, following the methods of Campbell et al. (2001). Median development times were calculated for each stage from 1 to 17 inclusively. These development rates were then grouped into the phases, to allow direct comparison with growth rates.

**MODEL FITTING**

We used allometric models to describe the effect of temperature on both growth and development rates. These models have previously been shown to be the most appropriate for other crustaceans, i.e. marine copepods (Forster, Hirst & Woodward 2011b) and planktonic larval species (O’connor et al. 2007). Further, applying exponential and Arrhenius functions to the data and comparing their fit using an information-theoretic approach (Akaike Information Criteria, Akaike 1974; Burnham & Anderson 2002) did not produce a better fit (see Appendix S2 in Supporting Information). Growth rates (g, day$^{-1}$) and development rates (D, day$^{-1}$) were modelled as functions of temperature (T, °C) using the allometric function:

$$\ln R = \ln a + b \ln T + \epsilon$$

where $R$ = rate, $a$ and $b$ are constants and $\epsilon$ is the error term. We centred the data for both growth and development rates around the mid-temperature of the experiment (25 °C). This improves the interpretability of each model (e.g. the intercept becomes the rate at 25 °C) and reduces the correlation between parameters (O’connor et al. 2007), thus ln$T$ became ln$(T - \ln(25))$, we term this centred temperature as $T_c$. Growth rates and development rates were available for different phases (e.g. stages 3–5, 6–8) and for 1st and 2nd generations. A linear mixed effect model was used to account for these differences in phase and generation number, incorporating phase as a random effect and generation number as a fixed effect (see Appendix S2). We applied multiple variations of the models in eqn 2 for growth and development, which allowed parameters $a$ and $b$ to vary with phase and/or generation number. Using a log-likelihood ratio test, we then discerned which variation of these equations best modelled data for each rate (see Appendix S2 for further details). These procedures were followed to determine whether ontogeny impacts the intercept of growth and development (ln$a$, eqn 2) and/or the slopes ($b$, eqn 2). Similarly, these procedures were used to determine whether the two generations show differences, both in their intercepts and in their slopes.

**COMPARISON OF WEIGHT WITHIN STAGE**

To estimate the temperature effect on weight at stage and to discern the importance of generation number on organism size, we described the effect of temperature on dry weights for each stage using a linear mixed effects model and an exponential equation form. A previous analysis of multicellular species data for weight vs. temperature has shown this equation form to best describe temperature-size data (Forster, Hirst & Atkinson 2011a). Further, we confirmed here, using Akaike weights, that other model forms (allometric and Arrhenius) did not provide a better fit to the data. We followed the same method used for modelling growth and development rates, except using individual stages (3, 4, 5 etc.), rather than phases. The equation applied was of the form:

$$\ln DW = \ln a + b T_c + \epsilon$$

where $DW$ = dry weight ($\mu$g) and $T_c$ = the experimental temperature (°C) minus 25. Having calculated the stage-specific slopes for all larval stages of *A. franciscana*, we converted these slopes to % change in weight per °C using the formula (exp(slope) – 1)*100. We collected the eggs produced as the second generation at each temperature (> 50 eggs for each temperature) and measured their diameter (E). Eggs were near-spherical and volume calculated as $4/3\pi(E/2)^3$. The slope of egg volume against temperature was calculated using generalized least squares regression and eqn 3.

**COMPARISON OF ONTOGENETIC TEMPERATURE-SIZE RESPONSE FOR CRUSTACEANS**

To compare the temperature-size response of *A. franciscana* with other species, we searched the wider literature for data on weight at stage vs. temperature in other crustaceans, measured through ontogeny. We searched the ISI Web of Knowledge using the search terms ‘larva* AND temperature AND (mass OR size OR weight)’ along with previous data collected for copepod species (Forster, Hirst & Woodward 2011b) and other multicellular organisms (Forster, Hirst & Atkinson 2011a). We included data where size had been measured for ≥3 temperatures at ≥2 larval stages. We applied a linear mixed effects model to each species in turn, using eqn 3 and following the same methods as applied to the *A. franciscana* data.

**Results**

*Artemia* were successfully reared over two generations at fixed temperatures ranging between 22.5 and 32.5 °C. Although those reared at 20 °C reached adulthood and reproduced at the end of the first generation, the 2nd generation did not reach maturity. Growth rate changed during ontogeny, with two distinct trajectories: the first being early larval growth during the formation of thoracic segments (stages 1–11) and the second trajectory during the formation of abdominal segments (stages 12–17); growth was distinctly faster during this second period (as demonstrated by the steeper slope of ln weight vs. time, Fig. 1a). Our phases were defined so as not to combine stages across this division. Such marked shifts through ontogeny were not present in development rates (Fig. 1b).

**MODEL FITTING**

The inclusion of generation number did not improve the fit for either growth or development, and therefore this identifier was removed from the linear mixed effects models. The lack of improved fit suggests no significant difference between 1st and 2nd generation for growth or development rates. For growth rates, the best fit model required the inclusion of phase as a random parameter within both the slope and intercept term. This suggests that growth rates vary between phases (intercept), i.e. some have faster weight-specific
growth than others, but also that different phases have a different temperature dependence (i.e. slopes, see Fig. 2). By contrast, the fit of the development model was not improved with the incorporation of phase as a random effect in the slope term, the best fit model required phase to be incorporated within the intercept term only (Fig. 3). This suggests that some phases develop more quickly than others, but they have the same temperature dependence regardless of phase (i.e. slopes are similar).

**COMPARISON OF WEIGHT WITHIN STAGE**

We compared the temperature-size response across different stages of *Artemia* using the linear mixed effects model applied to eqn 3. This revealed a temperature dependence of stage-specific weight, but no significant improvement of the model with the addition of generation number. This was confirmed in the results of the models applied to growth and development: generation number did not appreciably change the thermal response of these rates. To demonstrate this, we present the stage-specific best fit models in Fig. 4, but including the generation number term in the model, to show the slopes of these models are near-identical for the 1st and 2nd generation. The lowest temperature (20 °C) was excluded from this analysis as the reared individuals failed to reach maturity in the second generation. Furthermore, size decreased at this temperature in later stages (Fig. 5); thus, it was excluded to maintain the simplicity of the exponential model (eqn 3). The stage-specific temperature-dependent slopes revealed an inverse temperature-size response at early larval stages (Stages 1–7, Fig. 4). The temperature-size response became flat during intermediate larval stages (Stages 8–10), before establishing the more typical TSR at stages 11 onwards (Fig. 4), with a significant decrease in weight with increasing temperature for stage 12 onwards. We compared final larval size data to egg size in Fig. 5. Final larval size (excluding 20 °C) showed a significant negative slope of dry weight vs. temperature (−2.96% °C⁻¹, 95% CIs ± 0.62%), whereas the slope of egg volume vs. temperature was not significantly different from zero (0.08% °C⁻¹, 95% CIs ± 0.40%).

**COMPARISON OF ONTOGENETIC TEMPERATURE-SIZE RESPONSE FOR CRUSTACEANS**

Along with data for *A. franciscana*, we collected temperature-size data for 10 other crustacean species including 7 copepod, 2 crab and 1 daphnid species. The % change in weight °C⁻¹ is plotted for each species through ontogeny in Fig. 6. This revealed a general pattern of declining slopes with increasing stage. These patterns were very similar in all 11 crustacean species. Early larval stages show an inverse or no TSR, whereas later stages show a strong TSR, with weight changes varying between −1 and −4.5% per °C (Fig. 6). Data for 7 of the 8 species where very early larval or egg sizes were available (Stages 0–2, Fig. 6) show no significant change in progeny size with temperature. In many cases, the adult stage showed the strongest temperature-size response, but this was not always the case, and some showed a reduction in the response into the final stage(s).

**Discussion**

There were clear differences between the temperature dependence of growth and development rates in *A. franciscana*. While the temperature dependence of growth rates decrease with size/life stage (see slopes in Fig. 2), development rates had similar temperature dependence throughout ontogeny (see slopes in Fig. 3). Other development rate data from the crustacean literature support this outcome more widely. Copepods have been shown to maintain the temperature dependence of development during ontogeny; this is in effect the widely observed equiproportional development rule, where specific life stages occupy a fixed proportion of the total life cycle across different temperature regimes (Hart 1990). As earlier larval stages of *A. franciscana* show a greater temperature dependence of growth rate than later larval stages, this...
suggests these rates are stage/size dependent (see Fig. 2). Evidence for this in other crustaceans is scarce. Data for the crab *Carcinus maenas* follow a similar pattern, with growth rates of early zoeal stages being more temperature dependent than later larval stages (Dawirs, Puschel & Schorn 1986). There is also weak support from data for growth rates in marine copepod species (Forster, Hirst & Woodward 2011b); specifically, the slopes of early nauplius logged growth rates vs. temperature were found to be steeper than those of later copepodite stages, although this result was not significant (2 sample t-test, \(t = 1.94, P = 0.057\); Forster, Hirst & Woodward 2011b). A decrease in the temperature dependence of growth through ontogeny (with increasing stage/size) in *A. franciscana* has important implications for the TSR. Growth rates are more temperature dependent than development rates in the early larval stages (Figs 2 and 3), resulting in a reverse TSR: body size in early larval stages increases with increasing temperature (Fig. 4). Only during later stages, when the temperature dependence of growth is less than that of development, is the TSR established. Therefore, the appearance of the TSR is not determined solely by the temperature dependence of growth rates changing through ontogeny, but on growth having a lower temperature dependence than development rate in later larval stages. This highlights the importance of following changes in both growth and development rates throughout ontogeny.

![Fig. 2. *Artemia franciscana* weight-specific growth rates vs. temperature (°C) across 5 ontogenetic phases (stages 3–5, 6–8, 9–11, 12–14, 15–17). Triangles are mean values for the 1st generation, and squares are means for the 2nd generation. Panel 6 shows the best fit models (i.e. allometric, eqn 2) for each of the phases. All regressions are fitted through data from both generations combined. Regression equations are provided in each panel, where \(g\) = growth rate (day\(^{-1}\)) and \(T_C\) is temperature \(T\) (°C) centred around 25°C (i.e. \(T_C = \ln(T – \ln(25))\). Note the log10–log10 scale, error bars represent 95% confidence intervals.](image)
increasing size in *Artemia*? Previous mechanistic models to explain changes in growth rate with size and temperature have been based on the Von Bertalanffy (1957) growth equation:

\[
\frac{dW}{dt} = kW^n - lW^m
\]

where \( W \) = body weight, \( k \) is the coefficient of anabolism, \( l \) is the coefficient of catabolism and \( m \) and \( n \) are exponent parameters. Increasing temperature can alter maximal body size by changing either the coefficients or the exponents. Previous work by Perrin (1995) and Strong & Daborn (1980) has produced two mutually exclusive mechanisms based on the Von Bertalanffy (1957) growth rate model to explain changes in growth rates associated with temperature. Perrin (1995) showed optimal life history to follow the TSR when the temperature dependence of the catabolism coefficient \( l \) is greater than that of anabolism \( k \), assuming exponents \( m \) and \( n \) are constants (0·75 and 1, respectively). Conversely, Strong & Daborn (1980) used data for the isopod *Idotea baltica* to argue that smaller size is driven by a decrease in \( m \) (from approximately 1·0 to 0·7) and increase in \( n \) (from approximately 0·7 to 1·0) with increasing temperature, resulting in different allometries of anabolism and catabolism. Our results suggest that neither of these proximate mechanisms are sufficient to explain the change in growth rates in *A. franciscana*.
rates for any particular phase of *A. franciscana* growth decreases with increasing size remains elusive. A mechanistic explanation for why the temperature dependence of growth and development rates is inadequate at describing changes in growth rates across different phases and at different temperatures (Kozowski, Czarnoleski & Danko 2004). The lack of mechanistic explanation provided by the Von Bertalanffy (1957) growth equation are therefore inadequate at describing changes in growth rates in *A. franciscana*. Both coefficient terms and exponents would have to change to accommodate differences in growth rates across different phases and at different temperatures (Kozowski, Czarnoleski & Danko 2004). The lack of mechanistic explanation provided by the Von Bertalanffy (1957) highlights the problems associated with this model type; indeed, a mechanistic explanation for why the temperature dependence of growth decreases with increasing size remains elusive.

The temperature dependence of growth and development rates for any particular phase of *A. franciscana* did not change between the first and second generation, with similar ontogenetic patterns in the decoupling of growth and development rates in both (see Fig. 4). Further, generation number did not have a significant effect on body size through ontogeny (i.e. there were no size differences between first and second generation of organisms). We therefore suggest that acclimatory compensation of growth and development rates to novel thermal environments may be extremely rapid. Is this supported by data for other species in the wider literature? Although there is a lack of growth and development rates measurements over multiple generations, we can infer the acclimatory responses of these rates by examining available data for body size. Data for *Drosophila melanogaster* size vs. temperature showed the effect of generation (1st vs. 2nd generation) to have significant effects on organism size; however, these size changes were extremely small and explained only 0.23% of the variation in body weight found (compared to 82% of variation explained by temperature; Karan et al. 1998). Similarly, small but significant changes have been shown to occur in egg and adult size in *D. melanogaster*, driven by differences in the parental thermal environment (Crill, Huey & Gilchrist 1996). Fischer et al. (2003) showed

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**Fig. 4.** *Artemia franciscana* larval stage dry weights (µg, log<sub>10</sub> y-axis) vs. temperature (°C). Regression lines represent the best fit to the data using a linear mixed effect model and eqn 3. Early larval stages (1–8) show an inverse TSR, with TSR being established from stage 12 onwards (asterisks denote negative slopes, which are significantly different from zero). These slope values and confidence intervals are shown in the first panel of Figure 6. Best fit lines are given separately for the 1st and 2nd generation. There was no significant effect of generation on dry weight values, as including this within the mixed effects model did not improve the fit.

**Fig. 5.** (a) *Artemia franciscana* dry weight (µg, log<sub>10</sub> y-axis) vs. temperature (°C) for stage 17, the final larval stage. Triangles represent the 1st generation, and squares represent the 2nd generation. The best fit model (linear mixed effects model, eqn 3) was calculated and shows the TSR equivalent to −2.96% dry weight °C<sup>−1</sup>. (b) *A. franciscana* egg volume (mm<sup>3</sup>, log<sub>10</sub> y-axis) vs. temperature (°C). The best fit line (generalized least squares regression model, eqn 3) shows no TSR, with a change in volume equivalent to 0.08% °C<sup>−1</sup>.
that the butterfly *Bicyclus anynana* lays larger eggs at cooler temperatures, but that the effect of oviposition temperature does not significantly alter size at later larval stages when reared at a common temperature. Data for the yellow dung fly *Scatophaga stercoraria* showed maternal temperature did not have a significant effect on offspring growth rates (Blanckenhorn 2000). Similarly, data for the hawkmoth *M. sexta*, where eggs were hatched at different temperatures then reared at a common temperature, showed the hatch temperature to affect initial larval size, but that this disappeared by the fourth instar (Potter, Davidowitz & Woods 2011). These studies, and our own, suggest rapid acclimation of growth and development rates in ectothermic species.

We found size to decline in later stages at the lowest temperature (Fig. 5A). This concave thermal response of adult has previously been found in other ectotherm species, including *Drosophila* (data for both wing length and mass, David et al. 1997; Karan et al. 1998; Petavy et al. 2001; Ray 1960), aphids (Lamb et al. 1987), aquatic insects (Vannote and Sweeney 1980), leeches (Young and Ironmonger 1982), frogs (Smith-Grill & Berven 1979), copepods (Kimoto, Uye & Onbe 1986; Hansen et al. 2011) and a moth (Davidowitz & Nijhout 2004). This suggests there may be common temperature-size patterns in adult ectotherms, but that these are not simply linear or exponential terms. Indeed, applying empirical relationships between growth and development rates data have previously also resulted in the prediction of this concave shape (Davidowitz, D’amico & Nijhout 2004; Forster, Hirst & Woodward 2011b). We found *A. franciscana* did not attain adult stage at the lowest temperature in the 2nd generation, thus this lower temperature may be harmful over multiple generations. Low survivability, coupled with long generation times, make rearing ectotherms and obtaining data at lower thermal limits more difficult, which may explain why the majority of studies do not show a concave shape (Kingsolver & Huey 2008). Further, the low survivability associated with cold stress suggests that this aspect of the TSR may not be relevant in the field, as maintaining populations at these lower temperatures over multiple generations was not possible.

Examining the temperature-size response through ontogeny in *A. franciscana*, we found no relationship in eggs, an inverse TSR in early larval stages and a significant TSR established at stage 12 (Figs 4 and 6). Although the establishment of a significant temperature-size response

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**Fig. 6.** The temperature-size response through ontogeny in a variety of crustacean species; slopes are expressed as % change in weight per °C for a given stage. Species-specific life stages were assigned arbitrary values from egg (0) to first instar (1) onwards. Therefore, there is no relation, for example, between stage x of a crab species and stage x of a copepod species, but these are comparable within a group (copepod–copepod, crab–crab etc.). Arrows indicate the adult stage. *Daphnia pulex* data are embryonic stages (development within mother); therefore, adult stage is not indicated. Error bars represent 95% confidence intervals.
occurs at the same point as the shifts from slower to more rapid growth (and from thoracic segments being added to abdominal segments, Fig. 1), this appears largely circumstantial: the change from a negative temperature-size response is cumulative, with stage 1 showing the most negative temperature-size response, and this getting less negative with increasing stage, until a significant temperature-size response is established at stage 12. Other crustacean species follow similar patterns. Early larval stages show little or no temperature dependence of their size (and sometimes a reverse TSR), whereas later stages show the more typical TSR, with size declining with increasing temperature. This suggests that the temperature-size relationship is commonly ‘reset’ at the beginning of each generation (Forster, Hirst & Atkinson 2011a). Indeed, the data of Leandro, Tiselius & Queiroga (2006) for *Acartia tonsa* (see our Fig. 6) show that individuals acclimated for at least two generations to their thermal environment show this same pattern. This lends further support to the theory that crustaceans follow a common pattern of size responses to temperature, with initial size being relatively temperature independent even when organisms are maintained at temperatures for multiple generations.

Although we have restricted our analysis to crustaceans, data from other ectothermic groups have shown egg size to be less temperature dependent than adult size (e.g. see the synthesis of Forster, Hirst & Atkinson 2011a). Further support for the TSR emerging only in later larval stages comes from the insect *M. sexta* (Davidowitz, D’Amico & Nijhout 2004, Davidowitz & Nijhout 2004; Diamond & Kingsolver 2010). It should be noted, however, that some species have significant changes in egg and/or early larval size with temperature (Crill, Huey & Gilchrist 1996, Van Voorhies 1996; Ernsting & Isaaks 1997; Blanckenhorn 2000; Fischer, Brakefield & Zwaan 2003; Hassall et al. 2006; Steigenga & Fischer 2007), with size changes following the TSR. The fact that egg and early larval stages are temperature dependent in some ectotherms, but typically not in crustaceans, shows that different groups with different life history patterns respond to temperature in different ways. This suggests different proximate mechanisms bring about temperature-size changes in different taxa, which in turn gives weight to the idea that the TSR is an adaptive response (Atkinson 1994; Atkinson, Ciotti & Montagnes 2003), i.e. there is a fitness benefit to smaller size at warmer temperature and organisms achieve this through a variety of mechanisms.

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**References**


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Achieving temperature-size changes in a unicellular organism

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The temperature-size rule (TSR) is an intraspecific phenomenon describing the phenotypic plastic response of an organism size to the temperature: individuals reared at cooler temperatures mature to be larger adults than those reared at warmer temperatures. The TSR is ubiquitous, affecting >80% species including uni- and multicellular groups. How the TSR is established has received attention in multicellular organisms, but not in unicells. Further, conceptual models suggest the mechanism of size change to be different in these two groups. Here, we test these theories using the protist Cyclidium glaucoma. We measure cell sizes, along with population growth during temperature acclimation, to determine how and when the temperature-size changes are achieved. We show that mother and daughter sizes become temporarily decoupled from the ratio 2:1 during acclimation, but these return to their coupled state (where daughter cells are half the size of the mother cell) once acclimated. Thermal acclimation is rapid, being completed within approximately a single generation. Further, we examine the impact of increased temperatures on carrying capacity and total biomass, to investigate potential adaptive strategies of size change. We demonstrate no temperature effect on carrying capacity, but maximum supported biomass to decrease with increasing temperature.

Introduction

The temperature-size rule (TSR) is a ubiquitous intraspecific phenomenon affecting most (>80%) ectotherms: individuals reared at cooler temperatures mature at a larger size than those reared at warmer temperatures (Atkinson, 1994). The rule is common both in multicellular (Atkinson 1994; Forster et al., 2011a, b) and in unicellular organisms, having been found in bacteria and many protists (Montagnes and Franklin, 2001; Atkinson et al., 2003). Recently, reduced body sizes at both the species and community level have been identified as a universal ecological response to global warming (Dauresne et al., 2009; Gardner et al., 2011; Sheridan and Bickford, 2011). Therefore, we need to understand how the TSR is brought about, and how it impacts on the ecology of ectothermic species.

Despite the ubiquity of the TSR, the effect of temperature on organism body size remains poorly understood. There has recently been a significant amount of work on how the TSR is established in multicellular organisms, with studies investigating how and when size changes occur during the life cycle (Karan et al., 1998; Petersen et al., 2000; Forster and Hirst, 2011; Potter et al., 2011). Further, differences in the thermal sensitivities of growth and development rates during ontogeny have been shown to drive and maintain the TSR in multicellular organisms (Forster et al., 2011a, b). Currently, such empirical study of how the TSR is established in unicellular organisms does not exist.

A simple conceptual model has demonstrated that the mechanism underpinning the TSR must be different in unicellular and multicellular organisms (Forster et al., 2011a). This difference is highlighted by the equation that links size and rates

\[
g \frac{D}{D} = \ln \frac{M_A}{M_p}
\]

where \(g\) is the mass-specific growth rate of the individual (day\(^{-1}\)), \(D\) is the development rate (day\(^{-1}\), that is, 1/doubling time), \(M_A\) is the mass of
Unicellular temperature-size changes
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the adult and \( M_p \) is the mass of a single progeny. We use the term ‘adult’ here to refer to the mass of a mother cell at the point of division in unicells. Similarly, ‘progeny’ (referring to eggs in multicellular organisms) refers to a single daughter cell just after division of the mother cell. We use the exponential form of the model in Equation 1 (Forster et al., 1986). In multicellular organisms, changes in size have been shown to differ in adults and progeny. Size changes in acclimated adults are \( \sim 2.5\% \, ^\circ C^{-1} \) but \( <1\% \, ^\circ C^{-1} \) in progeny. This cannot be the case in unicells: dividing in half requires the TSR to equally impact mother and daughter size in unicells at acclimation. This in turn means the rates driving the TSR, growth and development, can only become temporarily decoupled during acclimation in unicells (Figure 1). This temporary decoupling suggests a fundamentally different mechanism of the TSR in unicellular compared with multicellular organisms, where rates remain decoupled (Forster et al., 2011a), despite both groups obeying the TSR. Currently, this disparity between uni- and multicellular organisms remains theoretical: we still require testing of changes in mother and daughter size during the acclimation phase in unicellular organisms. Studies of unicellular organisms typically allow species to acclimate to new temperatures before carrying out size measurements (for example, five generations; Montagnes and Franklin, 2001). However, we need to understand when, and for how long, mother and daughter sizes become decoupled. Such research will show whether fundamental life-history rates relevant to all living organisms, growth and development (Equation 1), respond differently to temperature in different groups. We carry out this research here by measuring cell size changes in the ciliated protozoan Cyclidium glaucoma during the thermal acclimation. Further, including parameters for temperature, time and population abundance within a general linear model (GLM), we can ascertain and account for the impact of population abundance on cell size during the acclimation and thus singularly determine the importance of temperature in determining cell size.

Along with understanding how size changes are brought about in unicells at an individual level, we need to understand the potential impact of the TSR on carrying capacity and biomass. There have been few studies examining the impact of temperature on these traits in unicellular organisms: previous ecological theory predicts carrying capacity (defined here as the number of organisms supported in a given volume) to decrease with increasing temperature, following an Arrhenius function (Savage et al., 2004), thus we may expect maximum number of individuals in a culture to decrease with increasing temperature. Further, mesocosm experiments investigating the impact of temperature on freshwater phytoplankton found higher temperatures to be associated with a reduced total biomass (Yvon-Durocher et al., 2011). However, this was not conducted at an individual species level, thus we do not know whether changes in biomass are related to shifts towards smaller species or intraspecific size changes. Here, we compare carrying capacity and biomass (the product of carrying capacity and mean cell volume (MCV)) in C. glaucoma across a range of temperatures to see whether these traits do indeed scale negatively with temperature.

Using the ciliate species C. glaucoma, we shall therefore address the following questions: How do mother and daughter cell sizes change when acclimating to a novel thermal environment? Does carrying capacity scale negatively with temperature in C. glaucoma? Finally, how does temperature impact on the maximum biomass of C. glaucoma populations?

Materials and methods

The protist species C. glaucoma was chosen due to its short generation times; having a standard...
geometric shape, such that cell volumes could be accurately determined from length and width measurements; and individuals undergoing fixation in formalin maintaining an excellent cell structure. Ten sterile culture flasks (Corning 30 ml flasks) were prepared for *C. glaucoma*, using 30 ml of sterilised 30% filtered sea water, passed through a 0.20-μm Minisart filter. To each flask, one wheat grain, cut into two, was added. Wheat grains were boiled vigorously for 3 min before their addition to sterilise them. In all, 500 μl inoculum was then added to each of the 10 sterile culture flasks. Batch cultures of *C. glaucoma* used to inoculate these flasks were originally isolated, cloned and established in culture for a previous investigation (Finlay et al., 2006). These batch cultures were maintained at 17 °C for 2 weeks before experimentation. To allow the initiation of growth of the cultures, inoculated *C. glaucoma* flasks were maintained at 17 °C for a further 10 days, in accordance with pilot experiments, which indicated this was the minimum lag phase associated with this species. After this time, cultures were placed at a range of temperatures (8, 13, 17, 21 and 25 °C), with two flasks as replicates at each temperature. In all, 500 μl subsamples were taken from each culture over the course of 2 weeks. During the first 3 days, samples were taken every 4 h to obtain high temporal resolution results, and during this period we assumed acclimation would be most likely. Frequency of subsampling was then gradually reduced over the remainder of the 2-week period, such that the final subsamples were taken 3 days apart. *C. glaucoma* samples were preserved with 50 μl formalin for later counting.

**Size, temperature and population abundance**

Subsamples were analysed to calculate population abundances and cell volumes. Each was placed on a Sedgewick Rafter cell, which divides each sample into squares with 1 μl3 volumes. For each subsample, the numbers of individuals were counted in 50 randomly selected squares, and the mean number of cells calculated per 1 μl3. Thirty individual cells were randomly selected, photographed under a ×100 magnification optical microscope and then measurements of length and width (μm) made from these photographs using QCapture Pro (QImaging Software). Cell volume was calculated for each individual, assuming a standard geometric shape (prolate spheroid). Having estimated cell volumes for each individual, MCVs were calculated for the 30 cells per sample. MCVs and population abundances were subsequently plotted against time for each temperature and replicate. Doubling rates were calculated during the log period of growth, assuming mortality to be negligible during this phase. Doubling rates were calculated for each replicate as the slopes of natural logged population abundance vs time divided by ln(2). Further, to determine the relationship between MCV and population abundance, temperature and time, we constructed a GLM:

\[
\text{ln MCV} = \text{ln } t + \text{ln } P + T + (\text{ln } P) \times T
\]

where MCV = mean cell volume (μm3), \(t\) = time (hours), \(P\) = population abundance (cells μl-1) and \(T\) = temperature (°C). MCVs, time and population abundance were natural logged to maintain the homoscedasticity of residuals. Further, these equation forms for each parameter were shown to fit the data well (Figure 2) and thus deemed appropriate models. Using these parameters, we determined whether each parameter had a significant effect on MCVs. When the interaction term (ln \(P\) \times \(T\)) was not significant (\(P<0.05\)), this was removed from the GLM.

Mother cell sizes were calculated from the mean size of the 6 largest cells per 30 cell sample. Similarly, daughter cell sizes were calculated from the mean size of the smallest 6 cells. As data were normally distributed in each sample of 30 cells at a particular time interval, this approximately estimates size data >1 s.d. away from the mean value (~20% of data in each tail). Moreover, the estimates for daughter cell sizes were similar to those for cells

![Figure 2](https://example.com/figure2.png)  
*Figure 2*  
*Cyclidium glaucoma* 3D scatter plots of ln MCV vs (a) ln time (t, hours) and temperature (\(T\), °C); (b) temperature (\(T\), °C) and ln population abundance (\(P\), individuals μl-1). Meshes indicate best fit models using equation forms applied to the data in the GLM, that is, ln MCVs vs ln \(t\), ln \(P\) and \(T\). Meshes provide good fits to the data (\(P<0.0001\) for all parameters).
on the verge of division, photographed and calculated in pilot studies. Using these measurements for mother and daughter volumes, we similarly applied the GLM (Equation 2) to the data. We found a clear impact of population abundance upon the cell sizes of the organisms in the cultures. During the temperature-acclimation period, population size was changing (log phase of population growth). We wished to remove the impact of this from the cell size change, such that the thermal acclimation could be calculated singularly. We corrected data to the population abundance, ensuring we were correcting only for population size and not temperature by using the slope of population abundance for the GLM applied to 17 °C only, as this was the culture in which temperature was in effect unchanged. Using the slope constant for population abundance (−0.233), cell volumes were all corrected to the temperature-independent carrying capacity (13.4 cells μL⁻³). Corrected cell volumes were subsequently plotted against temperature (across all treatments) at each observation time point, and the linear regression for each used to calculate percentage volume changes from that at 15 °C (following Atkinson et al., 2003). These percentage changes in volume were then plotted against time. Acclimated data were determined from working from the last observation point to the point at which 95% confidence intervals (CIs) for a prior observation point no longer overlapped. Using this point of acclimation, we calculated acclimated population abundance-corrected MCVs for each replicate. The time to cell size acclimation for each temperature and replicate was determined as being the first observation point where MCV was not significantly different from the acclimated MCV.

The TSR, carrying capacity and maximum biomass
To compare our temperature-size results with those for the many protists described in the meta-analysis conducted by Atkinson et al. (2003), we calculated acclimated MCVs at carrying capacity, and calculated the linear regression line between these values and temperature. We calculated the carrying capacity for each replicate, defining this as the point at which the slope of ln population abundance vs time was not significantly different from zero (one-way analysis of variance). We compared these carrying capacities across the different experimental temperatures. We also calculated the product of carrying capacity (cells μL⁻³) and MCV at this peak to determine the maximum biomass, and examined whether a decrease in cell size was associated with a concomitant decrease in maximum biomass.

Results
Size, temperature and population abundance
MCV increased with time when subjected to temperatures less than that at which they had previously been acclimated (that is, 17 °C), while MCV decreased with time at temperatures greater than this, thereby following the TSR. Changes in MCV were also associated with changes in population abundance. During the exponential growth phase, these changes in population abundance were accompanied by decreased MCV, suggesting a negative relationship between the two (see example in Figure 3). Application of the GLMs to ln MCVs revealed that all three factors (Equation 3) had a significant effect on MCV (P < 0.001 in all cases). Population abundance had a negative effect on size, though the interaction term between population abundance and temperature was not significant (P = 0.73). This suggests that the effects of population abundance and temperature on MCVs are additive and therefore independent of one another.

Mother and daughter size
Having applied corrected mother and daughter size data (to remove the effect of population abundance on cell sizes), the percentage change in volume (from that at 15 °C) showed temperature acclimation in both mother and daughter size. The temperature dependence of mother and daughter size was determined as being acclimated at ~70.5 and ~120 h, respectively (Figure 4). Once daughters became acclimated, there was no discernible difference between the percentage change in volume in mothers or daughters (Figure 4).

The TSR, carrying capacity and maximum biomass
C. glaucoma followed the TSR, with a linear MCV change of −3.6% (CIs ±0.45%) per °C (from the size at 15 °C, Figure 5a). The time at which MCV became acclimated corresponded to a point at or near the carrying capacity, except at 13 °C, where individuals

![Figure 3](https://example.com/figure3.png)
exhibited poor population growth in both replicates. These data were confounded by low population abundances and were therefore excluded from the TSR calculation. This linear decline in size pattern was present across the entire thermal range, from 8 to 25 °C (Figure 5a). Carrying capacities were reached at 8, 17, 21 and 25 °C. There was no significant regression slope between population abundance and temperature (Figure 5b). The peak biomass was similarly plotted against temperature (Figure 5c); the highest biomass was associated with the lowest temperature, showing a linear decrease with increasing temperature (Figure 5c).

Discussion

The unicellular organism *C. glaucoma* followed the TSR, with acclimated cells showing a negative linear decline in the volume (from the size at 15 °C of 3.6% (± 0.45% CIs)) per 1 °C increase in temperature (Figure 5a). This change in cell volume is not significantly different to the meta-analysis of protist size responses made by Atkinson *et al.* (2003), which showed cell size to change linearly by approximately −2.5% °C−1 (± 0.78% CIs) from the volume at 15 °C. Further, there was no significant difference between the TSR in autotrophic and heterotrophic species in their study. *C. glaucoma* therefore appears to be a reasonable unicellular organism in which to study the establishment of the TSR, on the basis that the degree to which it changes size with temperature is fairly typical.

Data for mother and daughter size revealed thermal acclimation to occur rapidly, this being
reached within \( \sim 70.5 \) and 120 h, respectively (assessed as the point when the temperature-size response was not significantly different from acclimated response). These acclimated percentage mass changes required the slope of temperature vs cell size to reach a constant. Given the doubling time at 8°C was 165 h, this lowest temperature treatment likely limited how long the TSR took to acclimate. Even so, it appears that size acclimation was intra-generational. Size changes were subsequently maintained beyond the point of acclimation. Although size changes are seen in both mother and daughter, we found that the daughters took longer to reach an acclimated state than did mothers (Figure 4). This is likely to be due to size changes in daughters being dependent on maternal cell size change, with a lag before the population of daughter cells became acclimated. This is the first study, of which we are aware, where the period over which thermal acclimation of size in a unicellular organism has been determined, and one of few studies which has focused on acclimatory changes associated with novel environments (salinity acclimation: Finlay et al., 2006). Our results have important implications with regard to Equation 1: we confirm that individual growth and development rates (doubling rates) do indeed become only temporarily decoupled, while the ratio of mother to daughter cell size becomes re-coupled after \( \sim 120 \) h (Figure 4).

The significant decoupling of growth and development during thermal acclimation (during 0–120 h, Figure 4) has not been recorded previously in unicells. The changes in cell size we have shown during the log phase of population increase of this protist may have important implications upon other studies relying upon data from this same phase. For example, growth rates of many protozoa are commonly calculated from measurements of increase in cell numbers, as doubling rates during the log growth phase (for example, Eppley, 1972; Rose and Caron, 2007). The implicit assumption is that a doubling of numbers is associated with a doubling of biomass (with constant cell size). This may not be a complete description of growth, as it does not consider the MCVs, which change considerably with conditions (temperature and population abundance here). Moreover, classic studies used to estimate field growth rates use dilution experiments and subsequent doubling rates (Landry and Hassett, 1982). Again, there may be problems here if cell size is changing and increase in numbers is no longer coupled to an increase in total biomass. Furthermore, the fact MCVs change with temperature and population abundance will cause discrepancies between growth rates estimated from fluorescence (which will presumably be impacted by cell size change) vs those estimated from cell numbers. This highlights the importance of identifying the decoupling of growth and doubling rate during the acclimation, as found in this study.

Our results regarding size and rate acclimation to temperature reveal both similarities and differences between uni- and multicellular organisms. Both groups display rapid thermal acclimation: \( C. \text{ glaucoma} \) appears thermally acclimated in size within one generation. Similarly, multicellular organisms appear to acclimate their size to their thermal environment within a single generation (Karan et al., 1998; Forster and Hirst, 2011). Further, these size changes are subsequently maintained in \( C. \text{ glaucoma} \) across multiple generations, which have also been found in multicellular organisms (Karan et al., 1998; Forster and Hirst, 2011; Potter et al., 2011). However, relative size changes differ between these two groups. \( C. \text{ glaucoma} \) shows acclimated size changes in daughters and mothers which are not significantly different from one another \(-4.07\% \text{ C}^{-1} \) (CIs ± 0.34%) and \(-3.76\% \text{ C}^{-1} \) (CIs ± 0.28%), respectively, Figure 4) whereas acclimated progeny size in multicellular organisms are commonly less temperature dependent than adult sizes (Forster et al., 2011a), resulting in acclimated development and growth rates having different temperature dependence (Forster et al., 2010a,b).

The TSR in unicellular organisms

What does this mean for our understanding of the TSR? Proximate mechanisms previously suggested to explain the TSR have been driven by either decoupling of growth and development rates (Sibly and Atkinson, 1994; van der Have and de Jong, 1996; Walters and Hassall, 2006), or different thermal sensitivities of anabolism and catabolism (Strong and Daborn, 1980; Perrin, 1995; Woods, 1999; Karl and Fischer, 2008). As the ratio \( \frac{M_s}{M_i} \) changes with temperature in multicellular organisms, growth and development (and possibly anabolism vs catabolism) have to be decoupled and have different temperature dependence across multiple (acclimated) generations (Forster et al., 2011a). However, this cannot be the case in protists, as sizes return to an acclimated state, and \( \frac{M_s}{M_i} \) becomes a constant (Figure 4). This requires \( g/D \) to also be a constant across temperatures when acclimated (Equation 1). Our results provide empirical evidence to support the conceptual scheme of Forster et al. (2011b), thus unicellular species achieve size changes associated with temperature through different mechanisms than those used by multicellular species. Across multicellular organisms, size changes have been shown to be brought about through changes in cell size (van Voorhies, 1996; Stelzer, 2002), changes in cell number (Noach et al., 1997; Arendt, 2007) or both (French et al., 1998; Blanckenhorn and Llaurens, 2005). Even within an organism, specific size changes have been shown to occur in some cell types but not others (Atkinson et al., 2006). These differences suggest there is no single universal proximate mechanism to explain the TSR in uni- and multicellular organisms, thus we are led to...
suggest that the TSR is an adaptive response, that is, that smaller size at warmer temperatures confers a fitness advantage to the majority of the species. The next important step is to determine why being smaller at warmer temperatures is advantageous.

Size, temperature and population abundance
Along with temperature-driven size changes in *C. glaucoma*, we found population abundance to significantly affect cell sizes. The negative correlation between cell size and population abundance was present across the entire exponential growth phase at higher temperatures (for example, Figure 3). If increased population abundances are associated with decreased MCVs in *C. glaucoma*, then how do we know there is a true temperature-size effect and that size changes are not simply driven by increasing population abundances? We find from the GLM that temperature has a significant effect on size, independent of population abundance, as there was no interaction between these two parameters. Therefore, these factors independently drive size changes. Indeed, along with *C. glaucoma*, decreasing MCV with increasing population abundance has previously observed in the dinoflagellates *Alexandrium ostenfeldii* (Jensen and Moestrup, 1997) and *A. tamarense*, and four diatom species (Prakash et al., 1973), suggesting this may be common in protists.

Why do MCVs change with population abundance in *C. glaucoma*? One factor potentially driving size changes is food concentration: as population abundances increase, food concentrations decrease and MCVs are reduced. Many studies have been conducted observing predator–prey interactions using protists as model organisms. In these predator–prey interactions, we find a cyclical population response, where protist populations increase to a peak and then crash as food becomes scarce (Sharon and Morin, 1993). If decreased food concentrations were driving the changes in size associated with the exponential growth phase seen in *C. glaucoma*, then we would expect to see a crash in population abundance following a peak. However, when we observe replicates 17°C B and 25°C A, where population abundances peak within the first 100 h of the experiment, we see no such crash in population abundance over the subsequent 500 h, suggesting food concentrations can support these population abundances and thus size changes are not driven by food shortages. Also, once cultures reached carrying capacity, MCVs are maintained (for example, from 200 h onwards, in Figure 3), whereas one would predict continued cell size reduction if food became more limiting. Further, a previous study investigating temperature-food interactions on cell volumes of *Oxyrrhis marina* (Kimmance et al., 2006) found an interactive effect between food and temperature, yet our GLM found no significant interaction between population abundance and temperature, again suggesting changes in size related to population abundance were not food driven.

The fact that cell sizes do change with population abundance in *C. glaucoma*, and have been shown to change during the exponential growth phase in a number of other protist species (Prakash et al., 1973; Jensen and Moestrup, 1997), has important implications for our understanding of the TSR. First, it shows a highly plastic response in cell volume in protist species; cell size can respond rapidly to changes in environmental conditions and population abundances. Second, it shows that measuring protist species’ size during the exponential growth phase is likely to be associated with high variability in MCVs, driven by the additive effects of changes in population abundance and temperature. As Jensen and Moestrup (1997) pointed out, this exponential growth phase is not indicative of “balanced growth in a constant environment”. A previous meta-analysis of protist data attempted to resolve this problem by only recording cell size data taken during the log phase of population growth (Atkinson et al., 2003), yet we suggest this phase to be associated with high variability of MCV, driven by population abundance.

The TSR, carrying capacity and maximum biomass
The carrying capacity data for *C. glaucoma* revealed no clear pattern relating this to temperature (Figure 5b). Although the temperature dependence of intraspecific carrying capacity has received little attention, previous theoretical work focused on carrying capacity (number of cells per volume) predicts this to scale negatively with temperature (Savage et al., 2004), in line with the relationship found at an interspecific level. Similarly, interspecific data have been used to predict a universal decline in carrying capacity with increased body mass (Belgrano et al., 2002), in line with the metabolic theory of ecology (Brown et al., 2004). Such predictions for the impact of temperature and body size on intraspecific carrying capacity are not supported in this study on *C. glaucoma*. We find carrying capacity to be temperature and size independent: we therefore suggest extrapolating this to the intraspecific relationships should be conducted with caution.

Although carrying capacity does not change with temperature, this is not the case for biomass. As biomass is a product of size (Figure 5a) and population abundance (Figure 5b), the lowest temperature was associated with the highest biomass values, with a linear decrease in maximum supported biomass as temperatures increased, due to the temperature-size response of MCV (Figure 5a). This provides potential clues as to the adaptive nature of the TSR in unicellular organisms. If the number of individuals which can be supported is temperature invariant, then maximising reproductive rate at the
cost of individual cell size with increased temperature will maximise fitness, as faster-reproducing individuals will make up a greater proportion of the final population (the law of ‘compound interest’, Atkinson et al., 2003).

This study demonstrates rapid, intra-generational responses in a unicellular species’ size to changing temperature. This shows fundamental differences between uni- and multicellular organisms. Further, population abundance is found not to be temperature dependent but maximum biomass decreases with temperature, driven by individual cell size change in line with the TSR. Data focusing on the interaction between cell size, population abundance and temperature during acclimation next require an extension beyond heterotrophic Protista, both at the intraspecific and at the food web level to determine the potential impacts of warming on size, population abundance and biomass.

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References


